

# PROCEEDINGS



Pour un secteur oléicole rénové, rentable et compétitif en Méditerranée

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For a renovated, profitable and competitive Mediterranean olive growing sector

Edité par:

B. KARRAY (IO, Tunisie)

J. KHECHAREM (IO, Tunisie)

S. ROUSSOS (IRD, France)



# **PROCEEDINGS OLIVEBIOTEQ 2009**

**SFAX-TUNISIE**

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## Présentation

Le premier séminaire sur les Biotechnologies et qualité des produits de l'olivier dans le bassin méditerranéen (Olivebioteq-2004) a eu lieu à Errachidia au Maroc en novembre 2004 ([www.olivebioteq.ird.fr](http://www.olivebioteq.ird.fr)). Le deuxième Olivebioteq a eu lieu à Palermo en Sicile Italie en octobre 2006 et le 3<sup>ème</sup> Olivebioteq aura lieu à Sfax Tunisie. L'Institut de Recherche pour le Développement (IRD) et plus particulièrement la Délégation à l'Information et à la Communication (DIC) ont participé à la création et à l'essor de ce séminaire de formation permanente des principaux acteurs méditerranéens en oléiculture.

L'histoire de la culture de l'olivier se confond avec celle de la méditerranée au point que l'olivier est devenu le symbole de cette région dans le monde. La gestion et la mise en valeur de l'espace agricole méditerranéen sont de fait fortement liées à la culture de l'olivier et aux conséquences économiques et sociales de la production, de la transformation et de la commercialisation de l'huile d'olive. L'oléiculture traditionnelle prédomine dans la plupart des pays méditerranéens, malgré l'émergence depuis le début des années 1980 d'une oléiculture moderne conduite en irrigué, en intensif et en hyper intensif.

L'évolution des filières huile d'olive dans les différents pays est liée au progrès technique et les politiques mises en œuvre pour favoriser l'organisation de ces filières afin de tirer le meilleur parti des potentialités des marchés. Elles ont bénéficié durant les dernières décennies de plusieurs types d'innovations technologiques résultant des travaux de recherche et des politiques de promotion, de formation, d'incitation et de vulgarisation. Ces innovations se retrouvent dans les principaux maillons des filières: exploitations agricoles, huileries, usines de conditionnement et circuits de distribution.

Malgré l'adaptation de l'olivier aux conditions naturelles et à l'écologie méditerranéennes et les efforts d'innovation déployés par la plupart des pays producteurs, le devenir de ce secteur reste tributaire notamment de la dynamique du marché mondial, des nouvelles dispositions en matière de politique agricole et commerciale au niveau multilatéral (PAC, Cycle de Doha, Accords de libéralisation bilatéraux et régionaux), ainsi que de la structure et du fonctionnement des systèmes de production et d'exportation des différents pays oléicoles.

La plupart des pays méditerranéens se trouvent actuellement dans un contexte économique incertain au sein duquel la prise de conscience des enjeux d'avenir, la rénovation des systèmes de production et d'exportation et l'identification de nouveaux usages et alternatives de valorisation des produits de l'olivier (nutrition, santé, cosmétique, énergie, environnement...) sont devenues impératives afin de renforcer le rôle économique, social et environnemental et la compétitivité de l'olivier.

Pour enrichir le débat autour de cette problématique, l'Institut de l'Olivier organise la troisième édition d'Olivebioteq sur le thème **"Pour un secteur oléicole rénové, rentable et compétitif en Méditerranée"**. L'objectif principal de cette édition est de faire connaître et de discuter les acquis de recherches et d'examiner les alternatives de leur valorisation pour l'innovation de la filière huile d'olive en Méditerranée.

La troisième édition d'Olivebioteq a lieu du 15 au 19 décembre 2009 à Sfax (Tunisie). Elle est organisée en collaboration avec des organismes nationaux (l'ONH, AVFA, DGPA, DGPCPQA, OTD, UTAP, CC, APIA, API et CEPEX) et internationaux (COI, IRD, FAO, Commission Européenne, CIHEAM, AFIDOL).

## Presentation

The first seminar on Biotechnology and olive products quality in the Mediterranean (Olivebioteq-2004) has been celebrated in Errachidia, Morocco in November 2004 ([www.olivebioteq.ird.fr](http://www.olivebioteq.ird.fr)). The second Olivebioteq has been celebrated in Palermo, Italy in October 2006 and the 3<sup>rd</sup> Olivebioteq will take place in Sfax, Tunisia. The IRD and the DIC (Délégation à l'Information et à la Communication) were the main participants in the creation and the design of this olive-growing training seminar of principal Mediterranean actors.

The history of the olive tree can be confused with the Mediterranean one at the point to become the symbol of this region in the world. The management and enhancement of agricultural Mediterranean area are strongly related to olive growing and the economic and social consequences of production, processing and commercialisation of olive oil. Despite the emergence since the early 1980s of modern olive cultivation regimes (intensive and hyper-intensive), traditional olive cultivation continues predominating in most Mediterranean countries.

The evolution of olive oil sector in different countries is basically related to technical progress and policies implemented to promote their organisation and to maximize their potential. In fact, in the recent decades, they have benefited from several types of technological innovations as a result of scientific research and policies of promotion, training, encouragement and technical support. These innovations can be observed in the major segments of the olive sector: farms, mills, packaging factories and distribution channels.

Despite the adaptation of the olive tree to natural conditions of the Mediterranean and despite the innovative efforts made by most producers, the future of this sector is particularly dependent on: i) the dynamics of the world market, ii) the new provisions of multilateral agricultural and trade policies (CAP, the Doha Round, bilateral and regional liberalisation agreements), and iii) the structure and functioning of production and export systems of olive producing countries.

Presently, most Mediterranean countries are in an uncertain economic situation in which the consciousness of future challenges, the renovation of production and export systems, the identification of new uses and the alternatives of olive products valorisation (nutrition, health, cosmetics, energy, environment ...) have become imperative to strengthen the economic, social and environmental performance and the olive competitiveness.

To enrich the debate around these issues, the Olive Tree Institute (Institut de l'Olivier) organizes the third edition of Olivebioteq on the theme "For a renovated profitable and competitive Mediterranean olive growing sector." The main objective of this edition is to expose and discuss the results of scientific research and the way to make them useful for the innovation of the Mediterranean olive oil sector.

The third edition of Olivebioteq will take place from 15 to 19 December 2009 in Sfax (Tunisia). It will be organized in collaboration with national organisations (ONH, AVFA, DGPA, DGPCQPA, OTD, UTAP, CC, APIA, API and CEPEX) and international ones (IOOC, IRD, European Commission, CIHEAM, AFIDOL).



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# Summary

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# **COMMUNICATION ORALE**

## **Thème 1**

**Patrimoine oléicole méditerranéen :**

**Biodiversité, potentialités et perspectives de préservation  
et d'amélioration**

## Origines et processus de domestication chez l'olivier, *Olea europaea* L., dans le sud ouest de la méditerranée : relations génétiques entre l'olivier au Maroc et dans la méditerranée

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### Résumé

Deux hypothèses sont formulées pour expliquer la diversification variétale de l'olivier au Maroc : i) une prédominance des variétés de l'est de la Méditerranée, ii) une sélection à partir des formes de l'est mais introgressées par le pool génétique local. Les relations génétiques entre 75 génotypes du Maroc, 343 accessions méditerranéennes et 43 variétés libanaises, en utilisant 12 marqueurs microsatellites et le polymorphisme de l'ADN chloroplastique, ont été étudiées. Les analyses bayésiennes à l'aide du programme « Structure » montrent que les variétés marocaines sont essentiellement classées dans deux groupes génétiques parmi les 6 identifiés au sein de l'olivier méditerranéen. Le premier groupe comprend essentiellement les variétés du sud et du centre du Maroc, alors que le second groupe correspond à des variétés du Maroc avec la « Picholine marocaine » ayant la lignée maternelle de l'est de la Méditerranée, mais également de l'Espagne. Ces résultats soutiennent l'hypothèse selon laquelle l'olivier cultivé au Maroc résulte à la fois d'une domestication primaire à partir de populations locales et d'une diversification secondaire à partir de formes de l'est de la Méditerranée introgressées par le pool génétique local.

**Mots clés:** *Olea europaea* L., structure génétique, SSRs, polymorphisme ADN chloroplastique. Méthodes d'analyse bayésiennes.

### Origins and domestication process of olive, *Olea europaea* L., in south west Mediterranean areas: A study of genetic relationships between Moroccan and Mediterranean olive

### Abstract

Two hypotheses are proposed to explain olive diversification processes in Morocco: i) the most introduced varieties from the East Mediterranean, ii) selection from the introduced Eastern varieties introgressed by a local gene pool. Genetic relationships between 75 genotypes from Morocco and 343 accessions from the Mediterranean olive germplasm and 43 varieties from Lebanon were studied using 12 microsatellite loci and chloroplast DNA markers. Based on the bayesian methodology using the Structure software, varieties from Morocco are classified in two clusters among the six identified in Mediterranean olive. The first one is mainly composed by varieties from the South and the centre of Morocco. The second includes varieties from Morocco with Eastern maternal lineage and from Spain. These results support the hypothesis that cultivated olive in Morocco results from local domestication and secondary diversification from introduced Eastern varieties introgressed by a local gene pool.

**Keywords:** *Olea europaea* L., domestication, genetic structure, SSRs, chloroplast DNA polymorphism, bayesian model clustering analysis.

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## 1. Introduction

Les oliviers cultivés et leurs parents sauvages "l'oléastre" représentent deux variétés botaniques d'*Olea europaea subsp. Europaea*, var. *europaea* et var. *sylvestris*, respectivement. Les études génétiques et archéobotaniques antérieures ont montré l'existence de populations d'oléastres dans l'Est et l'Ouest méditerranéen avant le néolithique (Lumaret et Ouazzani, 2001 ; Besnard *et al.* 2002; Terral *et al.* 2004; Khadari 2005; Breton *et al.* 2006). La domestication de l'olivier aurait eu lieu dans plusieurs foyers indépendants dans le bassin méditerranéen. La lignée maternelle du *pool* génétique de l'Est est majoritaire dans les variétés méditerranéennes, résultat de migrations humaines essentiellement d'Est en Ouest (Besnard *et al.* 2002; Khadari, 2005; Breton *et al.*, 2006).

En dépit de ces travaux, les origines et les processus de diversification à l'échelle locale demeurent méconnus. Au Maroc, Khadari *et al.* (2008) ont montré une diversité génétique importante des oliviers cultivés malgré la dominance d'une seule variété, « Picholine marocaine ». Sur la base du polymorphisme de l'ADN nucléaire et chloroplastique, les populations d'oléastres marocaines sont génétiquement distinctes des formes cultivées suggérant la coexistence entre des formes locales et introduites mais probablement introgressées par le *pool* génétique local (Khadari, 2005). Cependant, l'importance de cette introgression reste méconnue, ce qui nous amène à formuler ces deux hypothèses: i) les variétés du Maroc appartiennent aux différents groupes génétiques de l'olivier méditerranéen, ce qui traduirait des introductions du matériel allochtone sans échanges génétiques notables avec les populations locales; ii) les variétés du Maroc sont distinctes génétiquement des variétés méditerranéennes, résultat de la sélection locale des formes introduites de l'est méditerranéen introgressées par le *pool* génétique local.

Dans cette étude, nous présentons les résultats d'analyses génétiques de l'olivier cultivé au Maroc et des variétés méditerranéennes à l'aide des marqueurs microsatellites nucléaires et chloroplastiques selon une approche bayésienne. Nous discutons nos résultats en faveur de l'hypothèse d'une sélection locale et nous proposons un scénario sur les origines de l'olivier au Maroc.

## 2. Matériel et Méthodes

### 2.1. Matériel végétal

Les variétés méditerranéennes analysées correspondent à 343 accessions de la collection internationale implantée au domaine de Tassaout (INRA Marrakech) et à 43 variétés libanaises. Le matériel végétal marocain étudié comprend 60 génotypes précédemment identifiés par Khadari *et al.* (2008) et 31 oliviers échantillonnés dans la région d'Ouazzane et Chefchaoun (nord du Maroc). Pour ces derniers, la stratégie d'échantillonnage était ciblée sur la collecte d'oliviers distincts de la variété dominante « Picholine marocaine » en se basant sur des observations phénotypiques et des informations données par les agriculteurs locaux et les techniciens agricoles.

### 2.2. Analyses moléculaires

Douze locus microsatellites nucléaires ont été sélectionnés parmi les 18 proposés par Elioth-Smith (2007) et 3 marqueurs chloroplastiques de type insertion-délétion ont été utilisés (Besnard *et al.* 2005). La réaction PCR et le génotypage à l'aide d'un séquenceur semi-automatique ABI Prism 3130 XL sont décrits par Khadari *et al.* (2008). La lecture et le codage des données moléculaires ont été réalisés à l'aide du logiciel Genemapper v.3.7 (Applied Biosystems).

### 2.3. Analyses des données

L'analyse factorielle de correspondance a été réalisée à l'aide du logiciel Genetix 4.0 (Belkhir, 1999). Dans le but de définir des groupes génétiques sans a priori sur les origines géographiques des variétés étudiées, nous avons adopté un modèle de groupement, selon une approche bayésienne, implémenté dans le programme Structure v. 2.2 (Prichard *et al.* 2000). La validation du nombre de groupes K est réalisée à l'aide de la statistique d'Evanos *et al.* (2005).

### 3. Résultats et discussion

#### 3.1. Polymorphisme nucléaire et chloroplastique

L'analyse de 477 oliviers méditerranéens et marocains à l'aide de 12 locus microsatellites a permis d'identifier 437 génotypes correspondant à un total de 176 allèles et une moyenne de 14.6 allèles par locus (Tableau 1). Le nombre total d'allèles obtenus est similaire à celui observé chez les oléastres méditerranéens (Breton *et al.*, 2006), ce qui indique que la diversité génétique de l'olivier cultivé n'est pas réduite par rapport à celle de l'oléastre.

La majorité des variétés méditerranéennes (94%) et des formes cultivées au Maroc (74%) sont caractérisées par la lignée maternelle de l'Est de la méditerranée, ce qui confirme les résultats des travaux antérieurs (Besnard *et al.*, 2002; Khadari, 2005; Breton *et al.*, 2006).

#### 3.2. Structure génétique des variétés méditerranéennes et marocaines

L'analyse multivariable montre une structure génétique est-ouest de l'olivier méditerranéen (Figure 1). L'olivier cultivé au Maroc est relativement distinct du reste. Les analyses à l'aide du programme Structure ont été réalisées selon le modèle « No admixture » en considérant l'absence de corrélation entre les fréquences alléliques au sein des populations. La statistique d'Evanos *et al.* (2005) montrent que la structure en 6 groupes est la plus vraisemblable (Haouane *et al.*, résultats non publiés). Sur la base d'une probabilité d'assignation à 80 %, 371 parmi les 437 génotypes définis, ont été assignés aux 6 groupes (Tableau 2).

**Tableau 1.** Nombre d'allèles (Nb), taille attendue et hétérozygotie pour chacun des 12 loci SSR (Hobs = hétérozygotie observée ; He = hétérozygotie attendue non biaisée)

| Locus | Allèles |            |        |        | Fis (Wc) |
|-------|---------|------------|--------|--------|----------|
|       | Nb      | T.attendue | Hobs   | He     |          |
| DCA4  | 29      | 124-193    | 0.6538 | 0.8752 | 0.253    |
| DCA9  | 22      | 162-211    | 0.9076 | 0.8772 | -0.035   |
| DCA15 | 09      | 231-266    | 0.6748 | 0.6393 | -0.056   |
| DCA5  | 12      | 192-214    | 0.4942 | 0.4819 | -0.025   |
| DCA3  | 12      | 222-260    | 0.8951 | 0.8430 | -0.062   |
| UDO36 | 14      | 134-168    | 0.7890 | 0.7326 | -0.077   |
| GAP59 | 12      | 207-241    | 0.6683 | 0.6390 | -0.046   |
| DCA18 | 18      | 155-207    | 0.8383 | 0.8398 | 0.002    |
| DCA8  | 20      | 125-166    | 0.9289 | 0.8239 | -0.128   |
| GAP71 | 13      | 117-166    | 0.9108 | 0.8447 | -0.078   |
| DCA11 | 23      | 126-183    | 0.7535 | 0.8201 | 0.081    |
| EMO90 | 10      | 178-208    | 0.7586 | 0.6491 | -0.169   |

Cette structure génétique montre une spécificité génétique d'une part à l'est de la méditerranée par le groupe des variétés du Liban et de la Turquie, et d'autre part dans le sud ouest. En effet, l'olivier cultivé au Maroc est classé en deux groupes : i) le premier comprend essentiellement les formes cultivées dans le sud et le centre du Maroc dont la moitié est caractérisée par les 2 lignées maternelles caractéristiques de l'ouest de la méditerranée ; ii) le deuxième groupe comprend en plus la Picholine marocaine, des oliviers cultivés dans le nord du Maroc et des variétés espagnoles. La composition de ce deuxième groupe s'explique par les échanges du matériel entre le nord du Maroc et la péninsule ibérique depuis au moins la présence romaine au Maroc. En revanche, le premier groupe est spécifique de l'olivier marocain même si la moitié des génotypes ont une lignée maternelle de l'est.

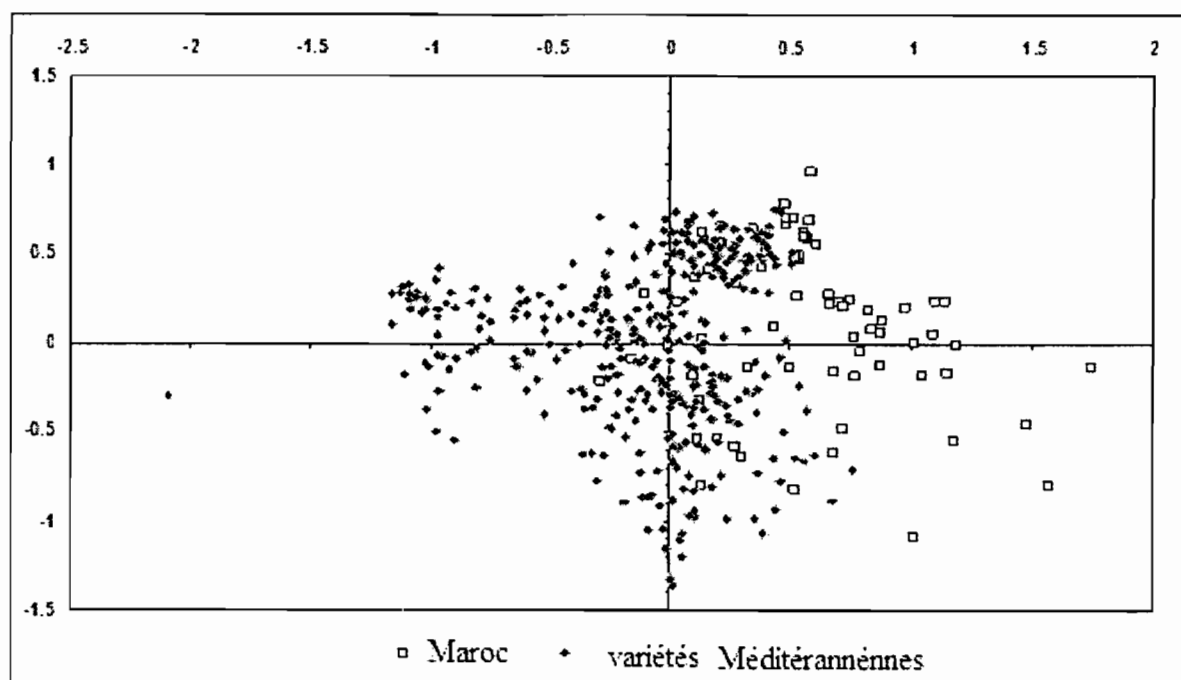


**Figure 1:** Analyse multivariable AFC (analyse factorielle de correspondance) basée sur les données SSR nucléaires. Comparaison des variétés marocaines et méditerranéennes.

Ce constat est probablement le résultat d'une sélection locale à partir de formes allochtones mais fortement introgressées par le pool génétique local.

**Tableau 2 :** Nombre de génotypes et proportion d'assignation des variétés par pays dans chacun des 6 groupes. Nombre de génotypes ; Na : nombre de génotypes assignés ; Ch-Tu : Turquie-Chypre.

| Pays     | N.  | Na. | Groupes de cultivars |    |    |    |   |    |
|----------|-----|-----|----------------------|----|----|----|---|----|
|          |     |     | 1                    | 2  | 3  | 4  | 5 | 6  |
| Maroc    | 69  | 68  | 40                   | 21 | 2  | 5  | 2 |    |
| Italie   | 160 | 139 | 15                   | 6  | 38 | 41 | 2 |    |
| Espagne  | 91  | 86  | 6                    | 64 | 9  | 5  |   |    |
| Grèce    | 14  | 6   | 1                    | 1  | 4  |    | 1 |    |
| Portugal | 13  | 12  | 1                    | 10 |    |    | 2 |    |
| France   | 13  | 12  |                      |    | 2  | 8  |   |    |
| Tunisie  | 6   | 4   |                      | 1  | 2  | 1  | 1 |    |
| Liban    | 23  | 21  |                      | 1  | 10 | 2  | 2 | 7  |
| Ch-Tu    | 25  | 23  |                      |    | 3  | 2  |   | 16 |

#### 4. Conclusion

Nos résultats suggèrent que l'olivier cultivé au Maroc est le résultat d'une domestication locale mais également d'une diversification secondaire. La validation de cette hypothèse nécessite d'analyser les relations génétiques entre l'olivier cultivé et l'oléastre et conduit à nous interroger sur le rapport entre la reproduction végétative et sexuée dans les processus de diversification locale de l'olivier.

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## Olive cultivar comparisons from around the world

Paul Vossen

### Abstract

Choosing the correct cultivar can make a huge difference in the profitability of an orchard. This review article shows that there are significant differences in cultivar performance based on: tree vigor, productivity, precocity, alternate bearing, oil content, fruit size, pit to pulp ratio, fatty acid composition, polyphenol content, sensory characteristics, cold hardiness, flowering and maturity dates, and disease susceptibilities. It shows how important it is to evaluate olive varieties from all over the world and to realize that there may be several cultivar choices with a potential to improve profits or create a different styled product. A review of the scientific literature from research in Spain, Italy, France, Morocco, Australia, and the USA shows that there are strong similarities in how most cultivars perform no matter where they are grown. This paper references work done in very diverse areas with a similar Mediterranean climate. The data indicates that some cultivars are consistently better than others and that many resident cultivars growing in specific geographical locations may be there only because other cultivars have not been adequately evaluated and subsequently adopted over time.

**Key Words:** olive oil, varieties, vigor, precocity, yield, flavor, hardiness, pollination, alternate bearing

### Résumé

Choisir le bon cultivar peut faire une énorme différence dans la rentabilité d'un verger. Ce compte rendu montre qu'il existe d'importantes différences dans la performance des cultivars basée sur: la vigueur des arbres, la productivité, la précocité, l'alternance de la production, la teneur en huile, la taille des fruits, la proportion de la pulpe au noyau, la composition en acides gras, la teneur en polyphénols, les caractéristiques sensorielles, la résistance au froid, les dates de la floraison et les dates de maturité, les sensibilités aux maladies. Cela montre qu'il est important d'évaluer les variétés mondiales d'olives et de réaliser qu'il existe plusieurs cultivars avec un potentiel d'améliorer les profits ou de créer un produit de style différent. L'étude de la littérature scientifique sur les recherches en Espagne, Italie, France, Maroc, Australie et Etats-Unis montre qu'il existe de solides similitudes dans la façon dont la plupart des cultivars se comporte, peu importe où ils sont cultivés. Ce document fait référence aux travaux effectués dans plusieurs endroits différents mais avec un climat méditerranéen similaire. Les statistiques indiquent que certains cultivars performant toujours mieux que d'autres et que les cultivars autochtones qui existent dans des emplacements géographiques particuliers ne sont là seulement parce que d'autres cultivars n'ont pas été suffisamment évalués, et en conséquence n'ont pas été adoptés.

## 1. Introduction

The choice of which variety/cultivar to grow is one of the most important decisions in growing any horticultural crop, but it is especially important in tree crops that live a long time and take several years to come into full bearing. That decision could haunt the farmer for many years and a great deal of time and energy could have been lost, if it was the wrong decision. Making the right choice, however, could mean years of profitable production from quality fruit. It is particularly difficult with olives since we know that the trees can live for a thousand years. Historically,



those long lived trees gave us many traditions and a passion for a certain style of oil or table fruit that was produced by our ancestors. The choice of what cultivar to plant, however, must be rational and take into consideration new information that has been developed over the last 25 years. It would be foolish to be content with what we had always done if there are better options. Previously, olive growers who were interested in planting a new orchard relied on a neighbor's advice or purchased plant material from a nursery, usually whatever the nursery had for sale. Over hundreds of years there was some selection that took place that weeded out the poor cultivars that did not have as many favorable characteristics. Much of that selection, however, was very local, without the benefit of having included other top selections from other regions or countries. Consequently, their real choices of cultivars were quite limited; see fig. A – the different look of ten cultivars from the germplasm repository in Córdoba, Spain.

Now we have many more choices due to greatly expanded worldwide information sharing and the diligent work by many researchers who have brought in cultivars from other areas and compared them side by side with their own traditional cultivars. This is a new phenomenon. Thirty years ago there were no Spanish cultivars in Italy or Greece or anywhere else outside Spain that were being compared to the regional cultivars and vice versa for Italian or Greek cultivars outside Italy or Greece. The Spanish had no idea how the Italian cultivars actually compared to their own and the Italians had never tried any Spanish cultivars. Everyone thought their own cadre of cultivars was somehow sacred. When cultivar trials were finally planted, it took a long time (20+ years) to really see how the trees performed over the long term. We are still learning from those cultivar trial plantings and many new ones have been established in the new world (California, Australia, Chile, Argentina, and South Africa). These non-traditional olive growing areas have no preconceived ideas as to which cultivars are better. Growers are simply choosing the cultivars that are the most productive, most cost efficient, and most desirable in the market.

The choice of cultivar often comes down to the bottom line, the economics of how much more profitable one might be than the other. Profitability can be influenced by how the cultivar yields and if it comes into bearing early or takes several more years while the farmer has no income. Yield is based on the raw tonnage of fresh fruit produced, but also the oil content of the cultivar and how much oil is easily extractable with the milling equipment that will be used. Oil sensory quality and keeping quality is also important. The market demand for specific oil styles and cultivars with certain characteristics can influence the choice of which cultivar to plant and grow. For table olives, producers need to be aware of fruit size, ease of hand harvest, pulp to pit ratio, ease of pit removal, appearance, and texture of the final processed product. Finally, the differences in the production costs from one cultivar to another can be extremely important since olives are a low margin crop and money wasted on unnecessary cultural costs is difficult to recover.

- |                                  |                      |                               |
|----------------------------------|----------------------|-------------------------------|
| • Vigor                          | • Oil Extractability | • Hand Harvestability (table) |
| • Precocity                      | • Oil Yield          | • Machine Harvestability      |
| • Fruit Yield                    | • Oil Flavor - style | • Drought Tolerance           |
| • Fruit Size (table)             | • Oil Stability      | • Cold Hardiness              |
| • Pulp to Pit Ratio (table)      | • Market Demand      | • Alternant Bearing           |
| • Oil Content Disease Resistance | • Pollination Needs  | • Appearance table            |
| • Salt Tolerance                 | • Maturity Date      |                               |

## 2. Factors affecting olive yield and oil yield

The amount of oil a producer gets per hectare depends primarily on the tonnage yield of fruit per unit area, which varies by cultivar, year, fruit set, irrigation, pruning, age of trees, etc. This seems straightforward, but it must be noted that oil yield and fruit yield do not necessarily increase at the same rate. This is because olive trees have an ability to produce more oil with an increased leaf-to-fruit ratio in years with a light crop. When there is less fruit on the tree, the greater numbers of leaves per fruit boosts the fruit's oil content, which partially makes up for lower fruit tonnage. In other words, there is not a perfect correlation between fruit yield and oil yield. The amount of oil that a producer gets from a given amount of fruit depends on many factors:

- **Oil content of the fruit** – varies by moisture content, year, amount of fruit on the tree, and cultivar
- **Extractability of the oil from the fruit** – varies by year, water content, fruit maturity, and cultivar
- **Extraction process** – varies by paste fineness; use of adjuvants, malaxation time and temperature; extraction equipment type, and efficiency - speed of the machinery.

## 3. Yield of olive

Yields per hectare can range from less than 1 to as high as 20 tons per hectare; a good consistent yield from year to year would be about 9 metric tons per hectare. Low yields usually can be related to a lack of shoot growth the previous year from poor tree vigor. This can be caused by inadequate irrigation or inadequate rainfall for dry farmed orchards, poor weed control, disease, very low fertility, inappropriate pruning, or a very high crop load the previous year. Low yields can also be caused by poor weather conditions during bloom, lack of chilling, frost damage, or inadequate flower pollination. Olives are strongly alternate bearing, so a low crop yield one year will likely promote more shoot growth, resulting in more flowers and higher yields the following year. High yields are produced consistently only from orchards that are very well managed. These yields all depend on the factors listed in table 1.

**Table 1:** Orchard yield projection scenarios for oil olives

| Fruit Yield               | Factors Affecting Yield                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
|---------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 2 TONS/ HA                | <ul style="list-style-type: none"> <li>• Medium-density orchard in the 5<sup>th</sup> – 6<sup>th</sup> year or older orchard with close spacing with shading problems.</li> <li>• Poor irrigation, weed control, pruning and nutrient management.</li> <li>• Excessively vigorous or weak growing conditions.</li> <li>• Poor pollination conditions from adverse weather during bloom, or inadequate pollinizer trees.</li> <li>• Alternate "off" year of production.</li> <li>• Super-high-density orchard in the 2<sup>nd</sup> year.</li> </ul> |
| 4 TONS/ HA                | <ul style="list-style-type: none"> <li>• Medium-density orchard in the 6<sup>th</sup> – 8<sup>th</sup> year or poor irrigation, weed control, pruning and nutrition.</li> <li>• Excessive shading. Alternate "off" year of production from very heavy production last year.</li> <li>• Excessively vigorous or weak growing conditions.</li> <li>• Poor pollination conditions from adverse weather during bloom, or inadequate pollinizer trees.</li> <li>• Super-high-density orchard in the 3<sup>rd</sup> year.</li> </ul>                      |
| 6 TONS/ HA                | <ul style="list-style-type: none"> <li>• Medium-density orchard in the 9<sup>th</sup> – 10<sup>th</sup> year without proper management.</li> <li>• Probable maximum yield from a coastal hillside orchard.</li> <li>• Some shading problems. Some poor weather during bloom or a lack of pollinizer trees.</li> <li>• Super-high-density orchard in the 3<sup>rd</sup> year.</li> </ul>                                                                                                                                                             |
| 9 TONS/ HA                | <ul style="list-style-type: none"> <li>• Medium-density orchard in the 10<sup>th</sup> year + with good irrigation, weed control, pruning and nutrition.</li> <li>• A great sustainable yield if everything is done right and nature cooperates.</li> <li>• Trees have the correct vigor and growing conditions.</li> <li>• Well managed super-high-density orchard in the 4<sup>th</sup> + years.</li> </ul>                                                                                                                                       |
| 11 TONS/ HA               | <ul style="list-style-type: none"> <li>• Medium-density orchard in the 10<sup>th</sup> year + with excellent irrigation, weed control, pruning and nutrition.</li> <li>• An excellent yield especially if it can be sustained each year.</li> <li>• Alternate "on" year of production from a low yield last year.</li> <li>• Perfect growing conditions and doing everything right.</li> <li>• Very well managed super-high-density orchard in the 4<sup>th</sup> + years.</li> </ul>                                                               |
| > 14 TONS/ HA             | <ul style="list-style-type: none"> <li>• Yield that cannot be sustained each year.</li> <li>• Medium-density orchard in the 10<sup>th</sup> year + with excellent irrigation, weed control, pruning and nutrition.</li> <li>• Alternate "on" year of production from a very low yield last year.</li> <li>• Perfect growing conditions and doing everything right.</li> <li>• Excellent management in a super-high-density orchard in the 5<sup>th</sup> + years.</li> </ul>                                                                        |
| Vossen, unpublished data. |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |

#### 4. Vigor – Precocity – Productivity – Fruit Weight – Oil content – Flesh to Pit Ratio

Vigor is the term used to define the amount or relative strength of the vegetative growth of the plant. It is measured in shoot growth, trunk diameter, tree volume, and sometimes in tree form. Precocity is the term used to define the length of time until the cultivar's first significant flowering and fruit set. It is measured in number of years from planting to harvest and the quantity of fruit from the first three harvests. Productivity is the amount of fruit or oil yield produced from a particular cultivar. It is measured in the average or maximum kilos per tree, tons of fruit per hectare, kilos of oil per hectare produced per year, average per year, or cumulative for a certain number of years. Sometimes the efficiency of production is measured based on kilos of fruit per cubic meter of tree volume, number of fruits per branch, or dry weight of fruit per branch. The measure of a cultivar's tendency to bear in alternate years is called the Alternance Index. It is a numerical value indicating the amount of increased or decreased crop yield to be expected from one year to the next; the larger the number, the more alternating the cultivar. Fruit weight is a measure of fruit size given in grams per fruit. Oil content is measured as a percentage on a dry or fresh (wet) weight basis. Flesh to pit ratio is a measurement of the pit size in relation to the amount of fruit flesh or pulp.

**Table 2:** Yield and alternance index of the picholine marocaine olive tree population and selected clones in marrakech, morocco (11 years - large 100+ year old trees)

| Cultivar                                                                            | Ave. Yield (kg/tree) | Alternance Index |
|-------------------------------------------------------------------------------------|----------------------|------------------|
| Haouzia                                                                             | 60                   | 0.67             |
| Menara                                                                              | 64                   | 0.60             |
| M26                                                                                 | 88                   | 0.50             |
| K26                                                                                 | 73                   | 0.81             |
| S19                                                                                 | 62                   | 0.70             |
| P. Marocaine                                                                        | 20                   | 0.90             |
| Belkassem Boulouha. 2006. National Agriculture Research Center, Marrakech, Morocco. |                      |                  |

**Table 3:** Evaluation of newly planted cultivar selections grown in marrakech,(1987-1997)

| Cultivar                                                                            | Ave. Yield (kg/tree) | Max. Yield | Oil Content (% dry basis) |
|-------------------------------------------------------------------------------------|----------------------|------------|---------------------------|
| Menara                                                                              | 31                   | 66         | 56                        |
| Haouzia                                                                             | 31                   | 60         | 48                        |
| Picholine                                                                           | 33                   | 70         | 47                        |
| Manzanillo                                                                          | 28                   | 80         | 36                        |
| Belkassem Boulouha. 2006. National Agriculture Research Center, Marrakech, Morocco. |                      |            |                           |

**Table 4:** Evaluation of several newly planted mediterranean olive cultivars grown in Marrakech Morocco (1988-1996)

| Cultivar                                                                            | Ave. Yield (kg/tree) | Max. Yield (kg/tree) | Foliar Disease (%) | Oil content (% dry basis) |
|-------------------------------------------------------------------------------------|----------------------|----------------------|--------------------|---------------------------|
| Arbequina                                                                           | 30                   | 54                   | 10                 | 37                        |
| Manzanillo                                                                          | 24                   | 56                   | 40                 | 42                        |
| Leccino                                                                             | 40                   | 83                   | 0                  | 36                        |
| Carolea                                                                             | 33                   | 55                   | 80                 | 37                        |
| P. Marocaine                                                                        | 25                   | 66                   | 70                 | 30                        |
| Picholine                                                                           | 30                   | 50                   | 0                  | 38                        |
| Blanqueta                                                                           | 30                   | 86                   | 20                 | 40                        |
| Sourani                                                                             | 05                   | 43                   | 0                  | 42                        |
| Ayvalik                                                                             | 04                   | 16                   | 20                 | 40                        |
| Belkassem Boulouha. 2006. National Agriculture Research Center, Marrakech, Morocco. |                      |                      |                    |                           |

Field observation and research in Morocco shows that the general population of 'Picholine Marocaine' cultivars could be greatly improved through clonal selection ('Haouzia' and 'Menara') giving greater productivity and less alternate bearing. These clonal selections also have a good content of oil ranging from 48-56% on a dry weight basis to about 23% on a wet weight basis. Several other introduced selections from Spain, Italy, Greece, and France showed superior overall productivity. New cultivars that have been recommended for Morocco, based primarily on productivity are 'Leccino', 'Picholine Languedoc', 'Blanqueta', and 'Arbequina' (Tables 2-7) (Boulouha, 1995, 2006; El Antari et al., 2003; Idrissi and Ouazzani, 2003).

**Table 5:** Various comparisons of mediterranean cultivars grown in Marrakech, Morocco

| Cultivar     | Olive Fly Damage | Fruit Wt. (g) | Free Acidity (%) | Total (ppm) Polyphenols | Oleic Acid (%) |
|--------------|------------------|---------------|------------------|-------------------------|----------------|
| P. Marocaine | 4-9%             | 3.78          | 0.37             | 300.2                   | 70-73%         |
| P. Languedoc | 0                | 5.12          | 0.36             | 234.8                   | 62-64%         |
| Manzanillo   | 4%               | 6.19          | 0.75             | 254.0                   | 64-68%         |
| Arbequina    | 0                | 1.47          | 0.23             | 156.7                   | 62-64%         |
| Leccino      | 1-23%            | 2.57          | 0.34             | 131.1                   | 70-73%         |
| Blanqueta dE | 0-49%            | 3.45          | 0.36             | 178.8                   | 61%            |

El Antari, El Moudini, and Ajana, 2003. Laboratoire de Technologie des Huiles Programme Olivier. 2003. Inst. Nat. de la Recherche Agronomique BP 533, Marrakech, Morocco

**Table 6:** Recommended new cultivar for morocco (Marrakech 2006)

| Cultivar  | Ave. Yield (kg/tree) | Oil content (% dry basis) | Disease Tolerance | Pollination Requirement |
|-----------|----------------------|---------------------------|-------------------|-------------------------|
| Leccino   | 40                   | 36                        | T                 | Auto-inc.               |
| Picholine | 30                   | 38                        | T                 | Partial                 |
| Blanqueta | 30                   | 40                        | T                 | Partial                 |
| Arbequina | 29                   | 37                        | T                 | Auto                    |

Belkassam Boulouha. 2006. National Agriculture Research Center, Marrakech, Morocco.

Researchers in Spain have been comparing their own local olive cultivars with introduced cultivars from Italy, Greece, France, Tunisia, Turkey, and Morocco. When comparing some Italian and Spanish cultivars grown at 204-286 trees/ha, they found differences in tree volume (vigor) with some trees being two to three times larger than others over a 9-10 year period (Tables 8-9). In Italy a comparison between several local cultivars found similar differences in overall growth and number of branches, but not in tree height or trunk diameter, which only varied by about 25-30% (Table 10) (Cimato, 2001; Del Rio et al., 2005; Tous, Romero, Plana, 2005 a).

**Table 7:** Olive cultivar precocity, productivity, alternance index and content in Meknes, Morocco grown at density of 178 trees/ha

Researchers in Spain have been comparing their own local olive cultivars with introduced cultivars

| Cultivar     | Ave. Yield* First 3 yrs. (kg/tree) | Ave. Yield Last 3 yrs. (kg/tree) | Cumulative Yield | Alternance Index | Oil content % wet wt. basis | Oil Yield Estimate kg/ha |
|--------------|------------------------------------|----------------------------------|------------------|------------------|-----------------------------|--------------------------|
| Arbequina    | 6.94                               | 29.17                            | 122.46           | 0.20             | 23                          | 1,040                    |
| Carolea      | 2.12                               | 19.53                            | 74.45            | 0.45             | 26                          | 903                      |
| Frantoio     | 2.28                               | 18.80                            | 70.85            | 0.33             | 27                          | 904                      |
| Haouzia      | 3.86                               | 17.17                            | 69.07            | 0.37             | 23                          | 703                      |
| Koroneiki    | 18.14                              | 25.19                            | 143.60           | 0.39             | 25                          | 1,150                    |
| Hojiblanca   | 5.93                               | 33.67                            | 131.49           | 0.29             | 20                          | 1,080                    |
| Leccino      | 1.16                               | 24.02                            | 87.05            | 0.63             | -                           | -                        |
| Manzanillo   | 2.17                               | 20.22                            | 77.99            | 0.52             | -                           | -                        |
| Mastoides    | 11.66                              | 16.95                            | 97.50            | 0.57             | 30                          | 905                      |
| Menara       | 5.46                               | 18.13                            | 75.75            | 0.41             | 23                          | 742                      |
| P. Languedoc | 5.62                               | 26.67                            | 103.79           | 0.32             | 22                          | 1,000                    |
| Picual       | 10.23                              | 38.60                            | 169.61           | 0.21             | 24                          | 1,580                    |

\* Starting three years after planting.

A. Idrissi and N. Ouazzani. 2003. Département d'Arboriculture, Unité de Génétique Ecole Nationale d'Agriculture, Meknès, Maroc

from Italy, Greece, France, Tunisia, Turkey, and Morocco. When comparing some Italian and Spanish cultivars grown at 204-286 trees/ha, they found differences in tree volume (vigor) with some trees being two to three times larger than others over a 9-10 year period (Tables 8-9). In Italy a comparison between several local cultivars found similar differences in overall growth and number of branches, but not in tree height or trunk diameter, which only varied by about 25-30% (Table 10) (Cimato, 2001; Del Rio et al., 2005; Tous, Romero, Plana, 2005 a).

Some of the same researchers measured the number of years it took for various cultivars to come into bearing, the yields from the first few years, yields once the trees were mature, and yield efficiency (kg fruit/m<sup>3</sup> of tree volume). In Cataluña, the cultivars 'Arbequina', 'Arbosana', 'Blanqueta', and 'Palomar' started bearing fruit in the second year, while all the other cultivars took at least a year or two longer, and some did not start fruiting until 5-6 years old. The yield efficiency for 'Arbequina', 'Arbosana', and 'Joanenca' were extremely high (4.0 to 4.8 kg fruit/m<sup>3</sup> of tree volume) compared to most other cultivars that were much lower (0.4 to 3.2 kg fruit/m<sup>3</sup>). Those same cultivars also had high yields the first three years of production and maintained those higher yields into the 7-10<sup>th</sup> years (Table 11). In Andalucía, the yield efficiencies ranged from 0.84 for 'Gordal' to 5.36 kg fruit/m<sup>3</sup> of tree volume for 'Manzanillo'. Some of the Italian cultivars had very good yields their first three years of production and into the 7-10<sup>th</sup> years; especially notable was 'Leccino' though it took an average of 4.5 years to come into production (Table 12). In Australia, researchers also found 'Arbequina' and 'Leccino' to come into bearing early along with 'Picual', 'Barnea', 'Hojiblanca', 'Koroneiki', and 'Manzanillo' (Table 13) (Del Rio et al., 2005; Sweeney, 2005; Tous, Romero, Plana, 2005 a).

**Table 8:** Olive cultivar vigor (tree volume) in the 9<sup>TH</sup> year at a density of 286 trees/ha in cataluna spain

| Cultivar       | Volume m <sup>3</sup> /tree |
|----------------|-----------------------------|
| Morrut         | 48.0                        |
| Changlot Real  | 47.0                        |
| Empeltre       | 42.0                        |
| Manzanilla     | 41.0                        |
| Blanqueta      | 39.0                        |
| Picual         | 34.0                        |
| Arbequina      | 34.0                        |
| Palomar        | 32.0                        |
| Joanenca       | 29.0                        |
| Santa Caterina | 25.0                        |
| Arbosana       | 24.0                        |
| i-50           | 23.0                        |

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**Table 9:** Olive cultivar vigor (tree volume) in the 10<sup>TH</sup> year at a density of 204 trees/ha in Andalucía Spain

| Cultivar           | Volume m <sup>3</sup> /tree |
|--------------------|-----------------------------|
| Moraiolo           | 66.7                        |
| Maurino            | 43.0                        |
| Blanqueta          | 42.2                        |
| Leccino            | 40.5                        |
| Frantoio           | 40.3                        |
| Gordal (Sevillano) | 31.0                        |
| Picudo             | 30.6                        |
| Empeltre           | 27.1                        |
| Manzanilla         | 24.2                        |
| Hojiblanca         | 22.5                        |
| Cornicabra         | 20.2                        |
| Coratina           | 16.8                        |

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**Table 10:** Olive cultivar vigor in Firenze, Italy

| Cultivar         | Tree height (cm) | Total vegetation (cm) | Number of branches per tree | Trunk diameter (cm) |
|------------------|------------------|-----------------------|-----------------------------|---------------------|
| Correggiolo      | 159.83           | 442.91                | 19.3                        | 7.6                 |
| Frantoio         | 142.08           | 304.58                | 15.6                        | 7.4                 |
| Grappolo         | 119.58           | 172.16                | 12.1                        | 7.2                 |
| Leccino          | 152.30           | 404.00                | 19.0                        | 6.5                 |
| Leccio del Corno | 112.00           | 158.5                 | 10.2                        | 6.0                 |
| Maurino          | 130.50           | 299.75                | 22.8                        | 7.1                 |
| Moraiolo         | 119.08           | 122.75                | 8.1                         | 5.6                 |
| Pendolino        | 134.16           | 315.91                | 20.5                        | 6.8                 |
| S. Caterina      | 121.66           | 199.66                | 9.9                         | 6.5                 |
| San Francesco    | 167.41           | 414.41                | 20.1                        | 9.4                 |

Antonio Cimato, Istituto sulla Propagazione delle Specie Legnose Consiglio Nazionale delle Ricerche Scaticci – Firenze, Italia

**Table 11:** Olive cultivar precocity and production 1991-2001 in cataluna spain

| Cultivar       | Years to produce | Yield first 3 yrs. kg/tree | Ave. yield 7 <sup>th</sup> -10 <sup>th</sup> yrs. kg/tree | Efficiency kg fruit/m <sup>3</sup> tree volume |
|----------------|------------------|----------------------------|-----------------------------------------------------------|------------------------------------------------|
| Blanqueta      | 2                | 27.6                       | 25.2                                                      | 3.2                                            |
| Arbequina      | 2                | 22.7                       | 27.1                                                      | 4.1                                            |
| Arbosana       | 2                | 18.0                       | 18.0                                                      | 4.8                                            |
| Palomar        | 2                | 9.0                        | 17.2                                                      | 2.6                                            |
| Picual         | 3                | 24.3                       | 19.9                                                      | 2.7                                            |
| Joanenca       | 3                | 23.4                       | 20.2                                                      | 4.0                                            |
| Manzanilla     | 3                | 10.7                       | 21.1                                                      | 2.0                                            |
| Empeltre       | 4                | 15.0                       | 14.0                                                      | 1.5                                            |
| Santa Caterina | 6                | 8.8                        | 3.6                                                       | 0.4                                            |

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**Table 12:** Olive cultivar precocity, and production 1987- 1997 in Andalusia spain

| Cultivar           | Years to produce | Yield first 3 yrs. kg/tree | Ave. yield 7 <sup>th</sup> -10 <sup>th</sup> yrs kg/tree | Efficiency kg fruit/m <sup>3</sup> tree volume |
|--------------------|------------------|----------------------------|----------------------------------------------------------|------------------------------------------------|
| Blanqueta          | 3                | 7.7                        | 38.1                                                     | 4.06                                           |
| Maurino            | 3.5              | 31.0                       | 21.5                                                     | 2.88                                           |
| Picudo             | 3.5              | 14.9                       | 23.4                                                     | 3.83                                           |
| Manzanilla         | 4                | 21.8                       | 26.1                                                     | 5.36                                           |
| Hojiblanca         | 4                | 21.5                       | 22.2                                                     | 4.71                                           |
| Cornicabra         | 4                | 14.2                       | 19.7                                                     | 4.60                                           |
| Coratina           | 4                | 8.1                        | 13.1                                                     | 3.74                                           |
| Leccino            | 4.5              | 27.4                       | 34.2                                                     | 3.65                                           |
| Frantoio           | 5                | 41.2                       | 25.3                                                     | 2.87                                           |
| Empeltre           | 5.5              | 32.8                       | 10.9                                                     | 2.49                                           |
| Moraiolo           | 5.5              | 30.7                       | 15.0                                                     | 0.86                                           |
| Gordal (Sevillano) | 6                | 18.6                       | 6.4                                                      | 0.84                                           |

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**Table 13:** Cultivar comparisons for precocity in Australia

|               |                                                                           |
|---------------|---------------------------------------------------------------------------|
| Early bearing | Picual, Barnea, Hojiblanca, Arbequina, Koroneiki, Leccino, and Manzanillo |
| Mid bearing   | Mission, Pendolino, Columella, FS-17, and Coratina                        |
| Late bearing  | Ascolano, Frantoio, Gordal, Kalamon, I-77, and Souri                      |

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Additional research in Spain at the Córdoba Germplasm Repository provided more comparisons between cultivars from all over the world. 'Arbequina' and 'Arbosana' were much higher in number of fruits per branch, but not as high as 'Manzanillo' or 'Picual' in fruit dry weight per branch. Arbequina and Leccino stood out as being the most productive during years 6-9. (Tables 14-15) (Caballero et al., 2005; Ramirez and Rallo, 2005).

**Table 14:** Olive cultivar productivity, fruit dry weight, and weight fruit load in andalucia Spain

| Cultivar           | Productivity - fruit dry wt./branch | Fruit dry wt. | Fruit load fruits/branch |
|--------------------|-------------------------------------|---------------|--------------------------|
| Manzanilla         | 0.53 g                              | 1.52 g        | 0.35                     |
| Picual             | 0.51 g                              | 1.32 g        | 0.41                     |
| Arbosana           | 0.39 g                              | 0.61 g        | 0.64                     |
| Frantoio           | 0.39 g                              | 1.04 g        | 0.38                     |
| Arbequina          | 0.38 g                              | 0.53 g        | 0.71                     |
| Picholine          | 0.35 g                              | 1.16 g        | 0.30                     |
| Cornicabra         | 0.32 g                              | 1.02 g        | 0.31                     |
| Hojiblanca         | 0.32 g                              | 1.28 g        | 0.25                     |
| Gordal (Sevillano) | 0.30 g                              | 2.93 g        | 0.11                     |
| P. Marocaine       | 0.30 g                              | 1.13 g        | 0.27                     |
| Leccino            | 0.26 g                              | 1.17 g        | 0.23                     |
| Blanqueta          | 0.20 g                              | 0.57 g        | 0.37                     |
| Moraiolo           | 0.20 g                              | 0.65 g        | 0.31                     |
| Koroneiki          | 0.19 g                              | 0.25 g        | 0.75                     |

Magdalena Ramirez de Santa Pau and Luis Rallo. 2005. Dept. de Hort., Serv. de Inv. y Desarrollo Tec., J. de Extremadura, Badajoz. Dept. de Agri., U de Córdoba, Spain

**Table 15:** Olive cultivar precocity, productivity, fruit weight, and oil content in cordoba, spain 1988-98

| Cultivar     | Yield* first 3 yrs. kg/tree | Productivity Ave. 6 <sup>th</sup> -9 <sup>th</sup> yrs. (kg/tree) | Fruit Weight g/fruit | Oil content % dry wt. basis |
|--------------|-----------------------------|-------------------------------------------------------------------|----------------------|-----------------------------|
| Arbequina    | 23.0                        | 24.5                                                              | 1.7                  | 55.1                        |
| Manzanilla   | 7.4                         | 10.8                                                              | 3.9                  | 51.9                        |
| Villalonga   | 24.9                        | 12.8                                                              | 4.6                  | 58.4                        |
| Carolea      | 16.3                        | 14.0                                                              | 4.6                  | 58.4                        |
| Koroneiki    | 20.5                        | 13.3                                                              | 1.1                  | 55.7                        |
| Leccino      | 28.7                        | 23.6                                                              | 3.0                  | 49.0                        |
| Picholine    | 21.6                        | 10.2                                                              | 3.8                  | 56.1                        |
| P. Marocaine | 16.4                        | 15.8                                                              | 3.9                  | 53.1                        |

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**Table 16:** Olive cultivar precocity, productivity, fruit weight, and oil content in Catalunya, Spain 1988-01

| Cultivar     | Yield*<br>first 2 yrs.<br>kg/tree | Average<br>Productivity 8 <sup>th</sup> -<br>11 <sup>th</sup> yrs. kg/tree | Fruit Weight<br>g/fruit | Oil content %<br>dry wt. basis |
|--------------|-----------------------------------|----------------------------------------------------------------------------|-------------------------|--------------------------------|
| Arbequina    | 23.4                              | 29.8                                                                       | 1.2                     | 50.5                           |
| Manzanilla   | 10.1                              | 19.3                                                                       | 3.7                     | 50.4                           |
| Villalonga   | 18.5                              | 25.7                                                                       | 3.5                     | 48.3                           |
| Ayvalik      | 1.2                               | 5.6                                                                        | 3.4                     | 52.4                           |
| Carolea      | 5.8                               | 23.2                                                                       | 4.4                     | 53.3                           |
| Koroneiki    | 4.1                               | 8.5                                                                        | 0.8                     | 49.3                           |
| Leccino      | 13.5                              | 31.8                                                                       | 2.5                     | 47.1                           |
| Picholine    | 10.2                              | 31.5                                                                       | 3.8                     | 50.7                           |
| P. Marocaine | 4.9                               | 13.5                                                                       | 3.6                     | 46.9                           |

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'Arbequina' was the most productive cultivar after the first two years in Cataluña, Spain and it continued to be very productive as 'Picholine' and 'Leccino' performed similarly well in years 8-11 for average productivity. (Table 16) (Tous, Romero, Plana, 2005 a).

In Australia, the highest average yield the first three years of production was from the cultivars 'Picual', 'Barnea', 'Hojiblanca', 'Arbequina', and 'Manzanilla'. The popular Italian cultivars, 'Leccino' and 'Frantoio' were poor producers very early, but 'Leccino' had the 2<sup>nd</sup> highest production after 'Hojiblanca', which was followed by the 3<sup>rd</sup> highest producing cultivar, 'Koroneiki' in the 5<sup>th</sup> year after planting. The world's most common table olive cultivars, when grown in Australia, had the following fruit weights (g/fruit): 'Gordal', 9.66; 'Ascolano', 7.06; 'Manzanilla', 7.46; and 'Kalamon', 4.53. The cultivars with the smallest fruit sizes, under 3 g/fruit, were: 'Koroneiki', 0.96; 'Arbequina', 1.89, 'Pendolino', 2.46, and 'Frantoio', 2.80. The range in oleic fatty acid content among the cultivars was from 63.4% for 'Columella' to 79.2% for 'Koroneiki'. Oil content on a dry weight basis ranged from 40.4% for 'Hojiblanca' to 61.9% for 'Arbequina', which was the highest of all the cultivars tested. (Table 17) (Sweeney, 2005).

**Table 17:** Cultivar comparisons for yield, oleic fatty acid content, oil content, fruit weight, and flesh to pit ratio in Australia (1998 - 2004).

| Cultivar   | Ave. yield 3<br>yrs. kg/tree | Yield 2004<br>kg/tree | Oil content %<br>dry wt. basis | Fruit Wt.<br>Fresh (g) | Flesh to pit<br>ratio |
|------------|------------------------------|-----------------------|--------------------------------|------------------------|-----------------------|
| Picual     | 7.58                         | 11.8                  | 52.1                           | 4.26                   | 8.34                  |
| Barnea     | 6.80                         | 10.9                  | 61.0                           | 3.64                   | 8.16                  |
| Hojiblanca | 6.39                         | 17.2                  | 40.4                           | 4.47                   | 8.98                  |
| Arbequina  | 5.95                         | 11.6                  | 61.9                           | 1.89                   | 6.07                  |
| Manzanilla | 5.42                         | 8.7                   | 41.5                           | 5.46                   | 10.86                 |
| Mission    | 4.41                         | -                     | 48.2                           | 4.03                   | 7.26                  |
| Pendolino  | 4.39                         | 7.8                   | 41.4                           | 2.46                   | 6.19                  |
| Columella  | 4.11                         | -                     | 56.8                           | 4.86                   | 8.79                  |
| Leccino    | 3.88                         | 15.2                  | 47.6                           | 3.55                   | 6.18                  |
| FS-17      | 3.31                         | -                     | 61.7                           | 3.40                   | 11.2                  |
| Coratina   | 3.29                         | 1.4                   | 57.1                           | 3.94                   | 6.32                  |
| Ascolano   | 2.58                         | -                     | 54.1                           | 7.06                   | 12.4                  |
| Frantoio   | 2.52                         | 6.4                   | 57.4                           | 2.80                   | 5.15                  |
| Gordal     | 2.28                         | 5.8                   | 54.0                           | 9.66                   | 11.52                 |
| Kalamon    | 1.81                         | 1.5                   | 54.1                           | 4.53                   | 8.45                  |
| I-77       | 0.85                         | 4.4                   | 54.4                           | 5.13                   | 6.57                  |
| Souri      | 0.00                         | 0.0                   | 60.2                           | 3.34                   | 8.11                  |
| Koroneiki  | -                            | 13.0                  | 47.8                           | 0.96                   | 4.51                  |

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**Table 21:** Yield of oil (kg/tree) 2<sup>ND</sup> to the 6<sup>TH</sup> year in a super-high-density system (1.35 x 3.75 m – 1,975 trees/ha) by cultivar. Trees planted 3/2000 in Andalucía Spain

| Cultivar (% oil wet basis -% water)                                                                                                                                                              | Year 2 | Year 3 | Year 4 | Year 5 | Year 6 | Accumulated |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------|--------|--------|--------|--------|-------------|
| Arbequina (14.7% oil – 60.0% water)                                                                                                                                                              | 0.00   | 1.26   | 1.25   | 1.54   | 0.65   | 4.70        |
| Arbequina I-18 (15.8% oil – 59.9% water)                                                                                                                                                         | 0.00   | 1.31   | 1.01   | 1.47   | 0.66   | 4.45        |
| Arbosana (19.1% oil – 55.5% water)                                                                                                                                                               | 0.04   | 1.40   | 0.85   | 2.18   | 0.71   | 5.18        |
| FS-17 (14.7% oil – 66.2% water)                                                                                                                                                                  | 0.00   | 0.33   | 0.22   | 0.65   | 0.08   | 1.29        |
| Koroneiki (18.3% oil – 54.0% water)                                                                                                                                                              | 0.26   | 1.90   | 0.53   | 1.34   | 0.43   | 4.47        |
| Raul. De La Rosa, L. León, N. Guerrero, L. Rallo, and D. Barranco - CIFA – Alameda del Obispo, IFAPA – Junta de Andalucía – Córdoba, Spain and Dept. de Agronomía, Universidad de Córdoba, Spain |        |        |        |        |        |             |

Recent comparisons in Spain between cultivars grown in the super-high-density system (1,975 trees/ha) show the superior and very early productive capacities of the 'Arbequina', 'Koroneiki' and 'Arbosana' cultivars. All of the cultivars tested, except 'FS-17', indicate that they are compact growing and have a very high precocity. Of the cultivars tested, 'Koroneiki' was the most alternate bearing and 'Arbequina' was the least alternate bearing. Both had very high fruit and oil yields, but 'Arbosana' yielded the most oil in liters per hectare of all the cultivars, because it has a slightly higher oil content and consistently good yields. 'FS-17' had ¼ the production compared to the other varieties. The two clones of 'Arbequina', 'Standard' and 'I-18', were not significantly different from each other in this trial. (Tables 20-21) (De la Rosa et al., 2007). The "IRTA-i-18" clone has been shown in trials in Cataluña, however, to be more productive than standard 'Arbequina' (Tous et al., 1999; Tous, Romero, Plana, 2005 b).

A generalized comparison of the majority of the world's most widely planted table and oil cultivars is given in Table 22. While the table cultivars are primarily chosen on the basis of their fruit size, yield consistency is very important, which is why 'Manzanillo' is popular. It also has a very good pit to pulp ratio and the pit is easily extracted. The clone of 'Manzanillo' called 'Cortijo de Cuarto' has been shown to root more easily, have a larger trunk diameter, set more fruit, come into bearing earlier, produce more, and have larger fruit size with a greater pulp to pit ratio than the standard 'Manzanillo' (Suárez et al., 2005). Oil cultivars are often chosen for high oil and polyphenol content, because of their greater yields of oil per hectare and long keeping quality (Vossen, 2005).

**Table 22:** Oil content, fruit size, and polyphenol content of selected olive cultivars

| Cultivar                | % oil (wet) | Fruit size | Polyphenol content |
|-------------------------|-------------|------------|--------------------|
| Arbequina               | 22-27       | Small      | Low                |
| Aglandau                | 23-27       | Medium     | Medium             |
| Arbosana                | 23-27       | Small      | Medium-High        |
| Ascolano                | 15-22       | Large      | Medium             |
| Barnea                  | 16-26       | Medium     | Medium             |
| Barouni <sup>2</sup>    | 13-18       | Large      | Medium             |
| Bosana                  | 18-28       | Medium     | High               |
| Bouteillan              | 20-25       | Medium     | Medium             |
| Chemlali                | 26-28       | Very Small | High               |
| Coratina                | 23-27       | Medium     | Very High          |
| Cornicabra              | 23-27       | Medium     | Very High          |
| Empeltre                | 18-25       | Medium     | Medium             |
| Frantoio                | 23-26       | Medium     | Medium-High        |
| Farga                   | 23-27       | Medium     | Medium             |
| Hojiblanca              | 18-26       | Large      | Medium             |
| Kalamon                 | 15-25       | Large      | Medium             |
| Koroneiki               | 24-28       | Very Small | Very High          |
| Leccino                 | 22-27       | Medium     | Medium             |
| Manzanillo <sup>3</sup> | 15-26       | Large      | High               |
| Maurino                 | 20-25       | Medium     | High               |
| Mission <sup>3</sup>    | 19-24       | Medium     | High               |
| Moraiolo                | 18-28       | Small      | Very High          |
| Pendolino               | 20-25       | Medium     | Medium             |
| Picudo                  | 22-24       | Large      | Low                |
| Picual                  | 24-27       | Medium     | Very High          |
| Picholine               | 22-25       | Medium     | High               |
| Sevillano <sup>2</sup>  | 12-17       | Very Large | Low                |
| Taggiasca               | 22-27       | Medium     | Low                |

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**Table 18:** Olive cultivar fruit weight, pulp to pit ratio, and oil content in Andalusia Spain

**Table 19:** Olive cultivar fruit weight, pulp to pit ratio, and oil content in Catalonia Spain

**Table 20:** Yield of olives (kg/tree) from the 2<sup>nd</sup> to the 6<sup>th</sup> year in a super-high- density system (1.35 x 3.75 m – 1.975 trees/ha) by cultivar trees planted 3/200 in Andalusia Spain

| Cultivar       | Year 2 | Year 3 | Year 4 | Year 5 | Year 6 | Accumulated |
|----------------|--------|--------|--------|--------|--------|-------------|
| Arbequina      | 0.00   | 8.43   | 8.72   | 10.91  | 3.21   | 31.27       |
| Arbequina I-18 | 0.00   | 7.47   | 7.11   | 10.19  | 3.13   | 27.93       |
| Arbosana       | 0.15   | 8.69   | 4.53   | 9.81   | 3.74   | 26.92       |
| FS-17          | 0.00   | 2.21   | 1.43   | 4.44   | 0.35   | 8.43        |
| Koroneiki      | 1.82   | 10.50  | 3.09   | 6.34   | 2.29   | 24.04       |

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## 5. Tree spacing and cultivar influence on productivity

Traditional spacing of more than 7 m between trees is no longer popular anywhere in the world except for desert dry-farmed areas. In modern olive culture trees are planted either at a medium density of about 500 – 865 per hectare, or in the high-density system (also called super-high-density) where trees are planted up to 2,220 per ha. The medium-density system using trunk shakers for harvest is used: to accommodate most cultivars, achieve an aesthetic look with larger trees, in situations where the ground is too steep, or for farms that are too small for over-the-row mechanical harvesting.



Medium-density plantings have been very popular over the last 40 years with hundreds of thousands of hectares planted all over the world. Common spacings usually range from (6 x 3 m), to (4 x 8 m) apart. If the trees eventually grow too close together, every other tree can be removed to reduce shading. With the medium-density system (Fig. B), inputs are much less critical, and cultural practices are not as exacting compared to the super-high density system (Fig. C). The more trees planted per hectare, up to a point, the faster the planting comes into bearing. One way to get more trees per hectare is to plant them in a hedgerow configuration in a North–South direction with less space between trees compared to between rows.

The super-high-density system is where the trees are planted from 1.5 x 4 m apart to 1 x 3 m apart in lower vigor situations. They are never allowed to exceed a size of about 2.5-3 m tall by 1.8-2 m wide through annual renewal pruning. Vigorous cultivars cannot be planted in this system, because they become too large, shade out the lower portions of the trees, and require severe pruning to keep their size down resulting in vigorous growth that is unfruitful. Only highly precocious cultivars will fruit consistently under the conditions of close spacing and heavy pruning.



The super-high-density system has only worked with three cultivars that we know of: 'Arbequina', 'Arbosana', and 'Koroneiki'. It also helps if the orchard manager can control soil water status to moderate tree vigor. It is anticipated that this system will be more difficult to manage in excessively vigorous sites with deep soils and high rainfall or in very low vigor situations with limited capacity to irrigate the trees. Its long-term performance is not known.

### Typical yields from standard cultivars in the medium-density system

- 1<sup>st</sup> to 4<sup>th</sup> years – insignificant
- 5<sup>th</sup> year ~ 1.0 t/h
- 6<sup>th</sup> year ~ 2.2 t/h
- 7<sup>th</sup> year ~ 4.5 t/h
- 8<sup>th</sup> year ~ 6.7 t/h
- 9<sup>th</sup> year ~ 9.0 t/h
- 10<sup>th</sup> year + ~ 11.2 t/h

### Typical yields from precocious cultivars in the super-high-density system

- 1<sup>st</sup> and 2<sup>nd</sup> years – insignificant
- 3<sup>rd</sup> year ~ 4.5 t/h
- 4<sup>th</sup> year ~ 6.7 t/h
- 5<sup>th</sup> year + ~ 11.2 t/h

Planting a new olive orchard is a big puzzle with many parts. Almost simultaneously, decisions need to be made regarding the type of production system, harvest method, desired style of oil, and market segment where the oil will be sold. These decisions need to fit into the suitability of the climate, the layout of the land, soil characteristics, irrigation availability, water quality, labor resources, long term viability of the orchard, and the necessity for a rapid return on the capital invested. Existing conditions and decisions made for each part of the puzzle will help lead to making good cultivar choices (Pastor et al., 2005).

Table 23 is a summary of several research trials being conducted in different countries with orchards planted to low, high, and super-high density spacings with different cultivars. There is good data to show that the three primary cultivars used in the super-high-density system ('Arbequina', 'Arbosana', and 'Koroneiki') planted at 1,975 trees per hectare provides the most rapid return on investment. In most cases these orchards are producing more tons of fruit per hectare in the third year alone compared to the cumulative production from 7-8 years with more vigorous cultivars planted at medium-density or wider tree spacings. The old wide spacing production system planted at 204 trees per hectare only produces about half of what the orchards produce at 555 trees per hectare and only a third to a quarter of the production of trees planted at 1,975 trees/ha, after ten years (Tous et al., 1999, 2003).

**Table 23:** Summary of early olive yield and oil yield (tons/hectare) – first ten years from different orchards in several countries 204 trees/h (7m X 7m), 555 trees/h (3m X 6m), and 1,975 trees/h (1.35m X 3.75m)

| Age                                      | trees/h | Arbequina<br>15-25% | Arbosana<br>20-22% | Koroneiki<br>18-25% | Leccino<br>18-24% | Frantoio<br>23-28% | Coratina<br>23-28% | Picual<br>23-28% | Manz<br>13-24% | P. Maroc<br>15-25% |
|------------------------------------------|---------|---------------------|--------------------|---------------------|-------------------|--------------------|--------------------|------------------|----------------|--------------------|
| Year 2                                   | 204     | 0                   | 0                  | 0.4                 | 0                 | 0                  | 0                  | 0                | 0              | 0                  |
|                                          | 555     | 0.1                 | 0.2                | 2.0                 | 0                 | 0                  | 0                  | 0                | 0              | 0                  |
|                                          | 1,975   | 0.2                 | 0.4                | 3.6                 | -                 | -                  | -                  | -                | -              | -                  |
| Year 3                                   | 204     | 1.7                 | 1.9                | 2.7                 | 0                 | 0                  | 0                  | 0                | 0              | 0                  |
|                                          | 555     | 4.5                 | 4.8                | 10.4                | 0                 | 0                  | 0                  | 0                | 0              | 0                  |
|                                          | 1,975   | 16.6                | 17.2               | 20.7                | -                 | -                  | -                  | -                | -              | -                  |
| Year 4                                   | 204     | 1.8                 | 1.0                | 3.3                 | 0.1               | 0                  | 0                  | 0.2              | 0.1            | 0.1                |
|                                          | 555     | 5.5                 | 2.7                | 4.8                 | 0.5               | 0                  | 0                  | 0.6              | 0.3            | 0.3                |
|                                          | 1,975   | 17.2                | 9.0                | 6.1                 | -                 | -                  | -                  | -                | -              | -                  |
| Year 5                                   | 204     | 2.1                 | 1.9                | 4.9                 | 0.3               | 0.1                | 0.1                | 2.2              | 0.6            | 0.6                |
|                                          | 555     | 6.3                 | 5.2                | 6.7                 | 0.7               | 0.4                | 0.3                | 5.1              | 1.1            | 1.7                |
|                                          | 1,975   | 21.5                | 19.4               | 12.5                | -                 | -                  | -                  | -                | -              | -                  |
| Year 6                                   | 204     | 3.0                 | 3.0                | 2.8                 | 4.0               | 1.0                | 0.9                | 3.9              | 2.0            | 1.9                |
|                                          | 555     | 8.1                 | 6.3                | 10.0                | 8.3               | 2.8                | 2.2                | 9.7              | 5.3            | 5.0                |
|                                          | 1,975   | 6.3                 | 7.4                | 4.5                 | -                 | -                  | -                  | -                | -              | -                  |
| Year 7                                   | 204     | 4.3                 | 6.5                | 2.8                 | 5.5               | 1.6                | 2.0                | 4.7              | 2.6            | 1.1                |
|                                          | 555     | 11.6                | 9.8                | 14.2                | 5.9               | 4.3                | 4.5                | 5.9              | 5.5            | 3.0                |
|                                          | 1,975   | 15.5                | 11.1               | 20.6                | -                 | -                  | -                  | -                | -              | -                  |
| Year 8                                   | 204     | 8.0                 | 7.4                | 1.2                 | 10.5              | 1.2                | 2.9                | 5.9              | 5.5            | 5.4                |
|                                          | 555     | 21.6                | 9.4                | 3.0                 | 15.0              | 3.3                | 6.7                | 10.9             | 11.2           | 10.6               |
|                                          | 1,975   | 14.4                | 9.5                | 7.8                 | -                 | -                  | -                  | -                | -              | -                  |
| Year 9                                   | 204     | 9.9                 | 6.2                | 9.0                 | 6.5               | 4.3                | 7.5                | 11.2             | 8.0            | 1.0                |
|                                          | 555     | 10.0                | 9.6                | 12.1                | 3.1               | 11.6               | 9.0                | 8.6              | 18.2           | 2.7                |
|                                          | 1,975   | 10.9                | 10.4               | 15.9                | -                 | -                  | -                  | -                | -              | -                  |
| Year 10                                  | 204     | 16.0                | 11.8               | 5.2                 | 12.7              | 6.1                | 6.7                | 6.5              | 6.8            | 3.4                |
|                                          | 555     | 17.9                | 16.0               | 10.9                | 11.2              | 4.2                | 11.2               | 6.8              | 9.0            | 5.1                |
|                                          | 1,975   | 18.3                | 17.5               | 11.5                | -                 | -                  | -                  | -                | -              | -                  |
| Yield<br>Cum.<br>Total                   | 204     | 46.8                | 39.7               | 32.3                | 39.6              | 14.3               | 20.1               | 34.6             | 25.6           | 13.5               |
|                                          | 555     | 85.6                | 64.0               | 74.1                | 44.7              | 26.6               | 33.9               | 53.0             | 50.6           | 28.4               |
|                                          | 1,975   | 120.9               | 101.9              | 103.2               | -                 | -                  | -                  | -                | -              | -                  |
| Yield<br>Ave.<br>8-10 <sup>th</sup> yrs. | 204     | 11.3                | 8.5                | 5.1                 | 11.5              | 3.9                | 5.7                | 7.9              | 6.8            | 3.3                |
|                                          | 555     | 16.5                | 11.7               | 8.7                 | 10.8              | 6.4                | 9.0                | 8.7              | 12.8           | 6.1                |
|                                          | 1,975   | 14.5                | 12.5               | 11.7                | -                 | -                  | -                  | -                | -              | -                  |
| Oil Yield<br>Cum. Total                  | 204     | 9.36                | 8.34               | 6.94                | 8.31              | 3.65               | 5.12               | 8.82             | 4.73           | 2.7                |
|                                          | 555     | 17.12               | 13.44              | 15.91               | 9.38              | 6.78               | 8.64               | 13.51            | 9.36           | 5.68               |
|                                          | 1,975   | 24.18               | 21.39              | 22.18               | -                 | -                  | -                  | -                | -              | -                  |

Paul Vossen – Unpublished data, University of California Cooperative Extension, Santa Rosa, California. Raul. De La Rosa, L. León, N. Guerrero, L. Rallo, and D. Barranco - CIFA – Alameda del Obispo, IFAPA – Junta de Andalucía – Córdoba, Spain and Dept. de Agronomía, Universidad de Córdoba, Spain. Joan Tous, Juan F. Hermoso, Joan Plana, and Agustí Romero. Dept. d'Arboricultura Mediterrània, Centre "Mas Bové", IRTA, Reus-Constantí (Tarragona); Estación Experimental del Ebro, IRTA, Amposta (Tarragona), Spain. N. Ouazzani. Département d'Arboriculture, Unité de Génétique Ecole Nationale d'Agriculture, Meknès, Maroc

At about ten years of age when the trees reach or approach full size in the medium-density system (555 trees/ha) the orchard yields are comparable to what they are at the density of 1,975 trees/ha; it just takes much longer. Documented establishment costs for a super-high-density orchard are about twice what they are for a medium-density system, but production costs once the trees are mature, is only about half. In several cases very high yields of 10-15 tons/hectare were reached in the medium-density system and alternate bearing was quite evident. In some cases very high yields have been achieved in the super-high-density orchards of over 15-20 tons/hectare. Those orchards also are quite alternate bearing with very low yields preceding and directly following years with very high yields. Both systems can produce consistent yields of 9-11 tons per hectare and very high quality olive oils. (De la Rosa et al. 2007; Ouazzani, 2005; Vossen et al. 2001, 2003, Vossen et al. 2004).

Super-high-density orchards need to be intensively managed and were designed for mechanical inputs like an over-the-row harvester, mechanical pruning for topping and skirting, sophisticated irrigation and fertigation systems, processing facilities at close proximity to limit fruit degradation during transport, and knowledgeable management. Short stature trees that bear heavily soon after planting, however, could also provide an advantage for small scale growers who might do most or all of the management by hand, including harvest (Vossen, 2002, 2004).

## 6. Oil extraction

Oil yield predictions are difficult because there are so many variables (Table 24). Oil extractability depends on the cultivar, fruit maturity, fruit moisture content, and the processing system. The quantity of oil in the fruit is a built-in genetic factor, but it can vary from year to year due to tree vigor, crop load, fruit maturity, and fruit moisture content. Oil content varies by cultivar from less than 10% to about 30% on a wet weight basis. Since oil accumulation peaks when the fruit is quite mature, delaying harvest until the fruit is ripe assures the highest yield of oil, though that will change flavor characteristics, and extractability if the weather is rainy.

In processing, the extractability of the oil from the fruit is heavily influenced by paste fineness, malaxation time and temperature, and extraction machinery type. The fruit's water content influences the percentage of oil relative to moisture, so drier fruit will have a higher percentage of oil by weight. Oil is easier to extract from low moisture fruit, conversely, it is more difficult to extract from fruit that has been over-irrigated or has received rain before harvest and if the fruit is washed (wet) just prior to crushing. High moisture fruit often forms an emulsion (watery gel) in the paste that cannot be captured, but escapes with the fruit-water or pomace solids. Oil content increases slightly and extractability usually improves as the fruit matures, becomes softer, and is easier to crush. Some cultivars give up their oil quite easily and others do not. (Vossen, unpublished).

The amount of oil that can be extracted from the crushed paste of fresh olives has been extensively studied all over the world and in the USA as long as 100 years ago (Table 25). In one trial in the Southwestern USA, the total extracted (pressed) oil varied by as much as 145% from the 'Rubra' cultivar producing 91 kg per ton from black ripe fruit to 'Mission' producing 220.6 kg per ton. When oil yield was compared between unripe green fruit or fruit just turning color from green to red with fully mature fruit that was black ripe, the amount of extracted oil increased by 53% for 'Ascolano' going from 95.5-145.2 kg per ton. For the 'Correggiolo', 'Mission', and 'Morinello' cultivars yield increased by 26%, 54%, and 55% respectively when fruit was allowed to fully ripen. For the 'Mission' cultivar, just letting the fruit ripen from green-yellow color to red blush increased yield from 142.9-150.5 kg per ton, an increase of 6%. When the 'Pendolino' cultivar was allowed to go from black ripe to shriveled black ripe in maturity the oil extraction increased by 28% from 158.4-203.5 kg per ton. Most of these increases in oil yield per ton of riper olives are believed to be due to a reduction in fruit weight due to water loss and improved extraction.

**Table 24:** Approximate oil yield from 1 ton of olives with different oil content & extractability (% wet weight basis)

| Olive Cultivar – Water Status - Ripeness                                                                 | Oil Yield                                     |
|----------------------------------------------------------------------------------------------------------|-----------------------------------------------|
| Green over-watered Sevillano                                                                             | 10 gal of oil/t - 4 % = 37.9 liters per ton   |
| Ripe Sevillano - Green Ascolano                                                                          | 15 gal of oil/t - 6 % = 56.8 liters per ton   |
| Very ripe Sevillano – Ripe Ascolano                                                                      | 20 gal of oil/t - 8 % = 75.7 liters per ton   |
| Over-watered, green Arbequina or Manzanillo<br>Very ripe deficit-irrigated Ascolano                      | 25 gal of oil/t - 9.5 % = 94.6 liters per ton |
| Ripe over-watered Arbequina or Manzanillo<br>Green over-watered Frantoio, Leccino                        | 30 gal of oil/t - 11 % = 113.5 liters per ton |
| Very ripe Arbequina or Manzanillo – Green over- watered Mission<br>- Ripe over-watered Frantoio, Leccino | 35 gal of oil/t - 13 % = 132.5 liters per ton |
| Ripe Frantoio, Leccino – Green Mission<br>Deficit-irrigated Arbequina or Manzanillo                      | 40 gal of oil/t - 15 % = 151.4 liters per ton |
| Ripe over-watered Mission<br>Ripe, deficit-irrigated Frantoio, Leccino                                   | 45 gal of oil/t - 17 % = 170.3 liters per ton |
| Ripe Mission, Picual                                                                                     | 50 gal of oil/t - 19 % = 189.3 liters per ton |
| Very ripe, deficit-irrigated Mission, Picual                                                             | 55 gal of oil/t - 21 % = 208.2 liters per ton |
| Paul Vossen, unpublished data.                                                                           |                                               |

Research has indicated that as the season advances the oil content of the olives increases by about 1-2% as they passed from one color stage to the next providing a total increase in oil content by about 4-8% from green to black fruit. It also found that the oil content of olives grown in sandy soils was about 3-4% less than if they were grown in sandy loam or clay soil types and that in general Northern California grown olives had a higher average oil content than did those from the south central or southern growing regions by about 1-4% (Table 25) (Coit, 1909).

Another trial conducted at the UC Riverside Experiment Station showed considerable difference in oil content by cultivar and location when the trees were grown under conditions of no irrigation and no cultivation. It found that oil content on a wet basis varied by about 23% (33.7 to 11.2%), the percentage of moisture in the fruit varied by about 32% (45.0% to 76.8%), and that the oil content on a dry weight basis varied by about 21% (63.3% to 42.5%) (Table 26) (Condit, 1940, 1946).

**Table 25:** Oil extraction from a press as influenced by cultivar and fruit maturity

| Cultivar                                                                     | Maturity                                | Gallons of Oil/Ton of Olives | Kg oil per metric ton |
|------------------------------------------------------------------------------|-----------------------------------------|------------------------------|-----------------------|
| Ascolano                                                                     | Half ripe – beginning to turn red       | 25.2                         | 95.5                  |
| Ascolano                                                                     | Fully ripe – black – some shrivel       | 38.3                         | 145.2                 |
| Columella                                                                    | Half ripe – mostly red color            | 39.4                         | 149.3                 |
| Correggiolo                                                                  | Half red –half green                    | 44.1                         | 167.1                 |
| Correggiolo                                                                  | Fully ripe – black to the pit           | 51.4                         | 193.7                 |
| Manzanillo                                                                   | Fully ripe – black to the pit           | 34.9                         | 149.3                 |
| Mission                                                                      | Green – yellow                          | 37.7                         | 142.9                 |
| Mission                                                                      | Half ripe – red color                   | 39.7                         | 150.5                 |
| Mission                                                                      | Fully ripe – black fruit                | 50.1                         | 189.9                 |
| Mission                                                                      | Fully ripe – shriveled fruit            | 58.2                         | 220.6                 |
| Mission                                                                      | Moldy pomace – 2 <sup>nd</sup> pressing | 11.2                         | 42.4                  |
| Morinello                                                                    | Unripe green                            | 29.2                         | 110.7                 |
| Morinello                                                                    | Fully ripe – black                      | 45.5                         | 172.4                 |
| Nevadillo                                                                    | Black ripe                              | 44.8                         | 169.8                 |
| Pendolino                                                                    | Black ripe                              | 41.8                         | 158.4                 |
| Pendolino                                                                    | Fully ripe – black and shriveled        | 53.7                         | 203.5                 |
| Razza                                                                        | Black ripe                              | 47.8                         | 181.2                 |
| Rubra                                                                        | Black ripe                              | 24.0                         | 91.0                  |
| Uvaria                                                                       | Fully ripe – black                      | 31.4                         | 119.0                 |
| J. Eliot Coit, 1909. Olive culture and oil manufacture in the arid SW - USA. |                                         |                              |                       |



**Table 26:** Oil content of olives from various California districts

| Cultivar                | Location     | Oil % wet | Oil % dry | Water % |
|-------------------------|--------------|-----------|-----------|---------|
| Correggiolo             | Chico        | 33.7      | 63.3      | 46.8    |
| Grappoli                | Chico        | 33.5      | 60.9      | 45.0    |
| Chemlali                | San Fernando | 28.6      | 52.9      | 46.0    |
| Moraioli                | Chico        | 26.9      | 56.5      | 52.4    |
| Mission                 | Lindsay      | 25.5      | 56.2      | 54.7    |
| Maurini                 | Chico        | 25.0      | 62.8      | 60.2    |
| Nevadillo               | Porterville  | 25.0      | 57.0      | 56.2    |
| Nevadillo               | San Fernando | 24.5      | 55.8      | 56.1    |
| Manzanillo              | Lindsay      | 24.0      | 60.0      | 60.0    |
| Lecci                   | Chico        | 22.8      | 58.9      | 61.3    |
| Barouni                 | Chico        | 21.3      | 51.7      | 59.8    |
| Columbella              | Fallbrook    | 21.0      | 58.6      | 64.2    |
| Mission                 | Riverside    | 19.0      | 56.7      | 66.5    |
| Picholine               | San Fernando | 18.6      | 54.7      | 66.0    |
| Ascolano                | Riverside    | 16.8      | 60.0      | 72.0    |
| Obliza                  | San Fernando | 16.7      | 52.8      | 68.4    |
| Barouni                 | Riverside    | 15.7      | 54.1      | 71.0    |
| Ascolano                | Lindsay      | 14.8      | 52.4      | 71.8    |
| Sevillano               | Lindsay      | 14.7      | 55.5      | 73.7    |
| Manzanillo              | Riverside    | 13.0      | 53.0      | 75.5    |
| Sevillano               | Corning      | 12.2      | 52.5      | 76.8    |
| Chemlali                | Riverside    | 11.2      | 42.5      | 73.7    |
| Condit and Cruess, 1940 |              |           |           |         |

## 7. Fatty acid composition

The triglyceride structure of olive oil varies as to the percentage of different fatty acid types by cultivar. The primary fatty acid of interest is Oleic acid (C18:1), which makes up the largest percentage of the fatty acid types in olive oil. Note that there are only slight differences in the oleic fatty acid content of the same cultivar grown in different locations. For example, the C18:1 content of 'Arbequina' oil in France is 68.3%, in Cataluña Spain it is 68.2%, in Córdoba, 65.8%, in Australia, 69.8%, and in Morocco it was measured as 70.8%. The importance of the different percentages of oleic fatty acid content by growing region is minimal since all of the cultivars meet the minimum requirement from the IOOC as extra virgin (Tables 27-31) (Moutier et al., 2004; Ouazzani, 2005; Sweeney, 2005; Tous, Romero, Díaz, 2005; Uceda et al., 2005).

**Table 27:** Olive cultivars percentage oleic fatty acid content in Cataluña, Spain 1993-98

| Cultivar                                                       | C18:1 (%) |
|----------------------------------------------------------------|-----------|
| Picual                                                         | 78.28     |
| Arbosana                                                       | 74.77     |
| Manzanilla                                                     | 71.97     |
| Hojiblanca                                                     | 73.70     |
| Arbequina                                                      | 68.20     |
| Empeltre                                                       | 68.61     |
| Blanquette                                                     | 61.23     |
| Tous, Romero, and Díaz IRTA, Reus-Constantí (Tarragona), Spain |           |

**Table 28:** Olive cultivars percentage oleic fatty acid content in Meknes, Morocco

| Cultivar     | C18:1 (%) |
|--------------|-----------|
| Picual       | 80.24     |
| Koroneiki    | 78.22     |
| Leccino      | 76.33     |
| P. Marocaine | 75.25     |
| Mastoides    | 74.99     |
| Kalamon      | 74.56     |
| Frantoio     | 74.23     |
| Arbequina    | 70.85     |
| Carolea      | 70.53     |

Noureddine Ouazzani, 2005. Département d'Arboriculture, Unité de Génétique Ecole Nationale d'Agriculture, Meknès, Maroc

**Table 29:** Olive cultivars percentage oleic fatty acid content in France 1995-2004

| Cultivar        | C18:1 (%) |
|-----------------|-----------|
| Aglandau        | 73.41     |
| Bouteillan      | 68.32     |
| Cailletier      | 76.34     |
| Cayon           | 79.03     |
| Grossane        | 68.72     |
| Lucques         | 73.12     |
| P. du Languedoc | 74.35     |
| Salonenque      | 64.55     |
| Tanche          | 79.44     |
| Arbequina       | 68.26     |
| Cornicabra      | 67.55     |
| Frantoio        | 75.21     |
| Manzanillo      | 72.96     |

Natalie Moutier, Christian Pinatel, André Martre, and Jean-Paul Roger. 2004. INRA de Montpellier, AFIDOL d'Aix-en-Provence; CBNM de Porquerolles - France.

**Table 30:** Olive cultivars percentage oleic fatty acid content in cordoba, Spain, Spain 1989-1997

| Cultivar     | C18:1 (%) |
|--------------|-----------|
| Picual       | 78.34     |
| Koroneiki    | 77.66     |
| P. Marocaine | 76.05     |
| Manzanilla   | 75.33     |
| Hojiblanca   | 74.61     |
| Kalamon      | 74.56     |
| Cornicabra   | 74.30     |
| Frantoio     | 73.91     |
| Pendolino    | 71.69     |
| Maurino      | 71.35     |
| Leccino      | 70.82     |
| Empeltre     | 69.29     |
| Arbequina    | 65.83     |
| Moraiolo     | 65.09     |
| Picudo       | 63.29     |
| Blanquette   | 59.01     |

Uceda, Beltran, Jiménez. Estacion de Olivicultura y Elaiotecnica, Junta de Andalucía, Mengibar (Jaén), Spain

**Table 31:** Olive cultivars percentage oleic fatty acid content in Australia 2000-04

| Cultivar     | C18:1 (%)  |
|--------------|------------|
| Arbequina    | 68.9-70.7  |
| Ascolano     | 75.8-77.8  |
| Barnea       | 72.8-75.1  |
| Blanqueta    | 57.8-75.2  |
| Bouteillan   | 73.8-74.1  |
| Columella    | 60.2-64.6  |
| Coratina     | 74.6-82.7  |
| Frantoio     | 72.8-74.2  |
| FS-17        | 72.2-75.5  |
| Gordal       | 68.3-70.2  |
| Hojiblanca   | 75.3-76.9  |
| I-77         | 78.3-78.4  |
| Kalamon      | 76.2-81.0  |
| Koroneiki    | 79.1-80.5  |
| Leccino      | 77.3-77.6  |
| Manzanillo   | 72.4-74.7  |
| Mission (CA) | 77.0-77.6  |
| Pendolino    | 72.7-73.6  |
| Picual       | 77.0 -78.7 |
| Souri        | 74.1-74.4  |

Susan Sweeney, Plant Research Center, Waite Research Precinct; Rural Industries Research and Development Corporation Hartley Grove, Urrbrae, 5064, Australia.

### 8. Polyphenol content – bitterness – stability

The total polyphenol content of olive oil, expressed in parts per million (ppm) or milligrams per liter (mg/L) based on the caffeic acid standard, is quite different by cultivar. There is most often a direct correlation between total polyphenol content, bitterness of an olive oil expressed as a spectrophotometer reading at 225 nm, and stability of an olive oil expressed as hours of oxidative stability from a Rancimat test at either 98° or 120° C. Table 32 shows the results of an evaluation of olive oils in Morocco ranking the oxidative stability of each cultivar's oil. Note that 'Arbequina', which is normally thought to have a low stability, actually ranked in the medium category above 'Manzanillo', 'Frantoio', 'Carolea', and 'Hojiblanca' (Ouazzani, 2005).

**Table 32:** Olive cultivars – oil oxidative stability in Meknes, Morocco

| Cultivar     | Hrs. O <sub>2</sub> Stab. 120°C |
|--------------|---------------------------------|
| Picual       | 14.0                            |
| Leccino      | 10.4                            |
| Koroneiki    | 9.22                            |
| Haouzia      | 8.68                            |
| Menara       | 8.30                            |
| P. Marocaine | 8.25                            |
| P. Languedoc | 6.83                            |
| Mastoides    | 6.68                            |
| Arbequina    | 6.55                            |
| Manzanillo   | 5.92                            |
| Frantoio     | 3.85                            |
| Carolea      | 3.35                            |
| Hojiblanca   | 1.58                            |

Noureddine Ouazzani, 2005. Département d'Arboriculture, Unité de Génétique Ecole Nationale d'Agriculture, Meknès, Maroc

The consistency in polyphenol content, oil bitterness, and oxidative stability can be noted when comparing tables 32-34 from olive oil experiments in three different growing areas. The high polyphenol and stable cultivars ('Picual' and 'Koroneiki') are consistently high and stable no matter where they are grown and vice versa for the low polyphenol cultivars ('Arbequina' and 'Hojiblanca'). Most of the other cultivars fall in the middle ranges. The Italian study (Table 35) shows the great range and variability possible in polyphenol levels that can sometimes be greater within the cultivar than between cultivars. (Cimato et al., 1996; Ouazzani, 2005; Pannelli et al., 2001; Sweeney, 2005; Tous, Romero, Díaz, 2005; Uceda et al., 2005)

The polyphenol content of olive oil and its correlated bitterness and oxidative stability have been found to be influenced by several factors. Cultivar is important as noted above as well as the level of moisture stress received by the trees, the maturity of the fruit at harvest, and elevation above sea level where the trees are grown (Cortesi et al. 2000; Fiorino, 1996; Ranalli et al. 1999; Tous et al. 1997; Tous, Romero, Díaz, 1999).

An experiment in California provides an example of how irrigation level can influence both the total polyphenol content and oxidative stability (Table 36). This experiment demonstrated that there can be as much as 3-4 times more or less total polyphenols or bitterness in a single cultivar (Arbequina) based on the amount of irrigation water the trees received (Berenguer et al. 2006).

**Table 33:** Polyphenol content, bitterness, and stability of selected cultivars in cataluna  
spain 1996-1998

| Cultivar   | Polyphenols ppm | K <sub>225</sub> Bitterness | Hrs. O <sub>2</sub> Stab. 120°C |
|------------|-----------------|-----------------------------|---------------------------------|
| Villalonga | 887             | 0.72                        | 10.28                           |
| Picual     | 509             | 0.42                        | 21.45                           |
| Blanquette | 491             | 0.43                        | 10.26                           |
| Joanenca   | 367             | 0.38                        | 13.40                           |
| Manzanilla | 321             | 0.27                        | 15.35                           |
| Hojiblanca | 273             | 0.27                        | 6.25                            |
| Arbosana   | 269             | 0.24                        | 12.90                           |
| Palomar    | 248             | 0.29                        | 8.41                            |
| Empeltre   | 238             | 0.21                        | 7.82                            |
| Arbequina  | 201             | 0.19                        | 7.80                            |

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**Table 34:** Polyphenol content, bitterness, and stability of selected cultivars in Andalucia  
Spain 1989-1997

| Cultivar     | Polyphenols ppm | K <sub>225</sub> Bitterness | Hrs. O <sub>2</sub> Stab. 98°C |
|--------------|-----------------|-----------------------------|--------------------------------|
| Chetoui      | 1,240           | 0.82                        | 75.1                           |
| P. Marocaine | 787             | 0.52                        | 94.8                           |
| Picual       | 664             | 0.45                        | 140.6                          |
| Cornicabra   | 464             | 0.48                        | 118.7                          |
| Manzanilla   | 461             | 0.2                         | 57.8                           |
| Empeltre     | 420             | 0.22                        | 57.5                           |
| Koroneiki    | 411             | 0.36                        | 71.9                           |
| Frantoio     | 382             | 0.21                        | 64.6                           |
| Maurino      | 334             | 0.21                        | 46.1                           |
| Kalamon      | 332             | 0.27                        | 49.3                           |
| Leccino      | 302             | 0.25                        | 56.3                           |
| Blanquette   | 293             | 0.25                        | 34.8                           |
| Picudo       | 246             | 0.19                        | 38.03                          |
| Hojiblanca   | 187             | 0.19                        | 51.0                           |
| Arbequina    | 181             | 0.16                        | 38.31                          |
| Moraiolo     | 175             | 0.13                        | 36.37                          |

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**Table 35:** Olive cultivars comparative polyphenol content

| Cultivar         | Region  | Polyphenols ppm | C18:1 (%)    |
|------------------|---------|-----------------|--------------|
| Ascolana         | Marche  | 478.22 ± 93.78  | 78.94 ± 1.06 |
| Canino           | Marche  | 432.75 ± 58.63  | 76.92 ± 1.16 |
| Frantoio         | Marche  | 450.68 ± 75.83  | 77.39 ± 1.08 |
| Leccino          | Marche  | 308.34 ± 51.01  | 77.47 ± 0.79 |
| Leccio del Corno | Marche  | 495.34 ± 7.83   | 82.12 ± 0.91 |
| Maurino          | Marche  | 324.76 ± 56.24  | 76.81 ± 2.38 |
| Moraiolo         | Marche  | 496.49 ± 38.68  | 76.97 ± 1.15 |
| Pendolino        | Marche  | 312.01 ± 78.22  | 75.94 ± 1.92 |
| Coratina         | Toscana | 80.8 – 968.5    | -            |
| Frantoio         | Toscana | 173.0 – 501.4   | 79.1         |
| Leccino          | Toscana | 146.0 – 354.0   | 79.9         |
| Maurino          | Toscana | 147.0 – 290.6   | 76.6         |
| Moraiolo         | Toscana | 190.0 – 499.0   | 78.9         |
| Pendolino        | Toscana | 152.0 – 490.3   | 79.2         |

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**Table 36:** Polyphenol content and oxidative stability of arbequina oils produced in 2002 and 2003 with different levels of tree irrigation in California

| Irrigation level     | Polyphenols (mg/L caffeic) 2002 | Polyphenols (mg/L caffeic) 2003 | Hrs. O <sub>2</sub> Stab. 120°C 2002 | Hrs. O <sub>2</sub> Stab. 120°C 2003 |
|----------------------|---------------------------------|---------------------------------|--------------------------------------|--------------------------------------|
| Low<br>10%-34% ET    | 275.3-432.4 a                   | 85.0-175.3 a                    | 8.8-11.4 a                           | 31.2-40.5 a                          |
| Medium<br>35%-59% ET | 102.9-165.2 b                   | 70.0-73.7 b                     | 4.8-5.4 b                            | 28.0-30.3 ab                         |
| High > 60% ET        | 53.3-98.2 c                     | 73.0-97.3 b                     | 3.2-4.6 c                            | 23.6-28.1 b                          |

Maria-Jose Berenguer, Paul Vossen, Steve Grattan, Joseph Connell, and Vito Polito 2006. University of California. Different letters indicate that the values are significantly different at  $\alpha = 0.05$ .

## 9. Sensory characteristics

Flavor components within each cultivar come from the water-soluble hydrocarbons, flavonoids, phenols, polyphenols, tocopherols, and esters that make up the bitter, pungent, and fruity flavor of fresh olives. Some of these compounds are naturally occurring antioxidants that extend oil shelf life by reducing rancidity; others are the source of the fruity character of the oil. High polyphenol content oils can cause a temporary burning sensation in the back of the throat when swallowed and a bitter taste noted on the back of the tongue. If the levels of some of these compounds are too high, the oils can be excessively strong and disagreeably bitter. Fruitiness can be either green (vegetative) or ripe (fruity) in character and possibly sweet (Di Giovacchino et al., 2002).

**Table 37:** Sensory attributes of oils from selected cultivars in Andalusia and Catalonia Spain evaluated over several years

| Cultivar   | Bitter |      | Fruity |      | Green |      | Pungent |      | Sweet |      |
|------------|--------|------|--------|------|-------|------|---------|------|-------|------|
|            | Cat    | And  | Cat    | And  | Cat   | And  | Cat     | And  | Cat   | And  |
| Manzanilla | 3.56   | -    | 2.65   | -    | 2.51  | -    | 2.63    | -    | 1.09  | -    |
| Blanqueta  | 3.19   | 2.47 | 2.52   | 2.50 | 2.28  | 1.82 | 2.91    | 2.60 | 1.29  | 1.13 |
| Picual     | 2.96   | 1.79 | 3.20   | 2.78 | 2.71  | 2.31 | 2.75    | 2.13 | 1.29  | 1.24 |
| Frantoio   | 2.60   | 1.65 | 2.95   | 2.71 | 2.00  | 2.25 | 2.60    | 2.40 | 1.35  | 1.48 |
| Picudo     | 2.06   | 2.50 | 2.28   | 2.94 | 1.67  | 2.52 | 2.44    | 2.73 | 1.50  | 0.70 |
| Hojiblanca | 1.98   | 1.37 | 2.90   | 2.77 | 2.67  | 2.02 | 2.09    | 1.92 | 1.58  | 1.36 |
| Empeltre   | 1.90   | -    | 2.66   | -    | 2.03  | -    | 2.31    | -    | 1.90  | -    |
| Arbosana   | 1.73   | -    | 2.45   | -    | 1.64  | -    | 2.05    | -    | 1.95  | -    |
| Arbequina  | 1.48   | 1.13 | 2.56   | 2.88 | 1.91  | 1.97 | 1.78    | 1.63 | 2.04  | 2.15 |
| P. Maroc.  | -      | 2.64 | -      | 2.88 | -     | 2.35 | -       | 2.42 | -     | 0.42 |
| Koroneiki  | -      | 2.01 | -      | 2.86 | -     | 2.35 | -       | 2.53 | -     | 0.97 |
| Kalamon    | -      | 2.22 | -      | 3.22 | -     | 2.36 | -       | 2.93 | -     | 1.29 |

Agustí Romero, Joan Tous, and Luis Guerrero. Dept. d'Arboricultura Mediterrània, Centre "Mas Bové", IRTA, Reus-Constantí (Tarragona) and Centro Tecnología de la Carne, IRTA, Monells (Girona). Marino Uceda and Ma Paz Aguiler. Estacion de Olivicultura y Elaiotecnica, Junta de Andalucía, Mengibar, Jaén), Spain.

**Table 38:** Sensory attributes of oils from selected cultivars in Australia 2002-03

| Cultivar  | Fruitiness |      | Bitterness |      | Pungency |      |
|-----------|------------|------|------------|------|----------|------|
|           | 2002       | 2003 | 2002       | 2003 | 2002     | 2003 |
| Arbequina | 5.0        | 2.8  | 4.2        | 3.6  | 4.7      | 5.2  |
| Barnea    | 4.8        | -    | 4.7        | -    | 4.7      | -    |
| Coratina  | 5.4        | 3.7  | 6.7        | 4.5  | 7.0      | 5.4  |
| Paragon   | 4.6        | -    | 3.8        | -    | 4.9      | -    |
| Pendolino | -          | 3.1  | -          | 5.3  | -        | 5.7  |
| Picual    | 5.1        | 3.5  | 4.7        | 2.8  | 4.6      | 3.8  |

Richard Gawel, P. Cox, and S. Sweeney. Recognose Pty Ltd P.O. Box 487, Unley, South Australia, 5061; Plant Research Center, Waite Research Precinct, Hartley Grove, Urrbrae, 5064; Rural Ind. R and D Corporation

**Table 39:** Intensity of sensory characteristics single cultivar Tuscan oils evaluated over several years

| Cultivar  | Fruity | Green | Bitter | Pungent | Sweet |
|-----------|--------|-------|--------|---------|-------|
| Frantoio  | *****  | **    | **     | *****   | **    |
| Moraiolo  | ****   | **    | ****   | ***     | **    |
| Leccino   | ***    | *     | *      | **      | ***** |
| Pendolino | ****   | *     | ***    | **      | ***   |
| Maurino   | ****   | **    | **     | ***     | ****  |

A. Cimato, Baldini, Caselli, Marrunci, & Marzi; Firenze, Italia. 1996

Tables 37-39 show numerical sensory ratings for oil from different cultivars grown in Spain, Australia, and Italy. In Spain the differences in sensory attributes are sometimes quite evident from one region to the next for the same cultivar, plus there are clear differences between cultivars. In Australia, the sensory ratings were much higher one year compared to the next and again there are clear differences between cultivars. The Italian example of five Tuscan cultivars provides a relative comparison of their flavor attributes. All of these sensory characterizations by cultivar indicate that different styles of oils can be made based on cultivar. (Cimato et al., 1996; Gawel et al., 2005; Romero et al., 2005).

Though the intensity of an olive oil's flavor characteristics may be influenced by the location where the trees are grown, experience has shown us that all of the typical varietal flavors are part of the genetic make-up of the cultivar. Oil made from a specific cultivar will taste like that cultivar regardless of where it was grown. It has been demonstrated that the amount of irrigation water given to trees can have a profound effect on oil flavor intensities, which might be more important than growing region. In table 40 the fruitiness, bitterness, and pungency were shown to be greatly reduced as the olive trees were given more irrigation water (Angerosa, 2002; Berenguer et al., 2006; Tous et al., 1997; Tura et al., 2000).

**Table 40:** Sensory attributes of California arbequina oils produced with different levels of tree irrigation

| Irrigation level            | Fruitiness | Bitterness | Pungency | Description                                                                                                  |
|-----------------------------|------------|------------|----------|--------------------------------------------------------------------------------------------------------------|
| <b>Low</b><br>10%-34% ET    | 3.2-4.1    | 1.9-6.0    | 1.9-4.9  | Very fruity, some bitterness and pungency. Balanced. Herbaceous, grassy, green apple, nettle, and artichoke. |
| <b>Medium</b><br>35%-59% ET | 2.7-3.2    | 0.7-1.7    | 1.1-1.2  | Very fruity, slightly bitter and pungent. Herbaceous, ripe fruits, and complex.                              |
| <b>High</b><br>> 60% ET     | 1.7-2.0    | 0.2-0.3    | 0.2-0.6  | Mildly fruity mild ripe fruit flavors. Not bitter or pungent. Unbalanced.                                    |

Maria-Jose Berenguer, Paul Vossen, Steve Grattan, Joseph Connell, and Vito Polito 2006. University of California. Different letters indicate that the values are significantly different at  $\alpha = 0.01$ .

## 10. Flowering – fruit maturity – pollenizers – cold hardiness

The selection of cultivars was thought to depend on when they bloom relative to each other for pollination. However, the range in full bloom for 18 selected cultivars listed in table 41 is only 8-9 days, 'Lucques' being the earliest and 'Pendolino' the latest. In table 42, Australian researchers rated the cultivars by early, mid, and late flowering. In most cases, the time of full bloom is preceded and followed by several days of light bloom, indicating adequate overlap for all cultivars in all locations. Proper selection of pollenizer cultivars known to improve set in the main cultivars is very important. The most commonly recommended pollenizer cultivars are given in table 44. Since olives are wind pollinated, pollenizers should be placed upwind within 60 m of the main cultivar. (Barranco and Rallo, 2005; Griggs et al., 1975; Sweeney, 2005; Vossen 2005).

The time of fruit ripening can help spread out the harvest season and is a consideration used to avoid frost, rain, or other weather related problems. In Andalusia, Spain the range in fruit maturity calculated over several years went from October 3 to January 5, which is a span of three months. Within a cultivar the variability could be as much as 2.5 months depending on the year and crop load. Heavy crops delay fruit ripening (Table 41) (Barranco and Rallo, 2005).

**Table 41:** Olive cultivar flowering and maturity dates in Andalusia Spain

| Cultivar           | Full Bloom | Maturity    |
|--------------------|------------|-------------|
| Lucques            | 5/1-4      | 10/3-11/20  |
| Picholine          | 5/3-6      | 10/23-1/1   |
| Frantoio           | 5/4-7      | 10/9-12/16  |
| Empeltre           | 5/4-7      | 10/8-11/26  |
| Gordal (Sevillano) | 5/5-8      | 10/15-11/24 |
| Tanche             | 5/5-8      | 10/15-12/10 |
| Picudo             | 5/5-9      | 10/11-12/12 |
| Manzanilla         | 5/5-9      | 10/10-12/24 |
| Arbequina          | 5/6-9      | 10/15-12/25 |
| P. Marocaine       | 5/6-9      | 10/11-12/22 |
| Aglandau           | 5/7-10     | 10/4-12/24  |
| Picual             | 5/7-11     | 10/17-10/21 |
| Blanquette         | 5/8-11     | 10/21-1/5   |
| Hojiblanca         | 5/8-11     | 10/25-12/18 |
| Leccino            | 5/8-11     | 10/3-11/20  |
| Moraiolo           | 5/8-11     | 10/7-11/25  |
| Kalamon            | 5/8-11     | 11/6-12/25  |
| Pendolino          | 5/9-12     | 10/11-12/4  |

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**Table 42:** Cultivar comparisons for time of flowering in Australia

|                                                                                                                                        |                                                                                 |
|----------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------|
| Early flowering                                                                                                                        | Pendolino                                                                       |
| Mid flowering                                                                                                                          | Leccino, Mission, Manzanilla, Gordal, Barnea, Frantoio, Hojiblanca, and Kalamon |
| Late flowering                                                                                                                         | Arbequina, Aglandau, Coratina, Columella, Koroneiki, and Picual                 |
| Susan Sweeney, Plant Research Center, Waite Research Precinct, Rural Industries R and D Corporation Hartley Grove, Urrbrae, Australia. |                                                                                 |

Cold hardiness of olive cultivars is very important in many locations because of occasional freezing temperatures that can defoliate trees, kill branches in larger trees, kill entire young trees, and eliminate fruit production. Various rating systems have been developed to evaluate the cold hardiness of olive trees. Table 43 provides a subjective rating based on one cold event in California in 1990 (Denney et al., 1993). Many books list the relative cold hardiness of cultivars, which has been done by rating cultivars exposed many times to freezing temperatures over many decades (Table 44) (Bandino et al., 2001; Barranco et al. 2000; Cimato et al., 1997; Moutier et al., 2004; Pannelli et al., 2001; Vossen 2005)

**Table 43:** Subjective rating of december 1990 cold (lowest temperature: 15°F [-9.4°C]) damage to olive cultivars in the U.S. dept. Of agriculture germplasm repository in winters, California

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>RESISTANT</b><br>'Ascolano Dura', 'Asioloni', 'Chitori', 'Grappolo', 'Grossane', 'Karydolia', 'Mavrela', 'Merhavya', 'Mission', 'Sir George Grey's Spanish (Nevadillo)', No. 63, No.65A, 'Redondillo de Lomgrona', 'Rigali', 'Saiali Magloue', 'Souri', 'Touffahl', 'Vassilika', 'Yuaca', 'Yullutt'                                                                                                                                                                                                             |
| <b>SUSCEPTIBLE</b><br>'Arbequina', 'Ascolano-Robinson', 'Azapa', 'Bidh el Hamman', 'Calamata', 'Columello', 'Cordovil', 'Gaidourelia', 'Galega', 'Grossa di Spagna', 'Hamid', 'Leiva', 'Memeli', 'Moraoli', 'Morcal', 'Nabali', 'Nuevo di Sicrone', 'Ogliarola', 'Piconia', 'Picual', 'Redding Picholine', 'Rouget', 'Salome', 'Sigoise', 'Syroglyolia', 'Verdale', 'Zitoun', 'Zoragi', '355-117'                                                                                                                  |
| <b>VERY SUSCEPTIBLE</b><br>'Ascolano – El Toro', 'Balady', 'Barouni', 'Bouteillon', 'Conservolia', 'Coratina', 'Criolla', 'Cucci', 'Dwarf-D', 'Ferruginea', 'Franklin – Coming', 'Frantoio', 'Gairaffa', 'Gigante de Cerignola', 'Grosse Aberkan', K18, 'Kadesh', 'Kalamon', 'Karolia Aghizi', 'Koroneiki', 'Liguria', 'Manzanillo-Hoag', 'Manzanillo-Sharpe', 'Maruini', 'Meski', 'Mission-Davis', 'Nevadillo', 'Nieland Banger', 'Ouslati', 'Picholine de Languedoc', 'Rubra', 'Sevillano-Stralock', 'UC52-24-1' |
| Visual field rating based on shoot tip dieback, defoliation and bark splitting. Combined numerical scores (0-5) as follows: none or light, "resistant" (0, 1); moderate, "susceptible" (2, 3); and heavy, "very susceptible" (4, 5).<br><i>Denney J. O., G. C. Martin, R. Kammereck, D. O. Ketchie, J. H. Connell, W. H. Krueger, J. W. Osgood, G. S. Sibbett, and G. A. Nour. 1993. University of California, USA.</i>                                                                                            |



**Table 44:** Cold hardiness, & recommended pollenizers for selected olive cultivars

| Cultivar                | Cold Hardiness | Pollenizer cultivars <sup>1</sup> |
|-------------------------|----------------|-----------------------------------|
| Arbequina               | Hardy          | Arbosana - Koroneiki              |
| Aglandau                | Hardy          | Bouteillan - Cayon                |
| Arbosana                | Hardy          | Arbequina - Koroneiki             |
| Ascolano                | Hardy          | Manzanillo - Mission              |
| Barnea                  | Sensitive      | Manzanillo - Picholine            |
| Barouni <sup>2</sup>    | Hardy          | Manzanillo - Ascolano - Mission   |
| Bosana                  | Hardy          | Tondo de Cagliari - Pizzé Carroga |
| Bouteillan              | Hardy          | Aglandau - Melanger Verdale       |
| Chemlali                | Hardy          | Chetoui - Gerboui                 |
| Coratina                | Sensitive      | Cellina di Nardo - Ogliarola      |
| Cornicabra              | Hardy          | Verdial - Manzanilla              |
| Empeltre                | Sensitive      | Arbequina - Blanqueta             |
| Frantoio                | Sensitive      | Pendolino - Moraiolo - Leccino    |
| Farga                   | Hardy          | Arbequina - Empeltre              |
| Hojiblanca              | Hardy          | Picual - Picudo - Arbequina       |
| Kalamon                 | Moderate       | Koroneiki - Mastoides             |
| Koroneiki               | Sensitive      | Mastoides - Arbequina             |
| Leccino                 | Hardy          | Frantoio - Pendolino - Moraiolo   |
| Manzanillo <sup>3</sup> | Sensitive      | Sevillano - Ascolano              |
| Maurino                 | Hardy          | Lazzerio - Grappolo               |
| Mission <sup>3</sup>    | Hardy          | Sevillano - Ascolano              |
| Moraiolo                | Sensitive      | Pendolino - Maurino               |
| Pendolino               | Hardy          | Moraiolo - Frantoio - Leccino     |
| Picudo                  | Hardy          | Hojiblanca - Picual - Ocal        |
| Picual                  | Hardy          | Picudo - Hojiblanca               |
| Picholine               | Moderate       | Aglandau - Bouteillan             |
| Sevillano <sup>2</sup>  | Hardy          | Manzanillo - Mission - Ascolano   |
| Taggiasca               | Sensitive      | Leccino                           |

Most olive cultivars are somewhat self-incompatible. They will usually set a better crop with cross-pollination especially under adverse weather conditions. Leccino, Pendolino, Moraiolo, and Maurino are self-sterile and require a pollen source from another cultivar.

Barouni and Sevillano are not compatible cross pollenizers for each other.

Manzanillo and Mission are not compatible cross pollenizers for each other.

Paul Vossen, University of California Cooperative Extension. 2005.

| Cultivar           | Full Bloom | Maturity    |
|--------------------|------------|-------------|
| Lucques            | 5/1-4      | 10/3-11/20  |
| Picholine          | 5/3-6      | 10/23-1/1   |
| Frantoio           | 5/4-7      | 10/9-12/16  |
| Empeltre           | 5/4-7      | 10/8-11/26  |
| Gordal (Sevillano) | 5/5-8      | 10/15-11/24 |
| Tanche             | 5/5-8      | 10/15-12/10 |
| Picudo             | 5/5-9      | 10/11-12/12 |
| Manzanilla         | 5/5-9      | 10/10-12/24 |
| Arbequina          | 5/6-9      | 10/15-12/25 |
| P. Marocaine       | 5/6-9      | 10/11-12/22 |
| Aglandau           | 5/7-10     | 10/4-12/24  |
| Picual             | 5/7-11     | 10/17-10/21 |
| Blanquette         | 5/8-11     | 10/21-1/5   |
| Hojiblanca         | 5/8-11     | 10/25-12/18 |
| Leccino            | 5/8-11     | 10/3-11/20  |
| Moraiolo           | 5/8-11     | 10/7-11/25  |
| Kalamon            | 5/8-11     | 11/6-12/25  |
| Pendolino          | 5/9-12     | 10/11-12/4  |

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## 11. Disease susceptibility

Choosing olive cultivars based on disease susceptibility is another consideration depending on the level of management in the orchard. The three major diseases of olive are Peacock Spot (*Spilocaea oleagina*), Verticillium Wilt (*Verticillium dahliae*), and Olive Knot (*Pseudomonas savastanoi*). Several researchers in different parts of the world have been evaluating the relative resistance of olive cultivars to these diseases. The *Spilocaea* and *Pseudomonas* organisms are only problems in wet cold climates and can be controlled with well timed applications of fixed copper sprays. Strong *Verticillium* resistance has only been shown in two cultivars (Frantoio and Empeltre). (Table 45) (Blanco et al., 2005; Civantos, 1999; Krueger and Vossen 2005; Peñalver et al., 2005; Trapero and López-Doncel, 2005; Vossen et al., unpublished).

**Table 45:** Olive cultivar susceptibility to *Spilocaea oleagina*, *Verticillium dahliae*, and *Pseudomonas savastanoi*

| Cultivar         | <i>Spilocaea oleagina</i> | <i>Verticillium dahliae</i> | <i>Pseudomonas savastanoi</i> |
|------------------|---------------------------|-----------------------------|-------------------------------|
| Aglandau         | S                         | R                           | -                             |
| Arbequina        | S                         | VS                          | VS                            |
| Arbosana         | VR                        | -                           | -                             |
| Ascolano         | S                         | R                           | R                             |
| Blanqueta        | VS                        | -                           | R                             |
| Bouteillan       | R                         | -                           | -                             |
| Cayon            | R                         | -                           | -                             |
| Coratina         | R                         | -                           | -                             |
| Cornicabra       | VS                        | VS                          | -                             |
| Empeltre         | VS                        | VR                          | S                             |
| Frantoio         | VR                        | VR                          | R                             |
| Gordal Sevillana | S                         | S                           | S                             |
| Hojiblanca       | S                         | VS                          | S                             |
| Koroneiki        | VR                        | R                           | S                             |
| Kalamon          | S                         | S                           | R                             |
| Leccino          | R                         | S                           | R                             |
| Manzanilla       | S                         | S                           | VS                            |
| Maurino          | R                         | -                           | -                             |
| Mission          | VS                        | S                           | R                             |
| Moraiolo         | R                         | S                           | S                             |
| Pendolino        | S                         | S                           | S                             |
| Picholine        | S                         | S                           | -                             |
| P. Marocaine     | VS                        | -                           | R                             |
| Picual           | VS                        | VS                          | S                             |
| Picudo           | S                         | VS                          | VS                            |

VR = Very Resistant, R = Resistant, S = Susceptible, and VS = Very Susceptible

Paul Vossen, William Krueger, Rachel Elkins, Ed Weber, Ken Churches, and Alexandra Kicenik-Devarenne, University of California, USA; Blanco-López, M. A. and F. J. López-Escudero. 2005. U de Córdoba, Spain; Civantos López-Villalta, M. 1999, U of Jaén; Peñalver, R., A. Garcia, J. Pérez-Panadés, C. Del Rio, J. M. Caballero, J. Pinochet, J. Piquer, E. A. Carbonell, and M. Milagros López. 2005; Trapero, A. and L. M. López-Doncel. 2005

## 12. Conclusions

Olive cultivars are not created equal. The perfect cultivar does not exist. It is even more difficult to expect a cultivar to be both excellent for table fruit and oil production (dual-purpose). But, no matter where they are grown, some cultivars are far superior to others, based on their vigor, productivity, regular bearing, oil content, fruit size, pit to pulp ratio, fatty acid composition, polyphenol content, sensory characteristics, cold hardiness, flowering and maturity dates, pollenizer needs, and disease susceptibilities. The basic characteristics of the cultivar are inherent in the genetics. Cultivars with high or low polyphenol content, certain specific flavor characteristics, big or small fruit size, high or low oil content, or whatever percent of each fatty acid type, tend to retain those same characteristics beyond the influence of the country in which they were grown or how they were grown. All of the data comes from countries with a Mediterranean-type climate. Manipulations such as tree water stress, or

fruit maturity can have a significant influence on the ultimate characteristics of the product, but the oil, or fruit still retain the basic characteristics of the cultivar.

Some cultivars consistently seem to come into bearing before others (more precocious) and tend to produce heavier yields each year. Some have more oil content, more polyphenol content, are more cold hardy, and have greater resistance to some diseases. This does not seem to be based on local adaptation or natural selection process, but more likely a provincial adoption process. Until now many regions thought they had the best cultivar in the world, but only because they had never tried anything else. Therefore, it behooves researchers and industry to take a close look at the potential benefits of introducing superior producing cultivars or cultivars that have superior quality characteristics that would likely lead to greater efficiency and profit. New world producers beyond the Mediterranean region have planted most of the world's well known cultivars and are still in the process of evaluating the real potential of each one. There are many others, however, that still need to be tried and have not been simply because they have not yet been introduced or propagated.

Early production (precocity) is very important economically and it is difficult or impossible to make up for with cultivars that come into bearing much later. For similar reasons, regular bearing is extremely important; cultivar choice can reduce the problem of alternate bearing, but it is also very heavily influenced by cultural practices, particularly the availability of water. In managed orchards, cultivars that have consistently shown superior productivity are: 'Arbequina', 'Arbosana', and 'Koroneiki', followed by 'Barnea', 'Blanqueta', 'Coratina', 'Leccino', 'Hojiblanca', 'Manzanillo', and 'Picual'.

Of course, the choice of cultivar does not end with yield performance. Oil content and oil extractability can be an extremely important economic factor in cultivar choice. Cultural practices that influence fruit water content, fruit ripeness, and processing systems all have an influence on oil yield. Choices need to be made that fit in with the prevailing production and processing methods. Cultivars with superior oil yield are: 'Coratina', 'Koroneiki', and 'Picual' – followed by 'Arbequina', 'Arbosana', 'Empeltre', 'Frantoio', 'Haouzia', 'Hojiblanca', 'Leccino', 'Manzanillo', 'Menara', 'Mission', 'Picudo', and 'Taggiasca'.

Market characteristics such as fruit size, texture, flavor, and appearance are very important for table fruit, though table olives can be made from any cultivar. Oil processors look for superior stability, flavor, and style, again any olive can be made into oil. The table cultivars with consistently recognized quality are 'Ascolana', 'Chalkidiki', 'Gordal-Sevillano', 'Kalamon', 'Manzanilla', 'Picholine du Languedoc', and 'Santa Caterina'. The oil cultivars with consistently recognized flavor and style characteristics are: 'Arbequina', 'Bosana', 'Frantoio', 'Koroneiki', and 'Picudo' – followed by 'Ayvalik', 'Chetoui', 'Cornicabra', 'Coratina', 'Hojiblanca', 'Leccino', 'Moraiolo', and 'Picholine Marocaine'. The main cultivars with consistently superior oxidative stability are 'Coratina', 'Picual', 'Koroneiki', and 'Mission'. The fatty acid profile of a cultivar's oil is not normally used as a selection criterion, but could be if they produced fatty acid levels that were outside the accepted range of the IOC standard for olive oil.

Extremely important is how a cultivar will fit into the climate and site where it is to be grown and how it will be managed. Choosing cultivars based on high vigor, rustic hardiness, and ability to withstand drought is a step backwards in terms of modern world productive competitiveness. Dry-farmed or rain-fed orchard sites should only be chosen if they have adequate winter rainfall and deep enough soils to hold the moisture. Dry-farming can be successful, but it is certainly a management system that puts the farmer at a strong competitive disadvantage. Irrigation water is really necessary in order to assure no plant stress, so that adequate shoot growth can provide the foundation for fruit production in the following years. Good cultural practices must be followed in order to prevent weed competition, shading within the tree, alternate bearing, nutrient deficiencies, or disease outbreaks.

Pollenizer cultivars should be chosen based on their traditionally observed value in the field and on their documented pollen viability. They should be placed within 60 m of the principal cultivar to assure adequate cross pollination in years with weather problems during bloom. There are not enough differences in bloom dates to choose cultivars based when they bloom. It would also be wise to plant several cultivars that mature early to late in order to spread out the harvest season and make the best use of labor.

Cultivars with superior cold hardiness are 'Arbequina', 'Arbosana', 'Leccino', 'Hojiblanca', and 'Picual'. The most disease resistant cultivars are 'Arbosana', 'Blanqueta', 'Bouteillan', 'Frantoio', 'Koroneiki', and 'Leccino'. Disease resistance cultivar choices should be based on the past severity of specific diseases in the growing region.

Admittedly there are many gaps in the data for cultivar evaluation. Much of what is presented here is based on good comparative trials in two different regions of Spain that was published and widely available. It was combined with limited information from Italy, France, Morocco, Australia, and the USA. Many varieties were missing from the comparison trials and some of the trials have only measured results for a few years. Unfortunately, this research is very costly and very time consuming work. It is very important, however, for each growing region to make these comparisons – otherwise cultivar selection becomes haphazard and based on non-scientific criteria.

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## Morphological and pomological characteristics of Jordanian olive cultivars

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### Abstract

Morphological and pomological characterization was performed for 13 local olive cultivars held in the national germplasm collection in Jordan.

Results of the study indicated the presence of significant differences between the olive cultivars regarding the studied morphological characteristics; Shami cultivar characterized by the highest fruit weight, length, width and stone weight. Nabali Muhasan gave the highest leaf length. Kfari Romi and Nabali Muhasan cultivars characterized by the highest inflorescence length and number of flowers per inflorescence.

Pomological characterization showed significant differences between the cultivars. Fruit oil content was the highest for Nabali Baladi, Kfari Romi, Kfari Baladi and Kanabisi cultivars. Regarding olive oil fatty acid composition, oleic acid content was the highest in Nabali Baladi, Kfari Romi and Kfari Baladi cultivars. Palmitic acid was the highest in Nasouhi Jaba and Souri cultivars. Linoleic acid was the highest in Souri cultivar. Stearic acid was the highest in Bathni and Kfari Romi cultivars. Total polyphenol content was the highest in Kfari Romi, Nabali Baladi and Souri cultivars. The data obtained can be used for construction of a database that could form the basis for the national design of breeding programs.

**Key words:** *Olea europaea*, genetic resources, local cultivars, morphological characteristics, pomological characteristics.

## Caractéristiques morphologiques et pomologiques des cultivars d'oliviers en Jordanie

### Résumé

La caractérisation morphologique et pomologique ont été réalisées pour 13 cultivars locaux d'olive détenue dans la collection de germoplasme nationale en Jordanie. Les résultats de l'étude ont révélé la présence de différences significatives entre les cultivars d'olive sur les caractéristiques morphologiques étudiés; Shami cultivar caractérisé par le plus haut le poids des fruits, longueur, largeur et le poids de la pierre. Nabali Muhasan accordait la plus grande longueur de la feuille. Kfari Romi et Nabali Muhasan cultivars caractérisés par la plus grande longueur de l'inflorescence et le nombre de fleurs par inflorescence.

La caractérisation pomologique a montré des différences significatives entre les cultivars. Fruit teneur en huile est le plus élevé pour Nabali Baladi, Kfari Romi, Kfari Baladi et cultivars Kanabisi. En ce qui concerne l'huile d'olive composition en acides gras, teneur en acide oléique est le plus élevé Nabali Baladi, Kfari Romi et Kfari cultivars Baladi. L'acide palmitique est le plus élevé Nasouhi Jaba et cultivars Souri. L'acide linoléique est le plus élevé cultivar Souri. L'acide stéarique est le plus élevé Bathni et Kfari cultivars Romi.

Teneur en polyphénols totaux est le plus élevé Kfari Romi, Nabali Baladi et cultivars Souri. Les données obtenues peuvent être utilisées pour la construction d'une base de données qui pourrait servir de base à la conception nationale de programmes de sélection.

**Mots-clés:** *Olea europaea*, les ressources génétiques, les cultivars locaux, les caractéristiques morphologiques, les caractéristiques pomologique.



## 1. Introduction

The olive, *Olea europaea* L. is adapted to the Mediterranean climate and produces table olives and olive oil, both of which are important commodities in world markets. The cultivation of olive trees started in the Mediterranean basin some 6000 years ago.

Jordan can be considered one of the homelands and natural habitats of cultivated olives. The olive tree has nutritional, social and economic importance in the life of the Jordanian people. Olive plantation makes up about 70% of the orchards of Jordan. Approximately 128000 hectares are planted with around 17 million olive tree, making it the country's top agricultural product. In 2008, the total production of olive oil was 18000 tons (Anonymous, 2008).

The original center of olive cultivation is Palestine, Syria and Cyprus (Loukas and Krimbas, 1983). The *Olea europaea* L. species include the wild olive var. *Oleaster* distributed in various areas in the Middle East and the cultivated olive var. *sativa*. The cultivated olive trees were selected from the wild. The propagation of the selected types had already been performed vegetatively, thousands of years ago (Zohary and Spiegel, 1975). Thus, specific types and clones were preserved locally in each area or village. Many of these old clones were lost over the generations but due to the high viability potential of the olive, many of these trees survived and were grafted with some common cultivars.

Many olive cultivars are grown in Jordan since centuries; the main autochthonous ones are 'Nabali Baladi', 'Rasei', 'Shami', 'Kanabisi' and 'Nasouhi Jaba'. Other cultivars have been introduced to Jordan many years ago from various olive growing regions of the world. The most common local olive cultivars grown in Jordan are Nabali Baladi and Rasei. However, several clones of these cultivars are widely spread in different areas of Jordan and several synonyms are used for these cultivars depending on the region they were grown.

It is of great importance to evaluate and characterize the existing genetic diversity of the crop species, mainly for those, such as the case of olive, which still have well preserved a great cultivar patrimony, in spite of the disturbance of the environments where they are cultivated. Within the olive species, more than 2000 cultivars have been already described for the Mediterranean area as a whole (Bartolini et al., 1998). The problem of olive germplasm classification is not only complicated by the richness of its genetic patrimony, but also by the absence of reference standards and by the confusion on the cultivar names, with numerous cases of homonymy and synonymy (Bartolini et al., 1998). The identification of olive tree cultivars has been carried out using methods based on morphological, agronomical or biochemical traits (Cantini, et al., 1999; Barranco et al., 2000; Fabbri et al., 1995). Morphological approach has traditionally been used for olive cultivar identification and continues to be the initial main step for the description and classification of olive germplasm (Rotondi et al., 2003).

Currently, according to our knowledge, no studies have been implemented in Jordan regarding the morphological or pomological characterization of local olive cultivars. Therefore the objective of this study was to evaluate the morphological and pomological characters of the local olive cultivars present in Jordan.

## 2. Materials and methods

### 2.1. Field conditions and plant material

This study was carried out in 2007-2008. Plant material were collected from 13 olive cultivars conserved at the National collection which was established in 1996 at Al-Mushaqar Research Station (Altitude: 790 m above sea level, longitude: 35° 47' East and latitude: 31° 46' North), located 28 Km to the South West of Amman.

The national collection consists of 47 olive cultivars, including 34 foreign cultivars and 13 autochthonous. Trees were planted in rows of 10 trees of each cultivar. Tree spacing was at 6 meters within rows and 7 meters between rows. The olive trees were trained with minimum pruning and grown under rain-fed conditions and standard cultural practices. These cultivars are grown in different areas of Jordan including Jerash, Ajloun, Irbid, Balqa, Tafleh, and Kerak. Passport data were recorded for the 13 local olive cultivars held in the national collection as shown in table 1.

**Table 1:** Passport data of the autochthonous olive cultivars held in the national collection.

| N° | Common name    | Synonyms    | Origin            | Distribution                               | Purpose           |
|----|----------------|-------------|-------------------|--------------------------------------------|-------------------|
| 1  | Nabali Baladi  | Romi, Souri | Jordan, Palestine | Widespread in rain-fed areas of Jordan     | Dual-purpose      |
| 2  | Rasei          | Muhasan     | South of Jordan   | Widespread in rain-fed and irrigated areas | Dual-purpose      |
| 3  | Nabali Muhasan | None        | Jordan            | Rain-fed and irrigated areas               | Table olive       |
| 4  | Nasouhi Jaba   | None        | Jordan, Palestine | Few plantations in the irrigated areas     | Black table olive |
| 5  | Shami          | None        | Jordan            | Few plantations                            | Table olive       |
| 6  | Souri          | None        | Jordan            | Few plantations in the rain-fed areas      | Olive oil         |
| 7  | Kfari Romi     | Romi        | Jordan            | North of Jordan / Irbid                    | Dual-purpose      |
| 8  | Kfari Baladi   | Baladi      | Jordan            | North of Jordan / Irbid                    | Dual-purpose      |
| 9  | Kanabisi       | None        | Jordan            | North of Jordan / Ajloun and Jerash        | Olive oil         |
| 10 | Arabi Altafila | None        | Jordan            | South of Jordan / Tafelah                  | Table olive       |
| 11 | Ketat          | None        | Jordan            | South of Jordan / Tafelah                  | Table olive       |
| 12 | Bathni         | None        | Jordan            | South of Jordan / Tafelah                  | Table olive       |
| 13 | Rosai          | None        | Jordan            | South of Jordan / Tafelah                  | Table olive       |

## 2.2. Morphological characteristics

Primary characterization of the Jordanian olive cultivars was carried out for the 13 olive cultivars held in the national collection. Morphological characterization was performed according to the "methodology for the primary characterization of olive varieties" adapted by the International Olive Council (IOOC, 1997).

Each individual tree was characterized by primary descriptors relating to leaf characters (Shape, length, width, longitudinal curvature of the blade), inflorescence (length, number of flowers/inflorescence), fruit (weight, shape, symmetry, position of maximum transverse diameter, apex, base, nipple, presence of lenticels, size of lenticels, location of start of color change, colour at full maturity) and endocarp characters (weight, shape, symmetry, position of maximum transverse diameter, apex, base, surface, number of grooves, distribution of the grooves, termination of the apex) (IOOC, 1997). Representative samples of leaves, shoots, inflorescences and fruits were taken from each of four plants. 10 random samples were evaluated for all characters. Leaf and flower samples were collected from the mid-shoot portion of the current year growth, while 1-year-old shoots were taken at random from fruiting branches following a rotation around the tree at approximately 1.5m from the ground. At harvest time (mid November), 100 fruits were picked from each group of four plants and studied for fruit and pit characters.

## 2.3. Agronomical and pomological characteristics

Rooting ability of leafy stem cuttings of each cultivar was studied during March 2008. Percentage of rooted cuttings, average number of roots per cutting and average length of roots was reported after 60 days under mist. The cuttings were prepared from the middle section of vigorous shoots taken from trees on an "off" year and were treated with 3000 ppm indolebutyric acid (IBA) for 5 seconds (Caballero, 1981).

Study of pomological characteristics includes flesh/stone ratio of the fruit, percentage of moisture and percentage of oil in the fruit. The fruits were characterized using homogeneous samples of black olives that were harvested when the Maturity Index reached 4. Average fresh weight of the fruit and average fresh weight of the stone was calculated from two sub-samples of 50 olive fruits. Percentage of moisture in the fruit was calculated from two sub-samples of 50g of crushed fruits, dried in the oven at 105°C for 24 hours. Percentage of the oil in the fruit was calculated from two sub-samples using soxhlet extraction method (AOAC, 1980).

For each olive cultivar under study, olive fruits were harvested and sent directly to small-scale olive mill (Mini-Olomio) for oil extraction. The extracted olive oil samples were stored in the refrigerator until analysis. Olive oil fatty acid composition was determined according to the method reported by

European Committee (2003). Total polyphenols content was determined using caffeic acid as standard (Gutierrez, et al., 2001).

## 2.4. Statistical analysis

Statistical data analysis was performed by analysis of variance (ANOVA). Duncan's Multiple-Range Test was carried out to test significance of differences between treatment means (cultivars) using the SPSS program for Windows, version 17.0.

## 3. Results and Discussion

Results of the morphological characterization indicating the presence of significant differences between olive cultivars under study (Table 2 and 3). Shami cultivar characterized by the highest fruit weight, length, width and stone weight. However, Nabali Baladi, Nabali Muhasan, Souri and Kfari Romi were the lowest in fruit weight (Table 2). The highest leaf length was recorded for Nabali Muhasan and Souri cultivars, and the highest leaf width was recorded for Shami, Rosai, Nabali Baladi, Nabali Muhasan, Souri and Kfari Romi cultivars (Table 3). Higher internode length was recorded for Kfari Romi as compared to other cultivars. The highest inflorescence length was recorded for Kfari Romi and Nabali Muhasan cultivars and the highest number of flowers per inflorescence was recorded for Nabali Muhasan cultivar (Table 3).

**Table 2:** Average mean values of the parameters used for morphological characterization and description of Jordanian olive cultivars.

| No. | Olive Cultivar | Fruit Weight (g) | Fruit Length (cm) | Fruit Width (cm) | Stone Weight (g) | Stone Length (cm) | Stone Width (cm) |
|-----|----------------|------------------|-------------------|------------------|------------------|-------------------|------------------|
| 1   | Nabali Baladi  | 2.78 ef*         | 2.17 ef           | 1.52 fg          | 0.54 c           | 1.67 def          | 0.75 b           |
| 2   | Rasei          | 3.95 c           | 2.24 cde          | 1.71 de          | 0.51 c           | 1.51 a            | 0.79 b           |
| 3   | Nabali Muhasan | 2.48 ef          | 2.13 ef           | 1.41 h           | 0.79 b           | 1.82 bc           | 0.85 b           |
| 4   | Nasouhi Jaba   | 4.62 c           | 2.81 b            | 1.68 e           | 0.81 b           | 2.11 a            | 0.80 b           |
| 5   | Shami          | 7.04 a           | 3.00 a            | 2.13 a           | 0.92 a           | 1.94 b            | 0.95 ab          |
| 6   | Souri          | 2.34 ef          | 2.06 fg           | 1.44 gh          | 0.52 c           | 1.62 defg         | 0.71 b           |
| 7   | Kfari Romi     | 3.13 e           | 2.27 cde          | 1.58 f           | 0.51 c           | 1.66 def          | 0.72 b           |
| 8   | Kfari Baladi   | 4.20 cd          | 2.41 c            | 1.82 c           | 0.61 c           | 1.72 cd           | 0.78 b           |
| 9   | Kanabisi       | 4.32 cd          | 2.46 c            | 1.79 cd          | 0.76 b           | 1.69 cde          | 0.88 b           |
| 10  | Arabi Altafila | 3.98 c           | 2.21 cde          | 1.85 c           | 0.51 c           | 1.49 a            | 1.58 a           |
| 11  | Ketat          | 4.14 cd          | 2.26 cde          | 1.81 c           | 0.55 c           | 1.57 efg          | 0.80 b           |
| 12  | Bathni         | 4.23 cd          | 1.99 g            | 1.88 c           | 0.72 b           | 1.28 h            | 0.95 ab          |
| 13  | Rosai          | 5.33 b           | 2.33 cd           | 2.00 b           | 0.80 b           | 1.54 fg           | 0.96 ab          |

**Table 3:** Average mean values of the parameters used for morphological characterization and description of Jordanian olive cultivars.

| N° | Olive Cultivar | Leaf        | Leaf       | Inter-     | Inflores.   | No. of Flowers per Inflorescence |
|----|----------------|-------------|------------|------------|-------------|----------------------------------|
|    |                | Length (cm) | Width (cm) | Node (cm)  | Length (cm) |                                  |
| 1  | Nabali Baladi  | 5.00 bc*    | 1.14 ab    | 2.09 b     | 1.79 de     | 17.0 b                           |
| 2  | Rasei          | 4.79 bcd    | 1.03 bc    | 1.45 def   | 2.63 b      | 16.2 bc                          |
| 3  | Nabali Muhasan | 5.61 a      | 1.10 abc   | 1.57 f     | 2.93 a      | 28.2 a                           |
| 4  | Nasouhi Jaba   | 5.10 b      | 0.88 d     | 1.37 ef    | 1.61 e      | 10.5 e                           |
| 5  | Shami          | 5.16 b      | 1.20 a     | 2.10 b     | 1.92 d      | 12.9 d                           |
| 6  | Souri          | 5.61 a      | 1.12 abc   | 1.81 bcd   | 2.52 b      | 16.1 bc                          |
| 7  | Kfari Romi     | 4.81 bcd    | 1.10 abc   | 2.57 a     | 2.95 a      | 17.1 b                           |
| 8  | Kfari Baladi   | 4.64 cde    | 1.05 bc    | 2.04 bc    | 2.57 b      | 10.8 e                           |
| 9  | Kanabisi       | 4.31 ef     | 0.80 d     | 1.75 bcde  | 1.43 f      | 6.5 f                            |
| 10 | Arabi Altafila | 4.12 fg     | 0.88 d     | 2.02 bc    | 1.75 de     | 9.8 e                            |
| 11 | Ketat          | 4.49 de     | 0.81 d     | 1.40 ef    | 2.18 c      | 14.5 cd                          |
| 12 | Bathni         | 3.69 h      | 1.02 c     | 1.92 bc    | 1.31 f      | 6.4 f                            |
| 13 | Rosai          | 3.84 gh     | 1.17 a     | 1.68 bcdef | 2.57 b      | 9.5 e                            |

\* Means within each column having the same letters are not significantly different at 5% probability level according to Duncan's Multiple-Range Test.

Rooting ability study indicated that Rasei cultivar gave significantly the highest values regarding rooting percentage (85.2%) and average number of roots per cutting (10.7) compared to the other cultivars. On the other hand, Nasouhi Jaba gave the lowest rooting percentage (15.3%) without being significantly different from Nabal Baladi, Ketat and Kfari Baladi (Table 4). Regarding average length of roots, it was the highest for Arabi Altafila (9.2cm) without significant differences from Rasei, Nasouhi Jaba, Souri, Kfari Baladi, Kfari Romi, Kanabisi and Ketat cultivars (Table 4). It was reported that some olive cultivars are easy-to-root, while others are difficult (Fouad *et al.*, 1990). Rasei cultivar is easy-to-root, however, Nabali Baladi is difficult-to-root and has a low rooting percentage of cuttings (Qrunfleh *et al.*, 1994; Ayoub and Qrunfleh, 2006).

**Table 4:** Rooting ability of leafy stem cuttings for 13 olive cultivars rooted during March 2008.

| Cultivar       | Rooting % | Average number of roots per cutting | Average length of roots (cm) |
|----------------|-----------|-------------------------------------|------------------------------|
| Nabali Baladi  | 19.3 ef * | 3.5 bc                              | 4.3 b                        |
| Rasei          | 85.2 a    | 10.7 a                              | 6.2 ab                       |
| Nabali Muhasan | 53.6 b    | 6.1 b                               | 4.5 b                        |
| Nasouhi Jaba   | 15.3 f    | 2.6 c                               | 7.0 ab                       |
| Shami          | 25.5 de   | 3.5 bc                              | 5.6 b                        |
| Souri          | 30.3 cd   | 3.2 bc                              | 6.4 ab                       |
| Kfari Romi     | 25.4 de   | 5.4 bc                              | 7.2 ab                       |
| Kfari Baladi   | 21.2 ef   | 2.5 c                               | 7.5 ab                       |
| Kanabisi       | 32.4 c    | 3.7 bc                              | 6.3 ab                       |
| Arabi Altafila | 56.3 b    | 5.4 bc                              | 9.2 a                        |
| Ketat          | 18.5 f    | 2.4 c                               | 7.2 ab                       |
| Bathni         | 32.9 c    | 6.5 b                               | 4.6 b                        |
| Rosai          | 58.7 b    | 5.7 bc                              | 4.5 b                        |

Results of pomological parameters showed higher values regarding flesh-stone ratio of the fruit for Arabi Altafila, Rasei, Shami and Ketat cultivars compared to the other cultivars. However, the lowest values were recorded for Nabali Muhasan and Souri cultivars (Table 5). These results indicated that cultivars with higher flesh-stone ratio are more suitable to be used for table olive purposes than those with low flesh-stone ratio. Percentage of moisture in the fruit was the highest for Rasei and Nabali Muhasan cultivars. Percentage of oil in the fruit was the highest for Nabali Baladi, Kfari Romi, Kfari Baladi and Kanabisi cultivars (Table 5). It was reported by Said *et al.* (2005) that percentage of oil in the fruit for the local Nabali Muhasan cultivar was 45% based on dry weight bases, which is similar to our finding in this study. There are no reports available regarding oil content for the other local cultivars.

**Table 5:** Average values of the parameters used for pomological characterization of Jordanian olive cultivars.

| N° | Olive cultivar | Flesh-stone ratio of the fruit | Percentage of moisture in the fruit (%) | Percentage of oil in the fruit (dry wt.) (%) |
|----|----------------|--------------------------------|-----------------------------------------|----------------------------------------------|
| 1  | Nabali Baladi  | 4.2 cd*                        | 45.0 cd                                 | 60.6 a                                       |
| 2  | Rasei          | 6.7 a                          | 60.5 a                                  | 46.4 bc                                      |
| 3  | Nabali Muhasan | 2.1 e                          | 60.1 ab                                 | 36.6 cd                                      |
| 4  | Nasouhi Jaba   | 4.7 cd                         | 52.0 abcd                               | 35.4 d                                       |
| 5  | Shami          | 6.6 a                          | 50.3 bcd                                | 45.5 bcd                                     |
| 6  | Souri          | 3.5 de                         | 49.4 cd                                 | 47.6 b                                       |
| 7  | Kfari Romi     | 5.1 abcd                       | 46.8 cd                                 | 63.2 a                                       |
| 8  | Kfari Baladi   | 5.9 abc                        | 43.8 d                                  | 65.7 a                                       |
| 9  | Kanabisi       | 4.7 cd                         | 42.0 d                                  | 59.1 a                                       |
| 10 | Arabi Altafila | 6.8 a                          | 45.9 cd                                 | 35.4 d                                       |
| 11 | Ketat          | 6.5 ab                         | 50.8 abcd                               | 43.6 bcd                                     |
| 12 | Bathni         | 4.9 bcd                        | 43.6 d                                  | 44.7 bcd                                     |
| 13 | Rosai          | 5.7 abc                        | 54.5 abc                                | 37.9 bcd                                     |

\* Means within each column having the same letters are not significantly different at 5% probability level according to Duncan's Multiple-Range Test.

The composition of fatty acids in olive oil is shown in table 10. Oleic acid content was the highest in Nabali Baladi (71.4%) without significant differences from Kfari Romi or Kfari Baladi cultivars. However, the significantly lowest oleic acid content was recorded for Nasouhi Jaba cultivar (56.4%) (Table 6). Palmitic acid was the highest in Nasouhi Jaba and Souri cultivars. Linoleic acid was the significantly the highest in Souri cultivar (19.4%). Stearic acid was the highest in Bathni and Kfari Romi cultivars (Table 6). The results show that olive oil fatty acid content in all studied cultivars were within the normal reference values and legal limits as established by International Olive Oil Council (IOOC, 2006). In a study on olive oil fatty acid content of two local olive cultivars, Tawalbeh (2005) reported that oleic acid content in olive oil obtained from Nabali Baladi and Romi cultivars were 75.7% and 76.5%, respectively. Al-Rousan (2004) reported that oleic acid content in olive oil extracted from Nabali Muhasan cultivar grown in the north of Jordan contains 69.9% oleic acid, 14.1% palmitic acid, 2.8% Stearic acid and 8.7% lenoleic acid.

**Table 6:** Fatty acid composition of olive oil for the 13 olive cultivars.

| N°. | Cultivar       | Oleic acid (C18:1) % | Palmitic acid (C16:0) % | Linoleic acid (C18:2) % | Stearic acid (18:0) % |
|-----|----------------|----------------------|-------------------------|-------------------------|-----------------------|
| 1   | Nabali Baladi  | 71.42 a*             | 10.87 de                | 11.40 f                 | 3.83 abc              |
| 2   | Rasei          | 68.69 cd             | 12.41 c                 | 13.51 bc                | 2.94 cde              |
| 3   | Nabali Muhasan | 68.26 d              | 11.07 de                | 14.34 b                 | 3.75 abc              |
| 4   | Nasouhi Jaba   | 56.35 g              | 15.41 a                 | 12.31 def               | 2.24 e                |
| 5   | Shami          | 67.13 e              | 14.24 b                 | 13.14 cde               | 2.37 e                |
| 6   | Souri          | 60.13 f              | 14.39 ab                | 19.42 a                 | 2.66 de               |
| 7   | Kfari Romi     | 71.48 a              | 10.10 e                 | 11.82 f                 | 4.23 ab               |
| 8   | Kfari Baladi   | 71.21 a              | 11.00 de                | 12.07 f                 | 3.24 bcde             |
| 9   | Kanabisi       | 69.08 cd             | 12.33 c                 | 13.24 cd                | 2.57 de               |
| 10  | Arabi Altafila | 69.61 bc             | 12.57 c                 | 11.92 f                 | 3.39 bcd              |
| 11  | Ketat          | 70.18 b              | 11.75 cd                | 12.25 ef                | 3.41 bcd              |
| 12  | Bathni         | 68.25 d              | 11.12 de                | 13.55 bc                | 4.56 a                |
| 13  | Rosai          | 66.78 e              | 13.65 b                 | 13.73 bc                | 2.88 cde              |

With regard to olive oil polyphenols content, results of this study showed that the highest values were reported for olive oil obtained from Kfari Romi (323 mg/kg) and Nabali Baladi (308 mg/kg) cultivars. On the other hand, the lowest value (187mg/kg) of total polyphenols content was found in olive oil obtained from Rosai cultivar (Table 7). Similar finding was reported by Tawalbeh (2005) who found that total polyphenols content in olive oil obtained from Nabali Baladi and Romi cultivars were 372 mg/kg and 322 mg/kg, respectively.

**Table 7:** Total polyphenols content in olive oil for the 13 olive cultivars

| N° | Olive cultivar | Total phenols content mg/kg |
|----|----------------|-----------------------------|
| 1  | Nabali Baladi  | 308 ab*                     |
| 2  | Rasei          | 252 def                     |
| 3  | Nabali Muhasan | 273 cd                      |
| 4  | Nasouhi Jaba   | 235 efg                     |
| 5  | Shami          | 210 ghi                     |
| 6  | Souri          | 294 abc                     |
| 7  | Kfari Romi     | 323 a                       |
| 8  | Kfari Baladi   | 285 bcd                     |
| 9  | Kanabisi       | 264 cde                     |
| 10 | Arabi Altafila | 226 fgh                     |
| 11 | Ketat          | 198 hi                      |
| 12 | Bathni         | 268 cd                      |
| 13 | Rosai          | 187 i                       |

\*Means having the same letters are not significantly different at 5% probability level according to Duncan's Multiple-Range Test.

The differences in morphological and pomological characteristics among olive cultivars under this study could be attributed to genetic factors of these cultivars since they were grown under the same ecological conditions. In conclusion and based on the results of this study, it is possible to find cultivars with valuable morphological, agronomical and/or pomological traits that can be immediately distributed to the farmers or employed in breeding programs.

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## Comportement de six variétés d'olivier à huile dans le biotope de Taous (Sfax.Tunisie) : Résultats de 4 campagnes de suivi

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### Résumé

Le suivi du comportement de six variétés d'olivier à huile (Chemlali de Sfax, Chemchali, Oueslati, Chetoui, Arbéquina et Koroneiki) plantées à Taous (Sfax, Tunisie) en 2002 à raison de 204 arbres par hectare durant quatre campagnes agricoles (2005-2008), nous a permis de mettre en évidence les qualités de chacune de ces variétés dans ce biotope particulier. En ce qui concerne la production moyenne en olives, ce sont les variétés Chemlali de Sfax et Arbéquina qui se distinguent par des productions moyennes par arbre et par an dépassant les 14 Kg. La teneur en matière grasse par rapport au poids frais est variable de 22 à 35.5% selon le stade de maturité et la variété, cependant c'est la variété Oueslati qui enregistre le taux le plus élevé (35.5%) pour des olives cueillies au stade de fin véraison. Quant aux phénologies de la croissance végétative et de la floraison, elles présentent certaines variations d'une campagne à une autre.

**Mots clés:** Olive à huile, croissance, floraison, taux d'huile, production

### Abstract

Monitoring the behaviour of six olive oil varieties (Chemlali Sfax, Chemchali, Oueslati, Chetoui, Arbequina and Koroneiki) planted at Taous (Sfax, Tunisia) in 2002 at the rate of 204 trees per hectare for four crop years (2005 -2008), allowed us that Chemlali and Arbequina are characterized by an average of olive production per tree per year exceeding 13 Kg. The fat content compared to fresh weight varies from 19 to 33% depending mainly on variety, however, Oueslati and Chemchali varieties recorded the highest rate. As to the phenology of vegetative growth and flowering, there are some variations from one cultivar to another and from one year to another.

### 1. Introduction

La Tunisie, malgré ces 66 millions d'oliviers souffre de la fluctuation de sa production annuelle qui engendrerait des répercussions très néfastes pour l'économie nationale. Plusieurs facteurs sont à l'origine de cette situation dont les conditions climatiques caractérisées par l'irrégularité et parfois l'insuffisance des précipitations (Trigui, 1993). Pour remédier à cette situation, de multiples mesures ont été préconisées dont l'encouragement de l'installation de vergers intensifs (Chaari et al, 2006 et même hyper intensifs (Larbi et al, 2006) qui, étant donné leur relative indépendance des conditions climatiques, assureraient une production annuelle minimale permettant à la Tunisie, entre autres, d'honorer ces engagements envers ces partenaires. Or, le comportement en intensif des variétés tunisiennes d'olivier les plus cultivées (Chétoui et Chemlali) est peu connu à travers la Tunisie et ce en dépit de leur parfaite adaptation aux conditions de culture tunisiennes. En plus, des variétés comme Oueslati ou Chemchali Gafsa qui sont très performantes aussi bien sur le plan agronomique que technologique (Kammoun et Khlif 2001 ; Gheriani, 2006), se sont très peu propagées à travers le pays et leur étude en collection pourrait aboutir à des bonnes découvertes. Les vertus des variétés étrangères comme l'Arbéquina ou encore Koroneiki les présentent comme de sérieux concurrents ou compléments pour les variétés locales.

Le travail objet de ce papier traite de l'évaluation de quelques performances agronomique et technologique de ces six variétés d'oliviers à huile (Chemlali de Sfax, l'Arbéquina, Koroneiki, Chétoui, Oueslati El Alla et Chemchali Gafsa) dans le biotope de Taous (Situé à 28 Km au nord de la ville de Sfax-Tunisie).



## 2. Matériel et méthodes

### 2.1. Le verger d'étude

Le verger d'étude est situé dans la ferme expérimentale de l'institut d'olivier, situé à 28 Km du Nord ouest de la ville de Sfax (centre de la Tunisie). Les oliviers ont été plantés au mois de Février 2002 avec une densité de 204 pieds à l'hectare et un espacement de 7 m/7 m, irriguée au goutte à goutte. Au cours des années d'étude, l'apport en eau et en éléments nutritifs a été calculé selon les besoins annuels d'oliviers.

### 2.2. Matériel végétal

- *Chemlali de Sfax* : C'est une variété tunisienne à huile qui occupe 60% de la surface oléicole. Sa floraison est précoce. Ses fruits mûrissent tard, sont petits de poids moyen de 1gramme. Le rendement moyen en huile est de 20 % (Grati- Kammoun et Khlif, 2002).

- *Chetoui* : C'est une variété tunisienne appelée aussi « Zayati ». Son entrée en production est précoce et son époque de floraison est tardive (Trigui et Msallem, 2002). Les fruits sont nombreux et d'un poids moyen de 2grammes. Le rendement en huile est généralement de 28 %. C'est la principale variété à huile du Nord tunisien.

- *Chemchali Gafsa* : Appelée encore « Zeitoun el Ouaha », c'est la variété la plus répandue dans la région de Gafsa, aussi bien en sec qu'en irrigué dans les oasis où elle est plus productive. Son entrée en production est moyennement précoce et son époque de floraison est précoce. Le poids moyen de ses fruits est de 2.26grammes et son rendement en huile est de 28 %.

- *Oueslati* : C'est une variété tunisienne appelée encore « El Leguim ». Peuplant avec « El Horr » les plantations ancestrales de la zone traditionnelle de Oueslatia. Son entrée en production et son époque de floraison sont précoces. Ces fruits mûrissent tard, sont nombreux et petits de poids moyen de 2.5 grammes. Le taux moyen en huile de ses fruits est de 31 % (Grati- Kammoun et Khlif, 2002).

- *Koroneiki* : C'est la principale variété à huile de Grèce. Les arbres fleurissent précocement et produisent un pollen abondant. La maturation des fruits est précoce à moyenne. Sa productivité est élevée. Le fruit pèse en moyenne 1.8 gramme et son rendement à huile est élevé.

- *Arbequina* : C'est une variété des provinces de Lerida et Taragane (en Espagne), elle est très productive. Les arbres sont généralement de vigueur moyenne, les fruits sont petits de poids moyen 1 à 2 grammes avec un rendement en huile de 17 à 20 %.

### 2.3. Evaluation de la croissance végétative

Pendant la période de croissance, l'évolution de l'allongement végétatif est suivie sur huit arbres de chaque variété. Sur chaque arbre on suit la croissance de 4 rameaux disposés dans les quatre orientations de l'arbre, ces rameaux sont généralement représentatifs de l'état de végétation de l'arbre. Les mesures effectuées concernent : la longueur total du rameau, son diamètre mesuré à l'aide d'un pied à coulisse à 5 cm du point de son insertion et le nombre de nœuds. On rapporte les accroissements relatifs moyens pour ces 3 paramètres c'est-à-dire : (Valeur Finale- Valeur Initiale) du paramètre/Valeur Initiale.

### 2.4. Evaluation des potentialités reproductives des variétés étudiées

Il s'agit de l'étude de la biologie florale qui consiste à déterminer les dates du début de la floraison (qui correspond à 10% des fleurs ouvertes), de la pleine floraison quand on a 50% des fleurs qui sont ouvertes et de la fin de la floraison correspondant au début de la chute des pétales.

On s'est également intéressé à l'évaluation de la production annuelle en Kg de fruits par arbre et par variété.

### 2.5. Technologie oléicole :

- Caractérisation pomologique des olives :



- Le poids moyen d'une olive est déterminé systématiquement pour chaque échantillon étudié par la pesée de 3x100 fruits frais.
- Rapport pulpe/noyau : On procède au dénoyautage de 100 fruits préalablement pesés. Après nettoyage et pesée des noyaux obtenus, le rapport pulpe/noyau est calculé selon la formule suivante :

$$\text{Pulpe/Noyau} = \frac{\text{Poids de 100 fruits} - \text{Poids de 100 noyaux}}{\text{Poids de 100 noyaux}}$$

- Teneur en matière grasse

Elle est déterminée par la technique de spectrométrie de résonance magnétique nucléaire (R.M.N) à l'aide d'un spectromètre du type « OXFORD 4000 ».

La détermination de ce paramètre est réalisée sur des prises de 50 fruits frais préalablement pesé et séché à 105°C. Le poids sec des olives est communiqué à l'appareil à l'aide d'une balance électronique. L'appareil est programmé pour effectuer 3 mesures successives et affiche une valeur moyenne. La valeur affichée par l'appareil représente la teneur en matière grasse par rapport au poids sec. Celle exprimée par rapport au poids frais est déterminée par le calcul suivant : MG/poids frais = (MG/poids sec) x (poids sec)

- Composition acide de l'huile

L'extraction de l'huile à partir des olives fraîches (2,5 Kg environ pour chaque échantillon) a été effectuée à l'aide d'un oléo doseur au laboratoire d'Oléotechnie de l'Institut d'Olivier à Sfax. La technique d'extraction de l'huile comporte quatre étapes : le broyage, le malaxage, la centrifugation et enfin la décantation naturelle basée sur la différence de densité entre les deux liquides, l'huile (0.915 à 0.920) et les margines (1.015 à 1.086) suite à laquelle la phase supérieure constituée d'huile est recueillie pour déterminer sa composition en acides gras.

### 3. Résultats et discussions

#### 3.1. Caractéristiques agronomiques

##### 3.1.1. Production en olives

Alors qu'en 2004, seul 20 % des arbres ont donné des fruits, l'année 2005 a enregistré l'entrée en production de la quasi totalité des oliviers (85%). La Chemlali, l'Arbéquina et la Koroneiki, après 4 années de production, présentent les moyennes les plus élevées (Tableau 1). Une alternance franche caractérise la Oueslati. La production moyenne à l'hectare est variable de 1,5 Tonne/ha (Oueslati) à 3 Tonnes à l'hectare (Chemlali et Arbéquina). Il faut noter que dans les mêmes conditions de culture, l'Arbéquina peut produire jusqu'à 6 T/ha (Lopez-Villalta, 1997).

**Tableau 1:** Production en olives en Kg par arbre par variété et par année.

| Variété        | 2005        | 2006        | 2007         | 2008         | Moyenne      |
|----------------|-------------|-------------|--------------|--------------|--------------|
| Arbéquina      | 4,1         | 9,69        | 14           | 24,7         | 13,1225      |
| Chemchali      | 5,78        | 2,32        | 4,9          | 18,8         | 7,95         |
| Koroneiki      | 5,39        | 4,17        | 19           | 18,18        | 11,685       |
| Chemlali       | 11,46       | 8,12        | 21,5         | 18,58        | 14,915       |
| Chétoui        | 7,72        | 4,06        | 5,45         | 12,7         | 7,4825       |
| Oueslati       | 7,72        | 0,1         | 22,2         | 0,1          | 7,53         |
| <b>Moyenne</b> | <b>7,03</b> | <b>4,74</b> | <b>14,51</b> | <b>15,51</b> | <b>10,45</b> |

### 3.1.2 Floraison

Le suivi de la floraison des 6 variétés laisse apparaître une légère variation d'une année à une autre (Tableau 2). Ainsi pour les années 2005, 2006 et 2009, la pleine floraison coïncide avec le début du mois de Mai, alors que pour 2007 et 2008, elle se passe vers la fin du mois d'Avril. Les conditions climatiques peuvent être à l'origine de ces fluctuations (Lavee, 1997).

Une autre remarque à faire est le comportement presque identique de toutes les variétés quant à l'apparition des fleurs. Ce ramassage dans le temps de la floraison ne pourrait qu'avoir un effet bénéfique sur la qualité et la quantité des fruits puisqu'il permettrait d'éviter certaines malformations et imperfections des drupes (Lavee, 1997).

**Tableau 2:** Date du début de la floraison (DF), pleine floraison (PF) et fin de la floraison (FF).

|           |    | 2005 | 2006 | 2007 | 2008 | 2009 |
|-----------|----|------|------|------|------|------|
| Arbéquina | DF | 25/4 | 18/4 | 10/4 | 16/4 | 30/4 |
|           | PF | 2/5  | 2/5  | 20/4 | 22/4 | 3/5  |
|           | FF | 15/5 | 15/5 | 5/5  | 2/5  | 9/5  |
| Chemchali | DF | 28/4 | 27/4 | 12/4 | 18/4 | 30/4 |
|           | PF | 5/5  | 2/5  | 20/4 | 28/4 | 7/5  |
|           | FF | 15/5 | 12/5 | 5/5  | 5/5  | 12/5 |
| Koroneiki | DF | 25/4 | 27/4 | 12/4 | 16/4 | 30/4 |
|           | PF | 2/5  | 2/5  | 20/4 | 22/4 | 3/5  |
|           | FF | 15/5 | 17/5 | 5/5  | 2/5  | 9/5  |
| Chemlali  | DF | 25/4 | 18/4 | 10/4 | 16/4 | 30/4 |
|           | PF | 2/5  | 2/5  | 20/4 | 22/4 | 7/5  |
|           | FF | 15/5 | 17/5 | 5/5  | 2/5  | 12/5 |
| Chétoui   | DF | 25/4 | 18/4 | 10/4 | 18/4 | 30/4 |
|           | PF | 2/5  | 2/5  | 2/5  | 25/4 | 7/5  |
|           | FF | 15/5 | 17/5 | 7/5  | 5/5  | 12/5 |
| Oueslati  | DF | 25/4 | 27/4 | 12/4 | 18/4 | 30/4 |
|           | PF | 2/5  | 2/5  | 20/4 | 25/4 | 7/5  |
|           | FF | 15/5 | 17/5 | 7/5  | 5/5  | 12/5 |

### 3.1.3. Croissance végétative

Les variétés Chemlali de Sfax, Arbéquina, Chemchali et Chétoui s'adaptent relativement bien à ce système de culture puisqu'elles présentent des croissances relatives assez importantes (Tableau 3).

La Koroneiki se distingue surtout par un accroissement relativement important du diamètre moyen et un allongement des rameaux plutôt faible avec un nombre moyen de nœuds élevé ce qui explique le port naturellement ramassé de cette variété et la conservation de cette caractéristique dans le biotope de Taous (Tableau 3).

La variété Oueslati présente des accroissements faibles ce qui pourrait être le résultat du fait que les arbres sont issus de boutures semi ligneuses donc franc de pieds alors que cette variété est généralement cultivée greffée dans son site d'origine.

Les années à venir nous permettront de vérifier toutes ces hypothèses et de se prononcer sur le degré d'adaptabilité de ces variétés aux conditions édaphoclimatiques de la région sfaxienne.

**Tableau 3:** Allongement Relatif Moyen des Rameaux (ARR), Diamètre Relatif Moyen des rameaux à 5 cm du point de leur insertion (DRR) et le Nombre de Nœuds relatif Moyen des mêmes rameaux (NRN) par variété et par campagne.

| Variété   | Paramètre | 2005/06 | 2006/07 | 2007/08 |
|-----------|-----------|---------|---------|---------|
| Arbéquina | ARR       | 0.62    | 0.32    | 0.71    |
|           | DRR       | 0.90    | 0.52    | 1.29    |
|           | NRN       | 0.87    | 0.41    | 0.86    |
| Chemchali | ARR       | 0.35    | 0.51    | 0.73    |
|           | DRR       | 0.64    | 0.82    | 0.69    |
|           | NRN       | 0.86    | 0.62    | 0.94    |
| Koroneiki | ARR       | 0.50    | 0.28    | 0.31    |
|           | DRR       | 1.19    | 0.61    | 0.53    |
|           | NRN       | 0.83    | 0.34    | 0.65    |
| Chemlali  | ARR       | 0.57    | 0.76    | 0.68    |
|           | DRR       | 0.86    | 0.96    | 0.80    |
|           | NRN       | 1.59    | 0.78    | 0.97    |
| Chétoui   | ARR       | 0.49    | 0.46    | 0.92    |
|           | DRR       | 0.75    | 0.56    | 0.62    |
|           | NRN       | 0.69    | 0.38    | 1.26    |
| Oueslati  | ARR       | 0.56    | 0.61    | 0.37    |
|           | DRR       | 1.02    | 0.70    | 0.37    |
|           | NRN       | 1.03    | 0.59    | 0.70    |

### 3.2. Caractéristiques technologiques

#### 3.2.1. Variation du Poids Moyen d'un Fruit (PMF) et du rapport pulpe/noyau (P/N).

Le PMF est, pour presque toutes les variétés, variable d'une saison à une autre (Tableau 4). On constate que pour Chemlali et Koroneiki, le PMF est inférieur par rapport à celui de leur site d'origine (SO) (Kotsaftakis et al, 2000 ; Grati Kammoun, 2007). Alors que pour les autres variétés, il est supérieur ou égal au PMF dans le SO (Grati Kammoun, 2007). Quant aux rapports P/N, ils sont variables durant les 2 campagnes 2005 et 2007, mais en 2008, on peut remarquer que les valeurs sont proches de ceux des sites d'origine sauf pour la Koroneiki dont le P/N demeure inférieur à sa valeur (5) dans le SO (Kotsaftakis et al, 2000).

**Tableau 4:** Le PMF et le P/N par variété et par année.

|           | 2005 |       | 2007 |      | 2008 |      |
|-----------|------|-------|------|------|------|------|
|           | PMF  | P/N   | PMF  | P/N  | PMF  | P/N  |
| Oueslati  | 1,78 | 6,215 | 1,4  | 4,47 | -    | -    |
| Arbéquina | 1,98 | 5,565 | 1,75 | 6,19 | 1,11 | 4,71 |
| Chemlali  | 0,82 | 3,995 | 0,94 | 5,09 | 0,94 | 5,34 |
| Chemchali | 1,86 | 5,575 | 2,45 | 5,6  | 2,23 | 8,27 |
| Koroneiki | 0,94 | 4,31  | 0,93 | 3,36 | 0,88 | 4,91 |
| Chétoui   | 2,52 | 7,075 | 2,38 | 7,89 | 1,89 | 6,74 |

#### 3.2.2. Teneur en matière grasse par rapport au poids frais (MGF) et au poids sec (MGS).

**Tableau 5:** La MGF et La MGS par variété et par année

|           | 2005  | 2007  |       | 2008  |      |
|-----------|-------|-------|-------|-------|------|
|           | MGF   | MGS   | MGF   | MGS   | MGF  |
| Arbéquina | 24,05 | 51,81 | 26,37 | 39,65 | 19   |
| Chemchali | 23,99 | 59,34 | 33,6  | 58,87 | 33   |
| Koroneiki | 24,92 | 50,05 | 27,2  | 47,49 | 23   |
| Chemlali  | 22,28 | 49,82 | 27,13 | 44,66 | 19,9 |
| Chétoui   | 27,04 | 53    | 26,2  | 46,88 | 21,5 |
| Oueslati  | 27,92 | 55,67 | 32,77 | --    | --   |

Les taux de matière grasse par rapport au poids frais varient de 19 à 33 % (Tableau 5). Ils sont très variables d'une campagne à une autre. Il est à remarquer aussi la distinction de la Chemchali par des taux élevés (2007 et 2008) et une diminution de ces taux en 2008 pour le reste des variétés. La stabilisation de ce paramètre pourrait avoir lieu dans les années à venir.

### 3.2.3. Variation du pourcentage moyen des principaux acides gras

L'examen de la composition en acide gras majeurs de l'huile d'olive (Acide Palmitique, Acide oléique et Acide linoléique, Tableau 6) des 6 variétés nous permet de constater que seule la Chemlali Sfax a présenté des taux meilleurs en acide oléique en comparaison avec celui du site d'origine estimé à 57%. Toutes les autres variétés ont vu leur taux en acide oléique diminuer, par rapport aux valeurs des sites d'origines, en faveur des taux des acides Palmitique (Arbequina) et surtout du linoléique (Arbequina, Chetoui, Chemchali et Koroneiki), ce qui pourrait altérer la qualité de ces huiles. Ces paramètres devraient être suivis les années à venir pour pouvoir se prononcer sur l'utilité de ces introductions dans la région de Sfax.

**Tableau 6:** Variation du pourcentage moyen des principaux acides gras par variété et par année.

| Variétés  | Acide Palmitique |       |       | Acide oléique |       |       | Acide linoléique |       |       |
|-----------|------------------|-------|-------|---------------|-------|-------|------------------|-------|-------|
|           | 2005             | 2007  | 2008  | 2005          | 2007  | 2008  | 2005             | 2007  | 2008  |
| Oueslati  | 12,92            | 12,5  | -     | 69,25         | 68,98 | -     | 14,02            | 15,8  | -     |
| Arbequina | 18,70            | 16,75 | 18,74 | 59,17         | 62,56 | 57,83 | 15,99            | 16,62 | 16,02 |
| Chemlali  | 18,11            | 16,67 | 17,94 | 61,56         | 62,13 | 59,1  | 14,34            | 16,94 | 16,94 |
| Chemchali | 16,65            | 16,12 | 16,49 | 66,40         | 62,81 | 62,7  | 12,02            | 17,6  | 15,97 |
| Koroneiki | 12,34            | 12,24 | 12,4  | 77,78         | 76,74 | 73,7  | 5,57             | 8,1   | 9,82  |
| Chetoui   | 14,51            | 12,54 | 13,21 | 62,79         | 61,47 | 67,33 | 18,30            | 22,93 | 15,57 |

Enfin, cette étude préliminaire laisse supposer que la Chemchali, la Chemlali et la Chetoui s'adaptent de mieux en mieux à ce système de culture car leurs performances en 2008 sont relativement meilleures qu'en 2006 (Gheriani, 2006). L'Arbequina par contre, malgré une plus ou moins importante production en fruits, régénère un faible taux d'huile (40%) inférieur à la moyenne habituelle dans son site d'origine.

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## Interaction between some environmental parameters and plant behavior of different varieties of olive cultivars in Tunisia

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### Abstract

Phenology refers to events that are periodic in the plant-life cycle. Our biannual survey (2008-2009) covered three olive varieties: Chetoui, Chemlali, Meski of Institut de l'Olivier germoplasm collection in Tunis. Our goal was to study the plant-environment interaction in terms of accumulation of cold (CUmodified), accumulation of heat (GDD) and phenological variability. Specific optimal thresholds temperatures have been carried out as far as the starting accumulation dates for CU and GDD in each cultivars. In 2008, Meski's requirements for cold and heat are quite larger than Chetoui and Chemlali moreover, for all cultivars, the C and D phenological stages respectively for floral clusters formation and corolla differentiation coincide with the GDD and CU accumulations. On the contrary during D stage (buds swelling), a significant difference has been calculated. A phenological comparison with the main olive South-Italy cultivars allowed us to evaluate behavior's differences.

**Key words:** *Olea europaea* L., Phenology, Chilling Unit, Growing Degree Day, Threshold Temperature.

## Interaction entre certains paramètres environnementaux et le comportement de plantes d'oliviers de différents cultivars en Tunisie

### Résumé

La phénologie étudie des événements périodiques du cycle de la plante. Notre étude biennale (2008-2009) a concerné trois variétés d'oliviers : Chetoui, Chemlali, Meski de la collection du germoplasme de l'Institut de l'Olivier de Tunis. Notre but était d'étudier l'interaction plante-environnement en terme d'accumulation de froid (CU modifié), d'accumulation de chaleur (GDD) et de variabilité phénologique. Les résultats étaient la détermination de températures seuils optimales et spécifiques ainsi que les dates de début d'accumulation aussi bien des CU que des GDD. De plus en 2008, les exigences en froid et en chaleur de la Meski se sont révélées supérieures à ceux de Chetoui et Chemlali. Enfin, les phases phénologique C et D, correspondant respectivement à la formation des grappes florales et à la différenciation de la corolle, coïncident en terme d'accumulation de CU et de GDD au contraire, la phase D (gonflement des boutons floraux) reflète une différence significative. Une comparaison phénologique avec les principaux cultivars du Sud d'Italie a permis d'estimer les différences de comportement et d'adaptation au climat.

**Mots clés :** *Olea europaea* L., Phénologie, Unité de Froid, Degré Jour, Température Seuil

### 1. Introduction

Phenology is the discipline that studies biological phenomena that occur periodically with obvious changes in the appearance of organisms describing in details the different development phases and correlating them with the environmental variables. The succession of different stages of the plant-life cycle is regulated and influenced not only by biotic forces (biorhythms) that are regulated by the genetic constitution of the species (Orlandi et al., 2007), but also by abiotic forces depending on climatic conditions among which the most important is the air temperature (Orlandi et al., 2004), soil moisture and finally the photoperiod (Galan et al., 2005). A detailed knowledge of crops phenology allows both to monitor the impact of the environment on different species and to make predictions about the impact of weather events more or less favorable or climate change and consequently to define the most appropriate strategies for cultivation considering the real productive potentials.

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Nowadays, phenology has taken an increasing importance in various disciplines and vegetative species, among these the olive (*Olea europaea* L.) is one of the more studied tree crop. The olive phenology has been utilized in bioclimatic models that have carried out interesting results in measuring the extent of climate change (Galan et al., 2005). Generally, olive pollen is considered one of the most important factors for allergy in the Mediterranean area. Determining flowering date and its extending is very important to establish preventive measures anti-allergy. In agriculture, bioclimatic models based on phenology have proved to be valuable tools for predicting olive yield (Fornaciari et al., 2005; Galan et al., 2008). The study of quantitative relationship between crop phenology and meteorological parameters has been addressed for many species of agricultural interest to guide management decisions in a rational crop management and for the determination of the vocation of cultivation areas. One of the principal factors that influence the growth and development of organisms is the temperature that is recorded in a certain stage of development when a definite amount of "heat" is accumulated. Other researchers have been interested by the olive which is one of the main crop in the Mediterranean area with the aim to quantify the effects of both high and cold temperatures on the plant cycle considering that olive is characterized by a late flowering period compared to other species of its range of cultivation and which depends strongly on the temperature during the months preceding anthesis. By a bioclimatic point of view it is very important to estimate the olive chill requirements and assess its accumulations. Some authors have reported the importance of chilling requirements evaluation in olive underlining its crucial role in breaking dormancy in previously-initiated buds.

Recently a new flowering indicator (modified chilling units: CUm) has been proposed in order to forecast the flowering timing in olive thanks to phenological and meteorological data. Moreover, in consideration of spring heat requirements we have to consider that for the olive species the thresholds for heat calculation vary from one region to another according to the bio-geographical characteristics and according to the altitude (Galan et al., 2005). The aim of this study was to evaluate the plant-environment interaction in a Tunisian olive growing area in terms of consecutive accumulation of chilling and heating units in reference to the recent research findings (Orlandi et al., 2006). Finally, a comparison between Italian and Tunisian olive growing areas and cultivars was realized regarding the amounts of classic Growth Degree Days (GDD) and their relative calculation thresholds.

## 2. Materials and Methods

The phenological study was conducted during two study years (2008-2009) on three olive (*Olea europaea* L.) cultivars: two oil cultivars (Chemlali and Chetoui) and one table olive cultivar (Meski) selected in the olive germoplasm bank of the Institut de l'Olivier at Tunis (IO). These cultivars were chosen on the base of their diffusion in the Tunisian olive-growing landscape and of their economic importance. Operationally the survey was made by IO technical staff and biological and meteorological data were sent to phenology laboratory of the Department of Applied Biology (University of Perugia) for statistical processing.

In order to obtain objective results we selected four plants for each cultivar in good phytopathological and nutritional status. The phenological surveys have been conducted every 7 days beginning on the season of awakening to post-anthesis buds along the scale Maillard (1975). During full bloom the measurements were conducted every two days. The phenological stages were represented by different letters (B to G) in a phenogramme and every stage represents the average expression of a twig bearing type for each of the four plants chosen and monitored for each cultivar. The biometeorological analysis was based on the calculation of thermal amounts required by each phenological stage, moreover the meteorological variables (max, min temperatures and rainfall) were considered as 7-day averages according to the phenological frequency. In addition, the temperature amounts were drawn utilizing various mathematical formulas that refer respectively to the calculation of modified Chilling Units (CUm) and Growth Degree Day (GDD) (Orlandi et al. 2006; Fornaciari et al. 2000 a, b). The CUm were calculated by introducing different threshold temperatures beyond which a value of -1 is accumulated, so the chilling and heating effects were considered contemporary by the modified formula and they were calculated consecutively from the winter to the spring periods. Three threshold temperatures (TT) were taken into consideration (8-9-10°C). CUm = -1 for hourly temperature > TT above which negative values (CU= -1, chilling negation) were recorded and interpreted as heat amounts. The chill unit (CUM) summations were calculated from three different start dates (1<sup>st</sup> December, 1<sup>st</sup> January and 1<sup>st</sup> February) till the beginning of each phenological stage determined by the precedent phenological survey. The GDD method considered the maximum and minimum daily temperatures using five threshold temperatures (9-10-11-12-13°C). The summations of

GDD were calculated considering two start dates: from 1<sup>st</sup> January and from 1<sup>st</sup> February to the beginning of each phenological stage. Statistical analysis considered the calculation of coefficients of variation (CV) of chill and heat amounts for the assessment of lowest variability between the two study years for each phenological stages and for the three cultivars. Finally, the tunisian spring temperature amounts (GDD) calculated to full flowering dates were compared with those calculated in 16 olive growing areas in central-south Italy where different olive cultivars are present (representative areas of different Italian Provinces: Perugia, Avellino, Benevento, Salerno, Bari, Brindisi, Foggia, Taranto, Lecce, Cosenza Catanzaro, Reggio C., Messina, Palermo, Trapani and Agrigento). The comparison was realized to evaluate the differences between Mediterranean olive growing areas in terms of GDD thresholds and temperature requirements to develop reproductive structures.

### 3. Results

Thermopluviometric graph shows no anomalies during 2008 compared to 2009 where we noticed more relevant climatic anomalies specially during the second week of January and April, where peaks of high rainfall led to a reduction of the maximum temperatures. Comparing the phenology of the three cultivars with reference to the cumulated rainfall recorded during 2008, we noted that the olive-oil cultivars reacted positively to rainfall from the first week of March till the fourth week more than the table-olive cultivar which showed a marked delay. Later in the season, Chetoui and Chemlali tended to homogenize their behavior specially during C, D, E and F1 Maillard phenological stages. In 2009 the exceptional rainfall event during the first week of April has prompted a slowdown in the development of the cultivars. The phenological survey during this year revealed that chemlali (olive-oil cultivar) was distinguished by an advanced cycle followed by table-olive cultivar Meski finally by Chetoui. The temperature trend affected the whole phenological cycle. Definitely the analysis of maximum temperature graph revealed that 2009 was pretty cool year compared to 2008 resulting in a delay of phenological-cycle beginning of the three cultivars (third week of March) rather than by the third week of February. This fact is also confirmed by the CUM accumulation values. In fact, during 2008 the CUM accumulations reached approximately, at the B phase date, 875 units on the contrary, in 2009, CUM amounts reached 950 units in the wake of the low temperatures recorded even during March that increased the positive chilling units. Moreover, the temperatures also affect the progress of phenological stages. Indeed, during 2008 we see that the minimum temperatures at the beginning of the cycle had very low impact on the olive phenology compared to maximum temperatures which since the first week of March till the third raised the differences between olive-oil and olive-table cultivars (Fig. 1 A-B; 2 A-B). The analysis of variance (CV) realized considering the CUM amounts between the two study years evidenced the lowest variability during the pre-flowering and flowering phases (E, F, F1). In correspondence of these phases all the three cultivars showed CUM summations very similar manifesting that the olive trees in the two years accumulated, in different manners, chilling and heating amounts arriving however to similar final amounts. The variance analysis carried out with GDD amounts obtained for the three cultivars evidenced for the B and C phenological stages a temperature of 9°C as optimal threshold. Further more we noted that the three cultivars registered the least variability, in the two years and considering all the thresholds, during F and F1 stages (full flowering); however the maximum homogeneity in this phase was obtained with the threshold of 12°C.

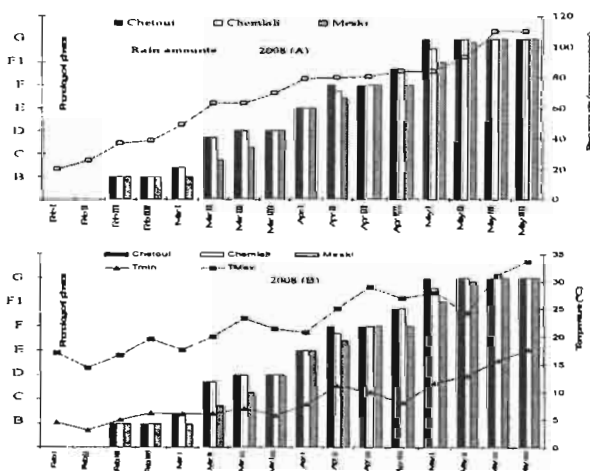


Figure 1 (A, B): Thermopluviometric graphs (2008) .

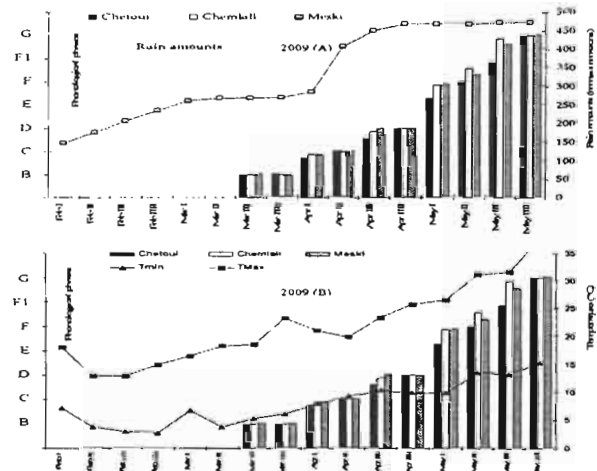


Figure 2 (A, B): Thermopluviometric graphs (2009) .



Meski cultivar, in particular, manifested the least variability for the accumulation of GDD for all the thresholds. The specific investigation at full flowering phase show that the three tunisian cultivars evidenced the best threshold temperature (which minimize the variance of GDD amounts) at 12°C; moreover, comparing the GDD requirements of the cultivars under study with Italian southern ones the same threshold values were recorded in Catanzaro, Palermo, Taranto, Messina provinces (Fig. 3). The average of GDD summation for each Tunisian cultivar is almost equal to that of Sicilian ones. In fact, the tunisian cultivars accumulate around 250 and 280 GDD (Fig. 4) in comparison to those of 320 and 350 GDD related to the cultivars growing at Messina, Agrigento and Trapani (the last two characterized by a threshold of 11°C).

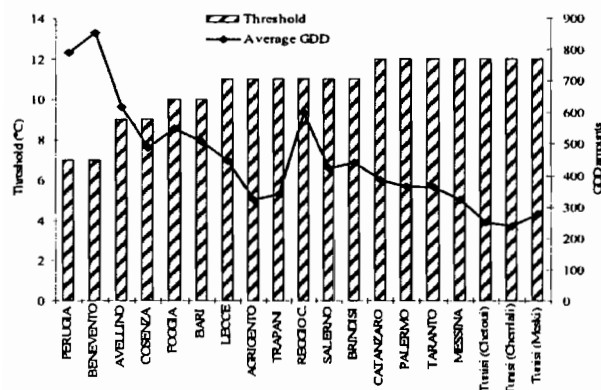


**Figure 3:** Optimal threshold temperatures of Tunisian and Italian cultivars.

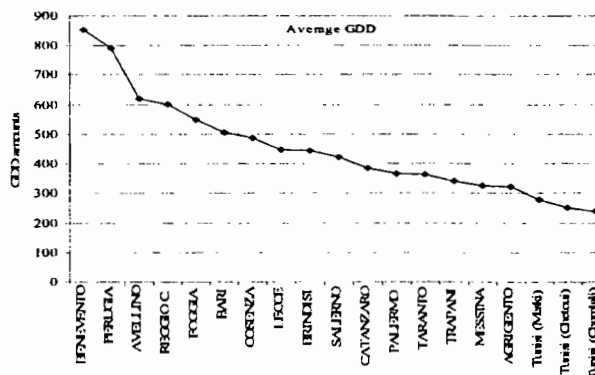


**Figure 4:** Average GDD amounts of Tunisian and Italian olive growing areas.

#### 4. Discussion

The present bio-meteorological analysis permitted to evidence the principal differences of vegetative/reproductive phases between olive cultivar grown under the same climatic conditions.

Temperatures affect the whole plant cycle and can cause variation in some phenological stages in fact, maximum temperatures excursions hold up the phenological advance of olive-oil cultivars compared to table-olive one instead, the minimum temperatures excursions tend to homogenize them. Meski cultivar presents chilling requirements superior then olive-oil cultivars Chetoui and Chemlali. However Chetoui showed some sensitivity to cold in fact, its phenological cycle was delayed in 2009 compared to 2008 where the same cultivar had an advanced cycle. Meski was the least affected by the cold accumulating in any case enough GDD to continue its phenological cycle. Finally, during the two years survey, chemlali confirmed its intermediate behavior. The best threshold temperature during pre-anthesis stages for the three cultivar was 9°C. Moreover during full blooming (F, F1), the best threshold was 12°C in accordance with the southern Italy cultivars growing specially in Sicily.

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## Latitude and altitude effect on flowering phenology of olive tree in two Mediterranean countries Tunisia and Spain

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### Abstract

Latitude and altitude are two of the main factors influencing plant phenology Orlandi et al. (2005). The present study shows the effect of these geographical parameters on olive flowering phenology in two Mediterranean countries Tunisia (Mornag (36° 39'N 10°16'E) and Chaal (34° 19', 10°04'E) and Spain (Cordoba (37°50'N 4°45'W) and Baena (37°37'N 4°20'W) during 2006-08. The floral phenology was studied by using the Hirst type volumetric spore trap, as an aerobiological monitoring method. The pollen emission trends showed a close relationship between phenological phases and some geographical parameters, i.e. altitude and latitude. Flowering start occurred before in Tunisia (Chaal and Mornag, 35m and 45m respectively) than in Spain (Cordoba and Baena, 118m and 420m respectively). Concerning the latitude, except 2008 there is a high correlation between the flowering start and the pick day with the latitude ( $R^2 = 0.9$ ).

**Keywords:** pollen emission, geographic parameters, phenology, *Olea europaea*

### Effet de l'altitude et la latitude sur la phénologie florale de l'olivier (*Olea europaea* L.) dans deux pays méditerranéens la Tunisie et l'Espagne

### Résumé

La phénologie de la plante est très influencée par les paramètres géographiques à savoir la latitude et l'altitude Orlandi et al., (2005). Ce présent travail montre l'effet de la latitude et l'altitude sur la phénologie florale de l'olivier dans deux pays méditerranéens la Tunisie (Mornag (36° 39'N 10° 16'E) et Chaal (34° 19' 10° 04'E)) et l'Espagne (Cordoba (37°50'N 4°45'W) et Baena (37°37'N 4°20'W)) durant 2006-08. La phénologie florale a été déterminée en utilisant le capteur volumétrique de pollen de type Hirst installé au milieu de l'olivieraie. Les résultats obtenus ont montré que les paramètres phénologiques sont en étroite relation avec l'altitude et la latitude. En effet, le début de la floraison en Tunisie (Chaal et Mornag, avec une altitude 35m et 45m respectivement), est plus précoce par rapport à l'Espagne (Corboda et Baena à altitudes respectives de 118m et 420m respectivement). De même la période de floraison varie proportionnellement selon l'altitude. Concernant la latitude, excepté l'an 2008, une forte corrélation ( $R^2 = 0.9$ ) existe aussi bien entre la latitude et le début de la floraison ainsi qu'avec le pic de la floraison.

**Mots clefs:** Emission pollinique, paramètres géographiques, phénologie, *Olea europaea*.

### 1. Introduction

The Mediterranean region is the main area in the world devoted to the olive tree crop (*Olea europaea* L.), where it is one of the most important agricultural activities COI (1996). i.e. In Tunisia the olive-tree occupied 1.7 million hectares whereas in Spain this species occupied 2.3 million hectares and this surface is continually in growing. The region of Andalusia accounts for 80% of total Spanish surface. Knowledge of olive tree phenology could greatly enhance a grower's ability to plan management practices in relation to the events occurring within the tree. Olive aerobiological data have been commonly used as a phenological tool for predicting the beginning of the pollination period, for crop forecasting Galán et al. (2008) and even as a bio-indicator of global climatic changes, Galán et al. (2005).

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Field phenology on plants allows to describe the flowering period taking into account different developing phases, manifesting the percentage of opened flowers with respect to the total tree canopy Maillard (1975). Likewise, several periods indicates the main phase of the olive reproductive cycle Galán et al. (2001). In fact, it has been demonstrated by different researchers a good relationship between the olive pollen season and the main flowering phenological phases Fornaciari et al. (2000) and Galán et al. (2001). Several factors affect plant development, temperature and photoperiod exert the strongest effect on vegetation development and especially on flowering Cenci et al. (1998). The variation in temperature and photoperiod due to altitude and latitude affect the spring generative phenophases that differ geographically Wielkerson (1999). i.e. a high temperature induce an advance in flowering start commonly with a short flowering phenological phases, however a low temperature cause a delay of flowering with a large period.

Two large cultivated areas were chosen because of their importance in olive oil production, the province of Córdoba in Spain and Tunisia. Aerobiological studies carried out previously reflect that pollen curve represents pollen grains released by different cultivars and, in some cases, point out the different crop flowering contributions. For that reason, phenological investigations were carried out during three years in those areas trying to verify the behaviour of pattern diversity within this species. The aim of this work was to study the spatial and temporal differences observed in the airborne *Olea europaea* pollen distribution in 4 sites placed at different latitude and altitude of two Mediterranean countries.

## 2. Material and methods

Phenological data were collected along a latitudinal gradient, from Chaal (34° 19' 10" 04'E), with an altitude of 35 m above sea level and situated in the southern part of Tunisia up to Cordoba (37°50'N 4°45'W), with an altitude of 118 m above sea level in the middle part of Andalusia.

On the other hand, other two olive cultivated areas were considered, taking into account also the different altitude Mornag (36° 39'N 10° 16'E) at 45 m above sea level in Tunisia, and Baena in Spain (37°37'N 4°20'W) at 420 m above sea level.

Cordoba and Baena climate is sub-continental, average annual rainfall is 536 mm and average annual temperature is 17.6°C. Chaal and Mornag climate is, however, sub-arid with 200 mm and 350 mm as average annual rainfall respectively, the average annual temperature is 18.5°C in both sites.

Different olive cultivars were cultivated in the monitored areas. In the two Tunisian areas the principal cultivars are Chemlali in the south (Chaal) and Chetoui in the north (Mornag); in Andalusia the cultivars Picual, Hojiblanca and Arbequina are the most abundant cultivars in the investigated areas.

The floral phenology was determined by using the Hirst type volumetric spore trap Hirst (1952). Pollen concentration in the atmosphere was expressed as the daily average of pollen grains per cube meter of air (pollen grains/m<sup>3</sup>). The pollen trap in the four sites was placed average 15 meters above ground level. The study was carried out in the years 2006, 2007 and 2008. The aerobiological data were collected in every monitoring station offering us information about the main phases of the reproductive cycle of olive tree. The phenological phases were defined as follows: SF: Start of Flowering, defined as the day in which one pollen grain/m<sup>3</sup> was reached wherever five subsequent days contained one or more pollen grains/m<sup>3</sup> Galán et al. (2001); PP: Pollen Peak day, maximum of flowering, defined as the day of maximum pollen concentration; EF: End of Flowering, defined as the first day with 0 pollen grains and at least 3 consecutive days with 0 pollen grains).

## 3. Results

Variations of the annual pollen concentration, expressed by the amplitude of the curves of pollen emission for the 4 sampling stations are shown in Figure 1 which shows the trends of pollen concentration during 2006, 2007 and 2008 in 4 sites.

As regards *Olea* spatial distribution, the start of the pollination period and concentrations varied depending on the study year and different sites. *Olea* pollen is generally present in the atmosphere of Tunisia from beginning of April to middle of May lasting, on average 42 days through the country. However, in Andalusia *Olea* pollen lasting on average 72 days, from beginning of April to end of June.

The day of maximum pollen concentration varied according the year and the geographical site. Nevertheless, the maximum of pollen emission was recorded in Tunisia on average 117<sup>th</sup> Julian day pass to 131<sup>st</sup> in the province of Cordoba (Figure 1).

Figure1 also shows a variation of pollen concentration, from year to year at different sites. Maximum of pollen concentration in Baena passes from the daily average of 3000 p/m<sup>3</sup> in 2007 to 1400 p/m<sup>3</sup> in 2008 whereas in 2006 the pollen concentration does not exceed the 2000 p/m<sup>3</sup>. In Cordoba variation of pollen concentration was registered from the daily average 630 p/m<sup>3</sup> in 2006 at 1400 p/m<sup>3</sup> in 2008.

In Tunisia a great variability of pollen concentrations between the two stations was recorded, indeed, the Chaal site presented during the three years the higher pollen concentration considering the abundance of the olive-tree in this area, whereas, the inter-annual variation is very weak. In Chaal the daily average of pollen concentration passes from 950 p/m<sup>3</sup> in 2006 to 1250 p/m<sup>3</sup> in 2008 However in Mornag the concentration passes from 200 p/m<sup>3</sup> in 2006 to 570 p/m<sup>3</sup> in 2008.

The curves of Figure 1 show a prior flowering period in Tunisian stations comparatively with those in Andalusia, this precocity is expressed by a beginning of flowering as well as pollen peak earlier in Chaal and Mornag sites comparatively with those in Baena and in Cordoba. In Chaal site the flowering start took place on average 97<sup>th</sup> Julien days, whereas in Cordoba it took place on average 115<sup>th</sup> Julien days. However the pollen peak took place in Chaal on average 111<sup>th</sup> Julien days, and in Baena it took place on average 133<sup>rd</sup> Julien days that means 22 days precocity due to the difference in altitude and latitude between the two sites. The numerical explication of this difference summaries in a delay of 6.43 days each degree of latitude and one day each 17.5 meters of altitude.

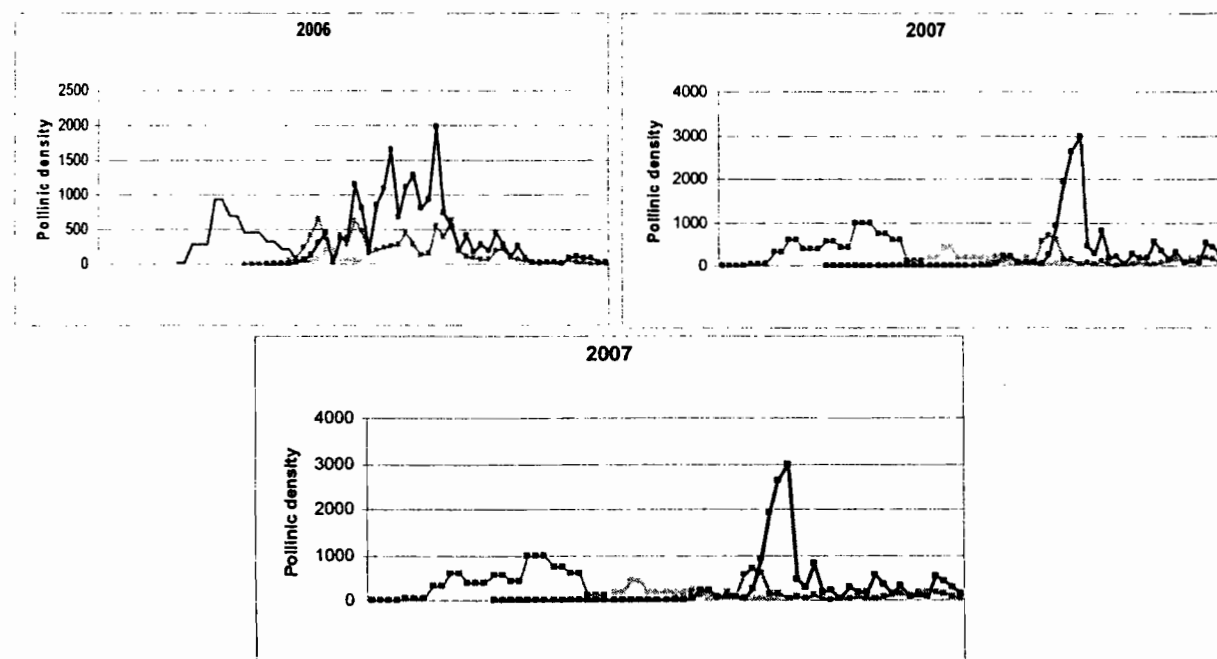


**Figure 1:** Comparison between the trends of pollen concentration during 2006, 2007 and 2008 in Chaal, Mornag, Baena and Cordoba.

In Tunisia the graphs show a prior flowering start in Chaal region on average 14 days comparatively with Mornag station, this precocity finds its explication in the low level in altitude of 10 meters and latitude of one degree of Chaal region comparatively of Mornag's. The combined effect of these two parameters makes that flowering in Mornag is two weeks later than in Chaal.

In Andalusia, the great difference in altitude between the two localities, Cordoba and Baena, (302 meters) made that pollen peak in Baena is earlier than in Cordoba in spite of the last site is located at higher latitudinal level.

Figure 2 represents the relationship between full flowering dates and geographic variables (altitude and latitude), explicated by linear regressions with R-Square 0.73 and 0.67 respectively,. On the other hand, good statistical correlation was found between the geographic variables and the flowering

period with R-Square 0.82 and 0.65 respectively altitude and latitude. The statistical results evidenced the high positive influence of latitude and altitude on flowering dates. Indeed a low altitude and latitude is often accompanied with advance in flowering start and a long period season. However a high altitude and latitude is accompanied with a delay in flowering start and a short period season.

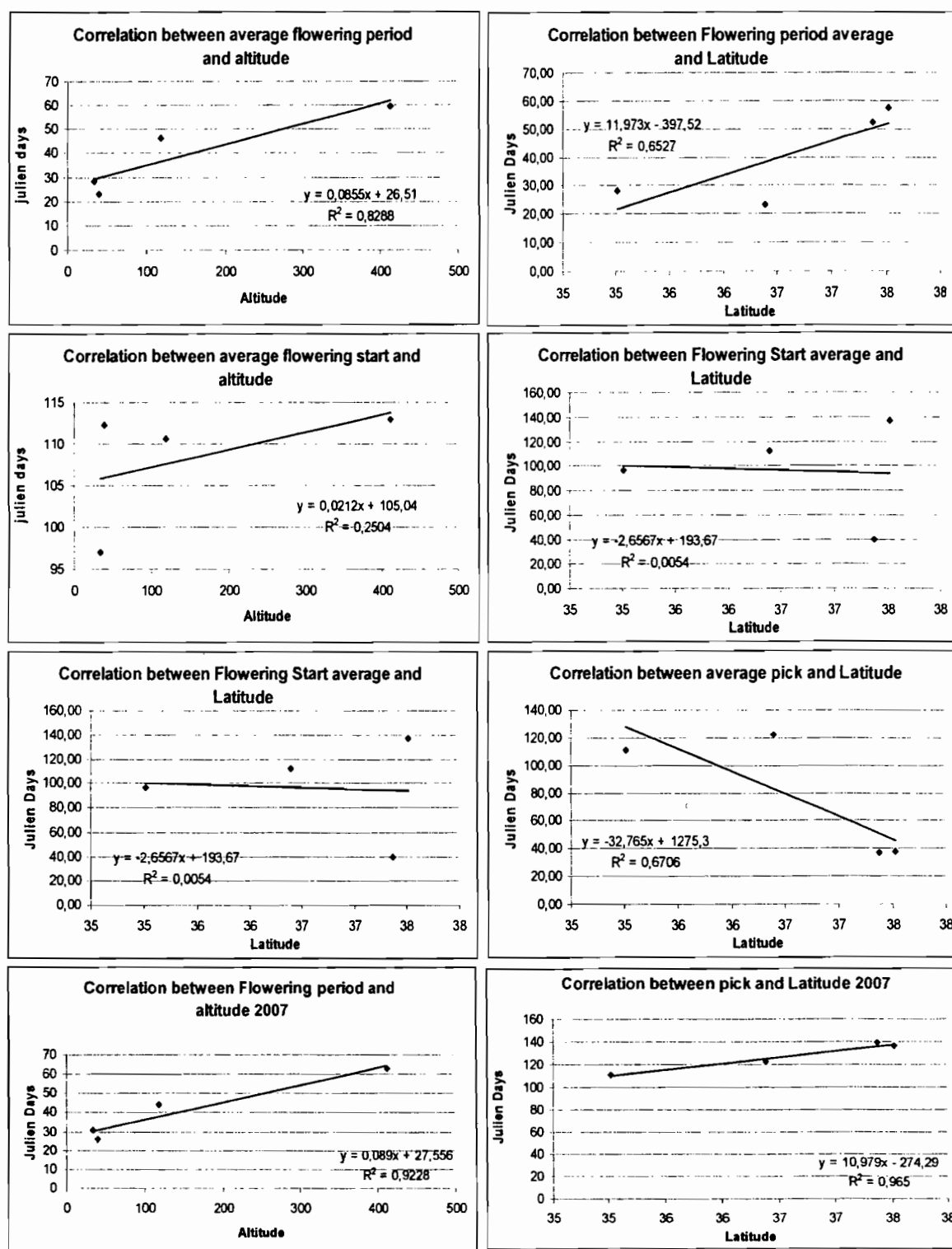


**Figure 2:** Linear regressions between flowering phenophases and geographical parameters of all the monitoring.

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## Functional genomics in *Olea europaea*: identification and annotation of differentially expressed transcripts in developing olive fruit

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### Abstract

Differently from other fruit tree species, a few sequences of genes and gene products are available for olive in the NCBI databases. This study deals with the identification and annotation of differentially expressed genes in developing olive fruit. Total RNA from fruit of the cv. Leccino sampled at three different developmental stages [i.e., 30 days after full bloom (DAF) (A), 90 DAF (pit hardening, B) and 130 DAF (veraison, C)] was used for the identification of differentially expressed genes putatively involved in main processes characterizing fruit growth, development and ripening. In order to isolate up- and down-regulated genes, four subtractive hybridization libraries were constructed: forward and reverse between A and B, forward and reverse between B and C. mRNA was isolated from total RNA using oligo-dT cellulose resin and forward and reverse subtractive cDNA libraries were constructed using a combination of Clontech's PCR-based subtraction kit and Rx Biosciences unique procedure. About 300 clones were isolated and sequenced for each library.

All sequences were preliminary analyzed through BlastX against non-redundant NCBI databases and about 60% of them showed similarity to known proteins. Library-specific cDNA repertoires were annotated according to the three main vocabularies of the gene ontology (GO): cellular component, biological process and molecular function, respectively. BlastX analysis, GO terms mapping and annotation analysis were performed using the Blast2GO software, a research tool designed with the main purpose of enabling GO based data mining on sequence sets for which no GO annotation is yet available. Furthermore, the olive fruit-specific transcriptome dataset was used to query all known KEGG (Kyoto Encyclopaedia of Genes and Genomes) metabolic pathways for characterizing and positioning retrieved EST records within the drupe development. On the whole, our approach led to the identification of differentially expressed sequences that proved to be significantly differentiated in terms of GO category sequence amounts among the three developmental stages. Similarly, the integration of the olive sequence datasets within the MapMan platform for microarray analysis allowed us to display them into pathways along with metabolic and regulation diagrams useful for the definition of key functional categories in time course analyses for gene groups. Such characterization is a first step toward both, functional genomics and systems biology research in olive, especially for understanding gene regulatory networks and metabolic pathways in fruit growth, development and ripening.

## Génomique fonctionnelle dans *Olea europaea*: identification et annotation des transcriptions, exprimées différemment, concernant le développement du fruit d'olive

### Résumé

Différemment des autres espèces fruitières, seulement peu de séquences géniques de l'olivier sont disponibles dans le *database* NCBI. Cette étude s'occupe de l'identification et annotation des gènes différemment exprimés dans le développement du fruit de l'olivier. Les fruits de la cultivar Leccino ont été cueillis en trois différents stades de développement (i.e., 30, 90 et 130 jours après la floraison) et l'ARN total des fruits a été utilisé pour l'identification des putatifs gènes impliqués dans la pousse, le développement et la maturation du fruit. Quatre bibliothèques SSH (*Suppression Subtractive Hybridization*) ont été construites et 300 clones environ ont été isolés de chaque bibliothèque. Les séquences ont été annotées avec les programmes informatiques BlastX et Blast2GO. En suite les séquences ont été analysées en utilisant le KEGG (*Kyoto Encyclopaedia of Genes and Genomes*) pour identifier les voies métaboliques dans lesquelles se place le transcriptome du fruit de l'olivier étudié dans ce travail.

**Mot-clés:** Bibliothèque SSH, expression génétique, qualité de l'huile.

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## 1. Introduction

Olive (*Olea europaea* L.) is an evergreen species, widely cultivated in the Mediterranean Basin and its oil is a predominant component of the worldwide known 'Mediterranean diet'. Health benefits and cancer-protective properties have been demonstrated for olive oil (Pérez-Jiménez et al. 2007) and these beneficial attributes are closely related to composition and concentration in olive oil biological active molecules resulting from catabolic and anabolic processes during olive fruit development. The oil content of olives can reach up to 30% on a fresh weight basis at full ripening (Sánchez 1994) and accumulates mainly in the mesocarp. Oil accumulation in the pulp increases slowly, reaching the plateau after veraison and a marked triacylglycerol accumulation in seed and pulp occurs after endocarp lignification. Olive oil fatty acid profile is important in relation to its nutritional properties and it varies during maturation and ripening, according to cultivars and environmental conditions (Zarrouk et al. 1996; Ayton et al. 2001). Other metabolites affecting olive oil quality and its technological and nutritional properties accumulate throughout olive fruit development and include polyphenols, carotenoids, chlorophylls, sterols and terpenoids.

The making of a fruit is a genetically programmed developmental process and is largely influenced by environmental factors. To identify and characterize these genes, different genomic approaches (ESTs, large-scale microarrays, deep transcriptome profiling, etc.) have been used (Seymour et al. 2008) and the information concerning transcriptional networks and regulatory circuits involved in fruit physiological and developmental processes have increased tremendously. However, the information concerning genetic regulation of fruit metabolic processes in olive is still limited. Among different strategies available for identifying differentially expressed genes, suppression subtractive hybridization (SSH) libraries have been successfully used in fruit science. This article deals with the identification via SSH of large repertoires of differentially expressed genes in developing olive fruits, and their computational annotation by means of different bioinformatic software. The identification and characterization of gene regulatory networks and key metabolic pathways during fruit growth and development represent a relevant step for improving olive oil quality and its health-related properties.

## 2. Material and Methods

Olive (*Olea europaea* L., cv Leccino) fruits growth was determined by monitoring fresh weight accumulation and samples were collected at 30 days after flowering (DAF) (initial fruit set, stage 1), 90 DAF (completed pit hardening, stage 2) and 130 DAF (veraison, stage 3). Total RNA was extracted from pericarp of about 16 fruits for each sampling date, using the RNeasy Plant Mini Kit (Qiagen). The RNA was quantified by both, spectrophotometer and gel electrophoresis on a denaturing agarose gel and mRNA was isolated from about 400 µg of total RNA for each sample using oligo dT cellulose resin.

The first strand cDNA was synthesized with reverse transcriptase using oligo dT primer, modified for a tail containing RsaI restriction site. The second strand was synthesized by incubating the first strand cDNA with DNA polymerase I, RNase H and DNA ligase at 16°C for 2.5 hrs. Subtracted and reverse subtracted cDNA libraries were constructed using a combination of a PCR-based subtraction kit (Clontech) and a unique subtraction procedure (Rx Biosciences). Four subtracted cDNA libraries have been constructed to compare samples collected at 30 and 90 DAF (libraries A and B) and samples at 90 and 130 DAF (libraries C and D). For the two forward libraries A and C, cDNA from samples collected at 30 and 90 DAF, respectively, were used as tester and cDNA from 90 and 130 DAF, respectively, were used as drivers, vice versa for the reverse subtractive libraries B and D.

The DNA sequencing was performed using ABI3700 automatic DNA sequencers using 25-50 ng of DNA template according to manufactures protocol. Nucleotide sequences retrieved by SSH were screened for vector contamination by using a home made BioPearl script. Once cleaned by vector residuals, all sequences were used for contig assembly by using a web interface of the CAP3 software (CAP3, <http://deepc2.psi.iastate.edu/aat/cap/cap.html>) and redundancy within and among libraries were calculated as ratio of sequences belonging to a contig out of the total sequences considered. Computational annotation of the four olive EST datasets was performed using the Blast2GO software v1.3.3 (<http://www.blast2go.org>). Annotation distribution among originated libraries was represented by Venn diagrams by computing all retrieved annotation with the VennMaster software. Enzyme mapping of annotated sequences was done by direct GO to Enzyme annotation and used to query the Kyoto Encyclopaedia of Genes and Genomes (KEGG - <http://www.genome.jp/kegg>) to define the main



metabolic pathways involved. MapMan (<http://gabi.rzpd.de/projects/MapMan/>) analysis was done using the olive dataset properly rearranged as input files. Quantitative Real-Time PCR experiments were carried out to validate some of the genes isolated by SSH and characterized by GO. Among the whole dataset of non-redundant sequences, 89 gene sequences belonging to key biosynthetic and metabolic pathways were selected.

### 3. Results

The preparation of cDNA libraries using SSH represents an efficient strategy to isolate genes with an antagonist expression pattern and enable to identify transcripts of genes differentially expressed among the three developmental stages of olive fruit studied. The number of differentially expressed sequences randomly chosen varied from a minimum of 236 to a maximum of 317 per library, with a total number of clones equal to 1,132 (Table 1).

**Table 1:** Library characteristics - clone singlets and contigs abundance and mean size.

| Library | Clones |           | Singlets |           | Contigs |           |
|---------|--------|-----------|----------|-----------|---------|-----------|
|         | No.    | Mean (bp) | No.      | Mean (bp) | No.     | Mean (bp) |
| A       | 293    | 522       | 139      | 464       | 40      | 767       |
| B       | 317    | 637       | 132      | 583       | 35      | 963       |
| C       | 286    | 694       | 114      | 639       | 43      | 835       |
| D       | 236    | 517       | 127      | 506       | 64      | 558       |

The average length of the cDNA clones was 597 bp, ranging from 48 up to 1,283 bp. Within each single library the redundancy was relatively low, ranging between 1.7% and 5.3%. Querying with cDNA sequences the non-redundant NCBI databases allowed the attribution of a BLAST hit and around 75% of the BLAST hits of the olive fruit cDNA sequences were homologous to coding sequences present in the rice, *Arabidopsis* and grapevine genomes, with more than 1,000 hits per species (Table 2).

**Table 2:** Number of BLAST hits retrieved from NCBI databases and their distribution among different plant species and different organisms.

| Organism Name                  | BLAST hits |
|--------------------------------|------------|
| <i>Oryza sativa</i>            | 1471       |
| <i>Arabidopsis thaliana</i>    | 1142       |
| <i>Vitis vinifera</i>          | 1083       |
| <i>Populus trichocarpa</i>     | 146        |
| <i>Nicotiana tabacum</i>       | 108        |
| <i>Glycine max</i>             | 83         |
| <i>Solanum tuberosum</i>       | 77         |
| <i>Mus musculus</i>            | 74         |
| <i>Zea mays</i>                | 68         |
| <i>Picea sitchensis</i>        | 67         |
| <i>Homo sapiens</i>            | 53         |
| <i>Medicago truncatula</i>     | 51         |
| <i>Olea europaea</i>           | 47         |
| <i>Gossypium hirsutum</i>      | 47         |
| <i>Triticum aestivum</i>       | 44         |
| <i>Shigella flexneri</i>       | 43         |
| <i>Lycopersicum esculentum</i> | 39         |

The computational analysis of the whole EST collection using the software Blast2GO allowed the annotation of the expressed sequences according to the terms of the three main Gene Ontology (GO) vocabularies, i.e. cellular compartment, molecular function and biological process. For cellular compartments, the most represented are plastids and mitochondria, with more than 50% of the total annotations, followed by cytosol, plasma membrane, endoplasmic reticulum and nucleoplasm, whereas other cellular compartments were represented at a much lower scale. Regarding the molecular function, the most represented categories were those of nucleotide binding proteins,

followed by proteins with transport, kinase and enzymatic activities. The other molecular functions were represented at a lower extent and concerning the biological process vocabulary more than 30 categories were found: carbohydrate metabolism, response to biotic and environmental stresses, generation of precursors, metabolites and energy, and catabolic processes, secondary metabolites, metabolism of lipids, synthesis of amino acids and derivatives, metabolites and their precursors, and protein modification process.

A number of olive fruit stage-specific GO terms were identified and among the 296 and 464 GO terms found in the A and B libraries, 75 and 101 were associated to down- and up-regulated genes, respectively. The most significant GO terms were encoding elements of hormone biosynthesis and signal transduction mediated by ethylene, jasmonic acid, salicylic acid, and abscisic acid, as well as biosynthesis of secondary metabolites, such as terpenoids. In the C and D libraries, a total of 375 and 549 GO terms were recovered, 78 and 183 of which were related to down and up-regulated genes, respectively. Among these, there are GO terms associated to environmental stress responses, catabolism of secondary metabolites, and auxin signal transduction. Quantitative Real-Time PCR experiments were carried out to corroborate the expression patterns of a subset of sequences and validated the results from SSH experiments for 42 out of 61 genes. The Kyoto Encyclopaedia of Genes and Genomes (KEGG) was queried for sequences encoding enzymes and the deduced gene products were associated to specific metabolic and/or biosynthetic pathways related to carbohydrates, fatty acid and secondary metabolism. As expected, several genes encoding enzymes related to carbohydrate and fatty acid compounds were transcriptionally up- or down-regulated during olive fruit development. The integration of the olive sequence datasets within the MapMan platform for microarray analysis allowed the identification of specific biosynthetic pathways useful for the definition of key functional categories in time course analyses for gene groups. Extensive data analysis can be found in Galla et al., (2009).

In conclusion the generation of the SSH library throughout olive drupe development and the bioinformatic annotation of all gene sequences recovered was useful to highlight the metabolic pathways and transcriptional aspects related to carbohydrates, fatty acids, secondary metabolites, transcription factors and hormones as well as response to biotic and abiotic stresses. These results are a first step toward functional genomics and systems biology research aimed to understanding the gene functions and regulatory networks in olive fruit growth and ripening.

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## Transcriptomic analysis and computational annotation of genes involved in olive flower development

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### Abstract

Little is known on the physiological and molecular basis of olive ovule abortion and flower abscission, both influencing plant yield. Recent findings suggest that both processes might be under genetic control and are influenced by both nutritional and environmental factors. Two different approaches have been carried out to unravel the molecular networks. The first deals with the identification of large sets of differentially expressed genes through subtractive hybridization libraries (SSH), in order to isolate up- and down-regulated genes, between two selected flower developmental stages in the cv Leccino. SSH approach originated a total of 1,127 clones which were analysed and computationally annotated. The second deals with expression studies of genes modulated during olive flowering on two cultivars: Dolce Agogia (abundant flowering and low fruit set) and Leccino (fruit set capacity).

**Key words:** *Olea europaea*; flower development; SSH; gene expression, Gene Ontology.

## Analyse transcriptomique et annotation microinformatique des gènes intégrés dans le développement de la fleur de l'olivier

### Résumé

Les connaissances des bases physiologiques et moléculaires de l'avortement de l'ovule et de l'abscission florale chez l'olivier ne sont pas abondantes, et toutes ces deux caractéristiques influencent le rendement. Deux approches différentes ont été effectuées pour dénouer le réseau moléculaire responsable de leur contrôle génétique. La première traite l'identification d'un vaste ensemble de gènes exprimés diversement, à travers une librairie de l'hybridation soustractive (SSH) pour pouvoir isoler des gènes régulés positivement et négativement entre deux phases développementales de floraison du cultivar Leccino. L'approche SSH a résulté en un total de 1127 clones qui ont été analysés et annotés bio-informatiquement. La deuxième approche traite des études de l'expression de gènes modulés pendant la floraison de l'olivier, en deux cultivars : Dolce Agogia (floraison abondante et nouaison basse) et Leccino (capacité élevée de nouaison).

### 1. Introduction

In olive inflorescence hermaphrodite flowers are paired to male flowers in which androecium is well conformed and gynoecium is not completely differentiated or necrotic. Fruit set is correlated to the percent of perfect flowers. Ovule abortion and flower abscission are influenced by genetic background, since different cultivars behave in a different way, and by nutritional and environmental conditions. Studying the flower development, it was found that Dolce Agogia had the highest percentage of aborted ovaries and flowers, while Leccino had the lowest percentage of aborted ovaries and flowers (Reale et al 2006). Cyto-histological studies show that pistil development in staminate flowers is interrupted after the differentiation of the megaspore mother cell and microspore resulted enveloped by a callose wall (Reale et al 2009), suggesting that, also in olive, callose has an important role in the programmed cell death (PCD) of the non functional microspores (Angenent et al 1996). At the same stage of development, starch grains were only detected in the ovary, style and stigma of hermaphrodite flowers. No starch was observed in the pistils of the staminate flowers. It is known that carbohydrate supply is of fundamental importance for development of floral organs (Yu et al 2000; Clément et al 1996; Rodrigo et al 2000) and fruit setting (Iglesias et al 2003). In grapevine, it has been shown that a nutrient deficiency in the inflorescences could be a cause of flower abscission (Lebon et al 2004) and carbon metabolism is thought to be involved in flower necrosis (Gu et al 1996).

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Little knowledges are available in the literature on the genetic and molecular aspects involved in the abortion of gynoecium; therefore the main objective of this research is the identification of a large sets of differentially expressed genes during the development of the flower and to study their expression in the diverse step of olive flowering in olive.

## **2. Materials and Methods**

### **2.1. Plant Materials and Growth Condition**

Field observations and experiments were carried out in an olive grove of the "Istituto Sperimentale di Olivicoltura" in Spoleto (42°49'N, 12°40'W) at 300 m a.s.l. During inflorescences development of cultivars Leccino and Dolce Agogia, flowers were collected at different stages of development from three weeks before anthesis to full anthesis.

### **2.2. Nucleic acid extraction, cDNA synthesis and library construction**

Total RNA was extracted from flowers with a NucleSpin RNA Plant Kit (Macherey Nagel). Reverse transcriptions (RT) were carried out using Ready-To-Go RT-PCR beads (Amersham Biosciences). cDNA aliquots equivalent to 80 ng of total RNA were used in each RT-PCR reaction. An amount of 500 µg of total RNA from Leccino samples were further purified with the RNAeasy Plus Mini Kit (Qiagen) and prepared for the construction of subtractive (SSH) libraries. Libraries were prepared at Rx Biosciences (Rockville, MD, USA) using a proprietary technology. Two cDNA libraries were constructed to compare Leccino samples collected the 22<sup>th</sup> and 29<sup>th</sup> of May: a forward library, where cDNA of 22<sup>th</sup> of May was subtracted with 29<sup>th</sup>, and a reverse library. Libraries were analysed by sequencing 1,127 clones at Rx Biosciences using an ABI3700 automatic DNA sequencer and a first bioinformatic evaluation was performed with a CLC Combined Workbench 3 software. Nucleotide sequences retrieved by SSH were screened for vector contamination by using a BioPerl script, and used for contig assembly by using the CAP3 software (<http://deepc2.psi.iastate.edu/aat/cap/cap.html/>).

Computational annotation of the two EST dataset was performed using the Blast2GO software v.2.3.6 (<http://www.blast2go.org>) using the b2g\_jun09 database. Enzyme mapping of annotated sequences was done by direct GO to Enzyme annotation and used to query the Kyoto Encyclopaedia of Genes and Genomes (KEGG – <http://www.genome.jp/kegg/>) to define the main metabolic pathways involved.

### **2.3. Novel gene isolation and expression analysis**

A PCR strategy was applied to isolate partial nucleotide sequences corresponding to sucrose synthase, beta-glucosidase, beta-mannosidase, mitochondrial ATPase, ribulose-biphosphate carboxylase, long chain fatty acid CoA ligase, ubiquitin protein ligase, mitochondrial elongation factor Ts<sub>mt</sub>. Actin was used as housekeeping gene to provide a reference for the global transcription level. For quantitative gene expression analysis (qRT-PCR) flower samples were collected 14 days before (16<sup>th</sup>, 22<sup>th</sup>, 26<sup>th</sup>, 29<sup>th</sup> May) and 4 days after anthesis (4<sup>th</sup> June), frozen in liquid nitrogen and stored at -80° C until analysis. Total RNA was extracted as previously described. The qRT-PCR tests were carried out on three biological replicates using the LightCycler platform (Roche Molecular Diagnostics). Amplification and detection were performed using the SensiMix Lite kit (Quantace). The 20 µl reaction consisted of 2 µl of cDNA, 80 ng RNA, and 18 µl of the master mix prepared using 4 µl of 5x SensiMix Lite, 3.5 mM final concentration of MgCl<sub>2</sub>, 0.4 µl of 50x SYBR Green solution, 1.5 µl of Enzyme Mix, 0.5 µM final concentration of the forward and reverse primers (Table 1). The cycling conditions consisted of 10 min denaturation at 95° C followed by 35 cycles of 95° C for 15 s, 62° C for 20 s, 72° C for 20 s. For the quantification of the target gene, we used a DNA standard obtained by PCR of the genes of interest as previously described and determining the concentration by UV spectrophotometry. An external absolute standard curve was constructed with a reference standard in the range 10<sup>7</sup>-10<sup>3</sup> copies/reaction. The amount of the target transcripts fell within the range tested, allowing the quantification of the target sample. In addition, parallel assays were run for the housekeeping gene encoding actin.

**Table 1:** Description of primers described in this study.

| Gene                               | Forward primer                 | Reverse primer                  |
|------------------------------------|--------------------------------|---------------------------------|
| Actin                              | 5'-TCCTGAGGTTCTTACCAGCCTTC-3'  | 5'-CTAGCGCTGTAATTTCC TTGCTCA-3' |
| Sucrose synthase                   | 5'-CACAGATGAACCGTGTGAGAAATG-3' | 5'-GCCTCAACAACAGTCAAACCAAAG-3'  |
| beta-glucosidase                   | 5'-GGTGAATGACTTCCAAAAGGGTTC-3' | 5'-AGACATTGTTGTTGCCATGAAGTG-3'  |
| beta-mannosidase                   | 5'-GGGAAACCAATCATGTTGACTGAG-3' | 5'-CCGCTTCTTGACAACGTAGATT-3'    |
| Fatty-acid-CoA ligase              | 5'-ATCAGTGCCTGAGATGGGATATGA-3' | 5'-CCATCCATCCACAACCTACACCTTC-3' |
| RuBisCO                            | 5'-GCATTCCGAGTAACTCCTCAACCT-3' | 5'-TAACGATCAAGGCTGGTAAGTCCA-3'  |
| ATPase                             | 5'-AGAACTTATAATCGGGGACCGACA-3' | 5'-ACAGTTGAGCGTTTCTGTCCAATC-3'  |
| Ubiquitin-protein ligase           | 5'-AAGCTCTTTCCTGTCTCCTCCAGA-3' | 5'-CACCCCAAGAAGCATTAGGGATAG-3'  |
| Elongation factor T <sub>srm</sub> | 5'-GATGCCTCCTGTGAAGAATGAAGA-3' | 5'-AAAGATATTCGGCCAGTCTCCGTA-3'  |

### 3. Results and discussion

A total of 1,127 clones were sequenced and, among them, 232 ESTs were selected for further analysis: 148 sequences were singletons while 84 were contigs generated assembling contiguous and overlapping clones. The average length of the sequences analysed was 842 bp with a range of variation from 20 to 2952 bp. BlastX analysis, GO terms mapping and annotation analysis were performed using the Blast2GO software. No BLAST hits for olive could be recorded while cDNA sequences showed similarity with coding sequences present in the genome of the species *Vitis*, *Populus*, *Oryza*, *Arabidopsis* and *Ricinus*, indicating that novel genes have been isolated. Library-specific cDNA repertoires were analysed according to the three main vocabularies of gene ontology: cellular component, biological process and molecular function. About 70% of the sequences were annotated: the forward library enclosed 14 non redundant ESTs that account for the 90% of the analysed sequences, indicating that these ESTs could be strongly regulated, while the redundancy in the reverse library was much lower. Concerning the biological processes and molecular function (Table 2), the most represented categories were connected with carbohydrate and starch metabolism, energy metabolism, vesicle-mediated transport and transmembrane transport, genetic information processing and transcription factor.

**Table 2:** Gene Ontology terms distribution of the differentially expressed ESTs in the vocabularies of biological process and molecular function.

| Biological process                                          | Molecular function                                                  |
|-------------------------------------------------------------|---------------------------------------------------------------------|
| Fructose metabolic process (1)                              | Endonuclease activity (1)                                           |
| Starch metabolic process (6)                                | Sequence-specific DNA binding (1)                                   |
| Sucrose metabolic process (6)                               | Hydrogen ion transporting ATPase activity, rotational mechanism (1) |
| Substituted mannan metabolic process (1)                    | Aminoacyl-tRNA ligase activity (3)                                  |
| Regulation of transcription DNA-dependent (1)               | RNA binding (8)                                                     |
| Nucleoside metabolic process (1)                            | Protein dimerization activity (1)                                   |
| Protein ubiquitination (5)                                  | ATP binding (21)                                                    |
| ATP synthesis coupled proton transport (1)                  | Glucan 1,3-beta-glucosidase activity (2)                            |
| Lateral root development (1)                                | Oxidoreductase activity (12)                                        |
| Fatty acid metabolic process (1)                            | Serine-type carboxypeptidase activity (1)                           |
| Response to cadmium ion (5)                                 | Ubiquitin-protein ligase activity (5)                               |
| Ubiquitin-dependent catabolic process (2)                   | Identical protein binding (4)                                       |
| Mannose metabolic process (1)                               | Transcription factor activity (1)                                   |
| Response to hormone stimulus (1)                            | Zinc ion binding (15)                                               |
| Response to nematode (1)                                    | Subtilase activity (1)                                              |
| tRNA aminoacylation for protein translation (1)             | Mannan endo-1,4-beta-mannosidase activity (1)                       |
| Protein folding (3)                                         | Long-chain-fatty-acid-CoA ligase activity (1)                       |
| Oxidative phosphorylation (4)                               | Cysteine-type endopeptidase activity (2)                            |
| Glucose metabolic process (4)                               | Xylan 1,4-beta-xylosidase activity (1)                              |
| Cellular carbohydrate biosynthetic process (4)              | Hydrolase activity, hydrolizing O-glycosyl compounds (6)            |
| RNA metabolic process (3)                                   | Cation-transporting ATPase activity (3)                             |
| Protein aminoacyl phosphorylation (7)                       | Obsolete molecular function (4)                                     |
| Oxidation reduction (12)                                    | Nucleotidyltransferase activity (3)                                 |
| Nucleobase, nucleoside and nucleotide metabolic process (4) | Signal transducer activity (3)                                      |
| Response to stress (5)                                      | Carboxy-lyase activity (3)                                          |
| Translation (3)                                             | Protein serine/threonine kinase activity (7)                        |
| Signal transduction (5)                                     | Inorganic cation transmembrane transporter activity (3)             |
| Pyruvate metabolic process (3)                              | Iron ion binding (5)                                                |
| Cellular aromatic compound metabolic process (3)            | Structural molecule activity (4)                                    |
| Ubiquitin cycle (4)                                         | Electron carrier activity (6)                                       |
| Vesicle-mediated transport (4)                              | Calcium ion binding (5)                                             |
| Intracellular protein transport (3)                         | DNA binding (6)                                                     |
| Proteolysis (6)                                             | Coenzyme binding (5)                                                |
| Regulation of catalytic activity (3)                        | Transcription regulator activity (3)                                |
| Response to biotic stimulus (3)                             | Aspartic-type endopeptidase activity (4)                            |
| Aspartate family amino acid metabolic process (3)           | UDP-glucosyltransferase activity (3)                                |
| Proton transport (5)                                        | Magnesium ion binding (4)                                           |
| Photosynthesis (3)                                          | Methyltransferase activity (3)                                      |
| DNA metabolic process (7)                                   | Tetrapyrrole binding (3)                                            |
| Organelle organization (4)                                  | Heterocycle metabolic process (3)                                   |
| Regulation of transcription (3)                             | Electron transport (7)                                              |
| Serine family amino acid metabolic process (8)              | Carbon utilization (3)                                              |
| Cellular macromolecule catabolic process (4)                |                                                                     |

Since it is known that the nutritional theory and assimilates partitioning has a great importance in flower development and ovary abortion, our attention was firstly focussed on the transcriptional profile of sucrose synthase (Fig. 1a). Transcript levels of this gene were high at the beginning of flower development and then decrease at anthesis. Sucrose plays a pivotal role in plant metabolism as signal molecule in assimilate partitioning. In olive, starch content plays a role in the pistil development and it has been observed that starch grains were only present in the ovary, style and stigma of hermaphrodite flowers but not in staminate flowers (Reale et al. 2009). These observation suggest that starch storage tended to be negatively correlated with sucrose synthesis by sucrose synthase. The presence of lower transcript levels for sucrose synthase in Leccino than in Dolce Agogia could be associated with an higher starch content in hermaphrodite flowers. Other genes involved in carbohydrate metabolism which seems to be regulated in flower development were some glucosidases (Fig. 1c, d). Transcript levels for beta-glucosidase declined during development until anthesis, while beta-mannosidase levels increased with an opposite behaviour. These enzymes catalyse the hydrolysis of polysaccharides releasing simple sugars which could be used in the energetic metabolism or in the synthesis of storage materials. The presence of these hydrolases suggests a very quick turnover of cell wall polysaccharides which could be associated with actively growing tissues as developing flowers. The presence of soluble sugars could give a contribution to the flower bud expansion, decreasing of the cellular osmotic potential with the increase of osmotic pressure.

A different transcriptional profile of the long-chain-fatty-acid-CoA ligase in both cultivars was observed (Fig. 1b). In Dolce Agogia the transcript levels increased during time and then decreased quickly at anthesis, while in Leccino they were more stable. This enzyme is involved in a key step of the fatty acid degradation that has, as final product, high amounts of acetyl-CoA. This central intermediate could enter the tricarboxylic acid cycle, generating energy, or could be used in biosynthetic pathways.

Our results showed that while in Dolce Agogia the expression levels of this ligase decreased rapidly, in Leccino, where the proportion of perfect flowers was higher, they were higher and constant during flower development. Moreover it was analysed the transcriptional profile of RuBisCO-large subunit (Fig. 1e) and it has been observed that this gene was down regulated during flower development. The comparison between the cultivars showed that transcript levels were higher in Dolce Agogia in the first phase of development and in Leccino at anthesis. Concerning the energetic metabolism, the mitochondrial ATPase gene (Fig. 1f) was expressed at high levels in both cultivars. Histological observation with *in situ* hybridization had emphasized that this gene is expressed in all the structures of hermaphrodite flowers, mainly in the ovary wall, ovules and embryo sac, and it is absent in staminate flowers (Sgromo et al. 2009). The ubiquitin-protein ligase transcripts gene were detected in both the cultivars analysed but were higher in Dolce Agogia (Fig. 1g). This observation indicated that post-transcriptional regulation could have a role in the control of flower development by activating or repressing numerous genes. Similar observations were reported during the identification of flowering-related genes in *Poncirus trifoliata* (Zhang et al 2009) and it was suggested that the ubiquitin/26S proteasome pathway, which comprise also the activity of the ubiquitin-protein ligase, could play a critical role in various aspect of plant growth and development with a mechanism similar to *Arabidopsis thaliana*. Post-transcriptional regulatory process allow the cells to respond rapidly to intracellular signals and change environmental conditions by adjusting the levels of key proteins.

Finally a gene with high similarity to a mitochondrial translation elongation factor (EF-Ts<sub>mt</sub>) from tomato was analysed, but its role was not clear (Fig. 1h). Transcript levels for EF-Ts<sub>mt</sub> decreased during flower buds development in Leccino, and were present at high levels only in the first sample of Dolce Agogia. In tomato, a functional characterization demonstrated that EF-Ts<sub>m</sub> encodes a functional elongation factor which was induced during fruit ripening.



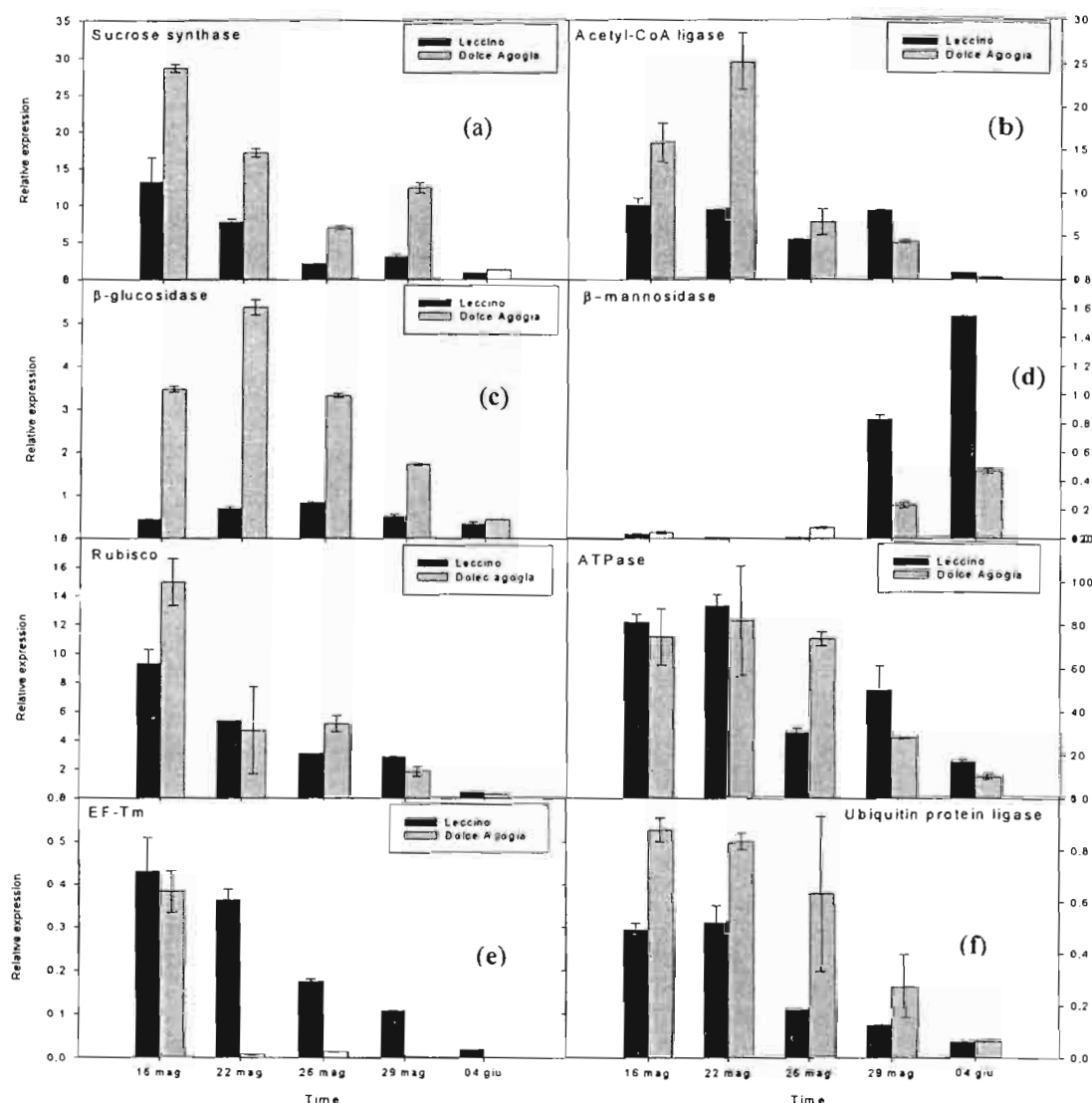


**Figure 1:** Relative quantities of sucrose synthase, fatty-acid-CoA ligase, beta-glucosidase, beta-mannosidase, mitochondrial ATPase, RuBisCO, mitochondrial elongation factor Ts and ubiquitin-protein ligase in cultivars Leccino (black bars) and Dolce Agogia (grey bars). Data points represent the mean  $\pm$  SE of at least three replication for the relative expression, which were calibrated by the amount of expression of the actin control.

#### 4. Conclusions

232 ESTs differentially expressed during flower development of Leccino using an SSH method have been isolated. We identified and analysed the time-course transcriptional profile of genes involved in carbohydrate and lipid metabolism, C-fixation in plant, energetic metabolism, genetic information and cellular processing. The transcript levels of most of the analysed genes decrease during flower development. This observation could indicate that before anthesis some metabolic processes slow down, probably waiting for fertilization and the subsequent developmental stage. During flowering transcript levels were differentially modulated in the cv Leccino and Dolce Agogia, providing new insight in understanding gene expression in hermaphrodite and staminate flowers. Taken together, the data we suggest the hypothesis that carbon deprivation and modification of carbohydrate pathways, induces abortion of gynoecium through the programmed cell dead pathway.

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## **Les huiles d'olive tunisiennes : Caractérisation chimique et sensorielle et valorisation en huiles mono-variétales et labels de qualité**

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### **Résumé**

Actuellement, la Tunisie s'est engagée à mettre en place des stratégies de développement axées sur l'amélioration de la productivité et de la qualité et de valoriser les spécificités des zones de production en vue de maintenir les marchés traditionnels et conquérir de nouveaux marchés.

De notre côté, nous avons oeuvré pour valoriser les potentialités oléicoles des ressources génétiques de notre pays par l'élaboration des huiles d'olive tunisiennes monovariétales et la contribution à la création du label « huile d'olive de Tunisie » et des premiers signes de qualité en Huile d'olive.

En effet, dans le cadre d'une convention de recherche-développement, nous avons élaboré dans le respect total des bonnes pratiques de fabrication et d'hygiène, cinq huiles monovariétales appartenant à 4 variétés : *Chétoui Medjez el Bab*, *Chemlali Sfax- Messouda*, *Chemlali Maknessy*, *Chemchali Gafsa* et *Oueslati El Alaa*. Les résultats montrent que les cinq huiles présentent des caractéristiques physicochimiques, un profil aromatique et des sensations agréables typiques propres à chaque variété et origine. Les tests de préférence réalisés auprès de 300 consommateurs montrent que la majorité des personnes enquêtées ont aimé le fruité de la Chétoui et malgré l'amertume et le piquant inhabituels à ces personnes, l'huile de cette variété a été très appréciée.

Le premier signe de qualité '« IP Huile d'olive Chemlali Monastir » dérive à la fois d'un terroir particulier et d'une variété autochtone bien adaptée depuis des siècles : la Chemlali de Monastir. C'est une huile vierge extra riche en tocophérols (760-1030 ppm) et en stérols (1630 - 2170 ppm). Cette huile est caractérisée par des odeurs de fleur de champs et d'amande verte. Les intensités de l'amertume et du piquant sont légères alors que celle du fruité est moyenne.

**Mots clés:** Huile d'olive, monovariétale, label, typicité.

### **Tunisian olive oils: Chemical and sensorial characterization and valorization at monovarietal olive oils and signs of quality**

#### **Abstract**

Despite the socio-economic importance of the Tunisian olive oil sector and the wide diversity of its genetic resources, Tunisian oils are not highly valued within the national and international markets.

In order to prove the excellent quality and diversity of Tunisian Olive Oil, we have produced, in the frame of the convention between the Technology and quality research unit at the Institut de l'Olivier and the private society's, the monovarietal olive oils: *Chetoui Medjez El Bab*, *Chemlali Sfax-Messouda*, *Chemlali Maknessy* and *Oueslati El Alaa*. These oils were strictly created according to rules of good practices of production and hygiene. Each stage of production was fully and carefully noted in a card to ensure the tractability of the product.

These monovarietal extra-virgin olive oils show physico-chemical characteristics, a flavor profile and pleasant aromas typical to each variety and origin.

The distinctive organoleptic sensations and the variation of taste and aroma of each variety were easily detected and appreciated by the Tunisian and foreign consumers. Each olive oil is distinguished by an origin, a variety and a specific know-how. The investigation consumers in the opinion of 300 persons show that the major part has appreciated the fruity of the Chetoui monovarietal olive oil despite its bitterness and pungency flavor taste.

In conclusion, these olive oils quality should be valued as a designed origin protected or protected indication and to represent their regions of origin.

Furthermore, in the frame of the convention between the "Regional Commissariat of Resources and Agriculture Développement" of Monastir and "Institut de l'olivier, we have cooperated to create the first quality sign of olive oil in Tunisia. Currently, the application is under consideration by the Ministry of Agriculture.

During this research, we have first started by identifying the variety of olive trees within the zones of olive plantation that are suited for establishing the quality sign 'Olive Oil Chemlali of Monastir'. These are: *Zeramdine*, *Ouardanine*, *Moknine*, *Manzel Kamel* and *Béni Hassen*. As a second step, we have proceeded to establish the pomologic, physiochemical and sensory characteristics of the oil. Finally, we have identified the optimum time of harvest that correspond to the second half of December.

The Olive Oil Chemlali of Monastir is the result of a particular region and specific variety which is cultivated in those zones for centuries. It is characterized by an aroma of flower fields, and green almond. The intensity of fruitiness is from medium to light while those of the bitterness and pungent bitter. It is an extra virgin olive oil characterized by: an acidic composition with medium rates of oleic acid (61.5-64%) and a high level of tocopherols ranging between 1030.33 and 757.85ppm. The polyphenols content of this oil varying between 177.14 and 210.4 ppm and a level of total sterols between 1623.82 and 2169 ppm.

**Keys words:** Monovarietal, olive oil, label, typicity

## 1. Introduction

L'oléiculture en Tunisie est un secteur stratégique sur le plan socio-économique et environnementale. Actuellement, l'oléiculture compte plus de 65 millions d'arbres, une superficie de 1,7 millions d'Hectares dont 115000hectares de cultures biologique certifiée, et une production annuelle moyenne de 150.000T d'huile d'olive.

Par ailleurs, la Tunisie se positionne en deuxième de point de vue superficie agricole cultivée en oléiculture. Le commerce international de l'huile d'olive assure 50 % du total des exportations agricoles, 5 % des exportations totales et constitue la cinquième source de devises du pays.

Toutefois, l'huile d'olive tunisienne ne dispose d'aucun signe de qualité. La majorité de la quantité exportée soit plus de 95% se fait en vrac laissant cette huile d'olive mal connue et parfois anonyme. Actuellement, notre pays s'emploie à doter l'huile d'olive tunisienne d'une notoriété internationale. Pour ce faire et afin de s'imposer face à la recrudescence de la concurrence, la Tunisie s'est engagée depuis le IX plan de mettre en place des stratégies de développement axées sur l'amélioration de la productivité et de la qualité et de valoriser les spécificités des zones de production en vue de maintenir les marchés traditionnels et conquérir de nouveaux marchés. Pour la réalisation de ces objectifs, nous nous sommes engagé pour contribuer à la valorisation des huiles d'olive tunisiennes à travers la constitution du premier signe de qualité en huile d'olive « huile d'olive Chemlali de Monastir », l'élaboration du cahier des charges du « label de l'huile d'olive de Tunisie » ce qui représente une première mondiale et la production et la valorisation des huiles d'olives tunisiennes monovariétales : Chétoui Medjez El Bab, Chemlali Sfax-, Chemlali Maknessy, et Oueslati El Alaa.

## 2. Matériel et méthodes

### 2.1. Matériel végétal étudié dans le cadre de l'élaboration du signe de qualité

Dans le but de contribuer à l'élaboration du signe de Qualité "Huile d'olive Chemlali de Monastir". L'Institut de l'Olivier et le CRDA de Monastir ont œuvré pour la création du premier signe de qualité en Huile d'olive:«l'Huile d'olive Chemlali Monastir ». Le dossier est soumis pour avis au Ministère de l'Agriculture, des Ressources Hydrauliques et de la Pêche.

Un cahier des charges du signe de qualité: « Huile d'olive Chemlali Monastir » est élaboré. Il refferme le nom du produit, la description physico-chimique et organoleptique de l'huile d'olive et la délimitation de son aire de production à savoir: Zeramidine, Béni Hassen, Moknine, Ouardanine, et Manzel Kamel (Figure 1), les éléments prouvant la provenance de l'huile d'olive de cet aire et la description de la méthode de production des olives de la variété Chemlali (les pratiques culturales, la période de récolte optimale, la méthode de l'élaboration de l'huile, le procédure de contrôle et les indications spécifiques de l'étiquetage.

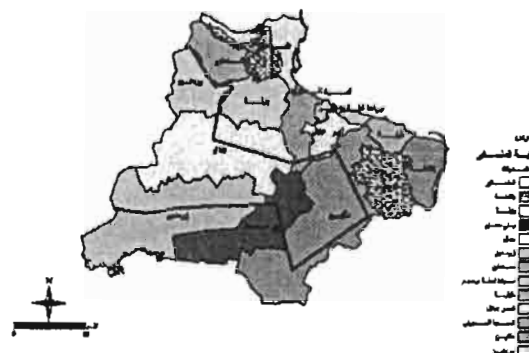


**Figure 1:** Les zones oléicoles délimitées de la région de Monastir pour l'IP «huile d'olive Chemlali Monastir».

## 2.2. Matériel végétal étudié dans le cadre de la production des huiles monovariétales

La valorisation des huiles d'olives tunisiennes monovariétales a été réalisée dans le cadre d'une convention entre l'Institut de l'Olivier et les sociétés SOCOHUILE et Huilerie Moderne. Les variétés choisies dans cette étude sont la Chemlali, la Chetoui et la Oueslati plantées dans quatre localités (Tableau 1).

**Tableau 1:** Caractéristiques des sites de plantation des variétés étudiées.

| Localité & caractéristiques |                                     |                |                 |                            |                 |
|-----------------------------|-------------------------------------|----------------|-----------------|----------------------------|-----------------|
| Variété                     | Localité                            | Superficie     | Nombre de pieds | % de la Variété recherchée | Date plantation |
| Chemlali                    | Messouda – Sfax                     | 152 hect       | 2600            | 99%                        | 1911            |
| Chemlali                    | Sidi Messoud –Maknessy              | 23 hect        | 400             | 99%                        | 1969            |
| Chetoui                     | Sidi Nasr – Chouache<br>Mjez El bab | 8 hect, 47 are | 813             | 98%                        | 1947            |
| Oueslati                    | El hseynia- El Alaa                 | 9 hect         | 200             | 100%                       | 1938            |

Chaque échantillon est constitué d'environ 2.5Kg d'olives provenant exclusivement de l'arbre et récoltés manuellement à hauteur d'homme sur toute la frondaison pour toutes les variétés étudiées. Aussitôt cueillis, les fruits sont transportés au laboratoire afin de préserver les caractéristiques de qualité. Ils sont effeuillés puis soumis aux différentes opérations pour la détermination de leurs caractéristiques pomologiques et pour l'extraction des huiles qui feront l'objet de différentes analyses physico-chimiques.

## 2.3. Les analyses effectuées

### 2.3.1. Analyses pomologiques des olives

**L'indice de maturité :** L'indice de maturité est un paramètre qui renseigne d'une façon globale sur la maturité des fruits, mais la valeur de cet indice ne reflète pas la réalité du mélange de couleurs d'un échantillon (El Antari et al, 2000).

**Le poids moyen du fruit (PMF) :** Le poids et les dimensions des fruits et des noyaux sont des caractéristiques variétales. Le patrimoine génétique de la variété a une incidence significative sur ces paramètres (Cimato, 1990 ; Grati Kamoun, 2007).

**La teneur en matière grasse:** Elle est effectuée par spectrométrie de résonance magnétique nucléaire (RMN). L'appareil est un spectromètre du type "Oxford 4000". Elle est exprimée en matière grasse par rapport au poids sec.

### 2.3.2. Caractérisation chimique des huiles

- Détermination de l'indice d'acide et de l'acidité dans les huiles, ISO 660 Troisièmes éditions 2009-06-15
- Préparation des esters méthyliques d'acides gras de l'huile d'olive et de l'huile de grignons d'olives COI/T.20/Document : 24 (2001).
- Analyse par CPG des esters méthyliques d'acides gras (ISO 5508)
- Analyses par spectrophotométrie dans l'ultraviolet de l'extinction spécifique aux longueurs d'ondes 232 nm (K232) et 270 nm (K270), COI/T.20/DOC n°19/r2V1.
- Détermination du pourcentage d'insaponifiable (ISO 3596)
- Détermination de la teneur en pigments (chlorophylles et carotènes) par spectrophotométrie : selon la méthode de Minguez-Mosquera *et al.* (1991).
- Détermination de la teneur des polyphénols par spectrophotométrie : Le dosage quantitatif des polyphénols est effectué selon la méthode Gutfinger (1981).
- Détermination de la teneur des tocophérols par HPLC (ISO, 9936)
- Détermination de la composition stérolique par CPG: (COI/T20/Doc. n°10)

## 3. Résultats et Discussions

### 3.1. Biodiversité des ressources génétiques tunisiennes : Production et Valorisation des huiles d'olives tunisiennes monovariétales

Le Tableau 2 récapitule les résultats obtenus pour les caractéristiques physico-chimiques et sensorielles des huiles monovariétales produites.

**Tableau 2:** Caractéristiques physico-chimiques et sensorielles des huiles monovariétales produites.

| Caractéristiques                            | Chemlali<br>Messouda | Chemlali<br>Maknessy | Chétoui | Oueslati |              |
|---------------------------------------------|----------------------|----------------------|---------|----------|--------------|
| Norme C.O.I. (2009)                         |                      |                      |         |          |              |
| Indice de Maturité                          | 2.98                 | 3.01                 | 2.94    | 2.87     |              |
| Acidité                                     | 0.20                 | 0.18                 | 0.20    | 0.20     | <0.8         |
| k232                                        | 2.05                 | 1.94                 | 1.96    | 1.94     | <2.5         |
| k270                                        | 0.08                 | 0.098                | 0.20    | 0.098    | <0.22        |
| %C16:0                                      | 17.1                 | 16.87                | 9.87    | 11.27    | 7.5 - 20.0   |
| %C18:1                                      | 60.76                | 64.04                | 73.62   | 74.11    | 55.0 - 83.0  |
| %C18:2                                      | 16.59                | 14.83                | 13.49   | 10.18    | 3,5 - 21,0   |
| Insaponifiables g/kg                        | 12.88                | 13.28                | 14.67   | 12.52    | <15          |
| Stérols totaux (ppm)                        | 1934.08              | 1638.42              | 1126.17 | 1228.57  | >1000        |
| Polyphénols (ppm)                           | 128,24               | 295,3                | 441,78  | 103,58   |              |
| Tocophérols                                 | 959.97               | 866.96               | 966.81  | 372.91   |              |
| Chlorophylles (ppm)                         | 3.47                 | 3.43                 | 5.75    | 4.05     |              |
| Carotènes (ppm)                             | 7.54                 | 8.29                 | 20.49   | 8.29     |              |
| Echelle organoleptique de 0 à 5 (fiche CCE) |                      |                      |         |          |              |
| Fruité                                      | 3                    | 3                    | 3.5     | 2.5      | Vierge Extra |
| Amer                                        | 1.5                  | 0.5                  | 3.5     | 1        |              |
| Piquant                                     | 1.5                  | 1                    | 3       | 2        |              |
| Doux                                        | 2                    | 2.5                  | 0       | 2        |              |
| Défaut                                      | 0                    | 0                    | 0       | 0        |              |
| Notation CCE                                | 7.5                  | 7                    | 7.75    | 7.25     |              |

Le patrimoine oléicole tunisien jouit d'une haute richesse en cultivars. Chaque variété se distingue par un profil chimique, aromatique et des sensations agréables propres à elle. Cette variation de goût et d'arômes peut entraîner une variation d'utilisation de l'huile et une création de nouvelles habitudes culinaires. Malheureusement, ces particularités et ces sensations agréables ne sont pas perçues par le consommateur tunisien à cause de certaines pratiques incorrectes et courantes dans la chaîne de fabrication de l'huile. En effet, une récolte inappropriée des olives, un long stockage à l'huilerie avant la trituration et système d'extraction mal conduit affectent considérablement la qualité de l'huile produite Montedoro (1989), Grati-Kamoun *et al.* (1999), Koutsaftakis *et al.* (2000), Grati Kamoun, (2007), Grati Kamoun, (2001) et Gomezp-Alonso *et al.* (2007).

### 3.1.1. Enquête consommateur

Dans le but de mieux valoriser les caractéristiques chimiques et sensorielles des huiles d'olive tunisiennes et afin de faire découvrir au consommateur ces sensations organoleptiques particulières (des notes d'amande verte, d'herbe fraîches,...), nous avons organisé au cours des Salons Méditerranéens d'Agriculture et des Industries Agroalimentaires « SMA 2008, 2009 et 2010 » des séances de dégustation des huiles d'olives monovariétales produites.

Une enquête auprès des visiteurs a été réalisée afin d'évaluer leurs appréciations pour les huiles produites et d'essayer de décrire le comportement du consommateur vis-à-vis des produits de qualité. L'échantillon est formé de 600 personnes aléatoirement choisies dont 76% sont des hommes, 54% appartiennent à la tranche d'âge 31 et 50 et 31% sont âgés entre 21 et 30 ans. La majorité des dégustateurs résident à Sfax environ 82%, 8% sont au Nord, 2% résident au Sahel et 8% sont des étrangers.

### 3.1.2. Habitudes de consommation chez les tunisiens:

Les analyses montrent que 87% des personnes de l'échantillon ont une tradition de consommation quotidienne de l'huile d'olive. 70% ont des connaissances concernant les différentes variétés d'olivier et ils sont conscients des bienfaits de l'huile d'olive et 74% sont prêts à payer plus cher pour avoir des huiles de qualité à condition que le surplus soit raisonnable.

### 3.1.3. Connaissances des signes de qualité par les consommateurs

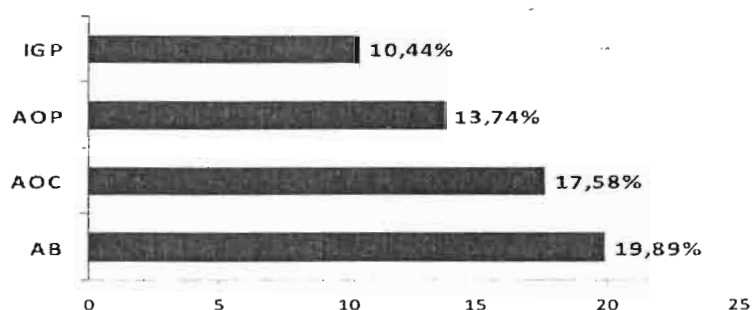


Figure 2: Notoriété des signes de qualité.

Ces résultats montrent que les consommateurs tunisiens ne connaissent pas les signes de qualité qui peuvent être apposés sur les produits alimentaires ainsi que « les promesses » derrière les labels et le taux de notoriété de ces labels sont globalement faibles. Nous constatons que pour l'AOC, signe de qualité qui existe en Tunisie depuis les années cinquante (vins et spiritueux), seuls 17.58% des personnes interrogées l'ont reconnue.

### 3.1.4. Confiance des consommateurs dans les signes de qualité

La confiance des consommateurs dans les signes de qualité se présente dans le graphique ci-dessous Malgré que les tunisiens connaissent peu les signes de qualité et ignorent la nature des promesses, ils peuvent leur faire confiance (figure 3). Au fait, ils font confiance à la certification en général qui Selon leur avis, elle est synonyme de la bonne qualité du produit.



Figure 3: Confiance dans les signes de qualité.

### 3.1.5. Appréciations des consommateurs concernant les huiles monovariétales

Les études ont montré que le goût, l'odeur et la couleur sont des critères de préférence importants pour les consommateurs tunisiens confirmant ainsi l'étude de Dekhil et D'Hauteville (2006). Ainsi, pour savoir les appréciations des dégustateurs sur les huiles monovariétales produites, on les a interrogé concernant ces trois attributs. L'idée de la production des huiles monovariétales a suscité un grand intérêt chez les visiteurs de différentes nationalités. Ces derniers, lors des séances de dégustation, ont beaucoup apprécié la qualité d'huiles produites. Ils ont rapidement perçu la différence et la spécificité de chaque produit et ils ont même pu distinguer les arômes d'amande verte et de la fraîcheur dans les huiles.

Tableau 3: Appréciation des dégustateurs pour les huiles monovariétales produites

| <i>L'huile monovariétale Chemlali Sfax-Messouda</i>       |                                                              |                                                              |
|-----------------------------------------------------------|--------------------------------------------------------------|--------------------------------------------------------------|
| <p><b>Goût</b></p> <p>■ Bon<br/>■ Moyen<br/>■ Mauvais</p> | <p><b>Couleur</b></p> <p>■ Bon<br/>■ Moyen<br/>■ Mauvais</p> | <p><b>Odeur</b></p> <p>■ Bon<br/>■ Moyen<br/>■ Mauvais</p>   |
| <i>L'huile monovariétale Chétoui Medjez El Bab</i>        |                                                              |                                                              |
| <p><b>Goût</b></p> <p>■ Bon<br/>■ Moyen<br/>■ Mauvais</p> | <p><b>Couleur</b></p> <p>■ Bon<br/>■ Moyen<br/>■ Mauvais</p> | <p><b>Odeur</b></p> <p>■ Bon<br/>■ Moyen<br/>■ Mauvais</p>   |
| <i>L'huile monovariétale Onestati d'El Alaa</i>           |                                                              |                                                              |
| <p><b>Goût</b></p> <p>■ Bon<br/>■ Moyen<br/>■ Mauvais</p> | <p><b>Odeur</b></p> <p>■ Bon<br/>■ Moyen<br/>■ Mauvais</p>   | <p><b>Couleur</b></p> <p>■ Bon<br/>■ Moyen<br/>■ Mauvais</p> |

Plus de 50% des dégustateurs ont décrit les huiles monovariétales de la Chemlali Messouda- Sfax, la Chemlali Maknessy et la Chétoui Medjez el Bab comme des huiles ayant un bon goût, une bonne odeur et une belle couleur. Les pourcentages les plus élevés ont été attribués à l'odeur de la Chétoui de Medjez El Bab, la couleur de la Chemlali Maknessy et le goût de la Chemlali Messouda-Sfax. Ils

ont aussi aimé la fraîcheur de la Chétoui et malgré l'amertume et le piquant inhabituels au sfaxiens, l'huile a été très appréciée. Pour finir, on conclut qu'une huile produite dans le respect total des bonnes pratiques de fabrication et d'hygiène ne peut que plaire à son goûteur.

### 3.2. Contribution à l'élaboration du signe de Qualité « Huile d'olive Chemlali de Monastir »

La description du produit est capitale lors de l'élaboration du cahier des charges d'un signe de qualité. De ce fait, cette partie est consacrée à l'étude des caractéristiques de l'huile d'olive provenant des aires de production délimitées par le signe de qualité ainsi qu'à la détermination de l'époque de récolte optimale permettant l'obtention d'une huile de qualité supérieure.

Les résultats obtenus à partir des analyses pomologiques, physico-chimiques, sensorielles et statistiques effectuées sur les échantillons d'olives et des huiles provenant des zones délimitées au gouvernorat de Monastir à savoir : *Manzel Kamel*, *Moknine*, *Béni Hassen*, *Zeramidine* et *Ouardanine*, sont présentés dans le tableau 2.

**Tableau 4:** Caractéristiques pomologiques, physico-chimiques, et sensorielles de «l'huile d'olive Chemlali Monastir».

| Caractéristiques         | Zeramidine | Ouardanine | Béni Hassen | Moknine | Manzel Kamel |                                                          |
|--------------------------|------------|------------|-------------|---------|--------------|----------------------------------------------------------|
| PMF                      | 0.96       | 0.97       | 1.07        | 1.02    | 0.83         |                                                          |
| % Pulpe                  | 81.13      | 79.67      | 82.33       | 82.33   | 79.12        |                                                          |
| %Humidité                | 51.60      | 53.10      | 52.80       | 56.69   | 53.48        |                                                          |
| MG/PF                    | 23.70      | 22.44      | 23.43       | 19.66   | 20.55        |                                                          |
|                          |            |            |             |         |              | <b>Norme C.O.I.</b>                                      |
| Acidité                  | 0.19       | 0.18       | 0.15        | 0.18    | 0.17         | <0.8                                                     |
| k232                     | 1.62       | 1.58       | 1.56        | 1.61    | 1.49         | <2.5                                                     |
| k270                     | 0.15       | 0.14       | 0.13        | 0.13    | 0.13         | <0.22                                                    |
| %C16:0                   | 16.87      | 17.37      | 17.37       | 17.48   | 16.34        | 7.5 - 20.0                                               |
| %C18:1                   | 62.85      | 62.89      | 61.88       | 61.48   | 64.12        | 55.0 - 83.0                                              |
| %C18:2                   | 15.54      | 14.51      | 16.26       | 15.78   | 14.34        | 3.5 - 21.0                                               |
| Polyphénols (ppm)        | 177.14     | 235.26     | 196.96      | 267.14  | 210.4        |                                                          |
| Tocophérols totaux (ppm) | 893.40     | 757.07     | 757.85      | 790.90  | 1030.33      |                                                          |
| Chlorophylles (ppm)      | 2.19       | 4.50       | 2.18        | 1.92    | 4.01         |                                                          |
| Carotènes (ppm)          | 15.87      | 15.64      | 17.03       | 15.75   | 16.11        |                                                          |
| Insaponifiables g/kg     | 11.0       | 13.8       | 13.2        | 11.6    | 10           |                                                          |
| Stérols totaux (ppm)     | 2169.78    | 1623.82    | 1939.5      | 2091.76 | 2039         | >1000                                                    |
|                          |            |            |             |         |              | <b>Echelle organoleptique de 0 à 5 (Norme CCE, 2007)</b> |
| Fruité                   | 2.28       | 2.61       | 2.43        | 2.46    | 2.6          | Vierge Extra                                             |
| Amer                     | 1.02       | 2.31       | 1.07        | 1.66    | 1.1          |                                                          |
| Piquant                  | 0.94       | 1.65       | 0.87        | 1.12    | 1.1          |                                                          |
| Doux                     | 1.71       | 0.3        | 1.73        | 1.03    | 1.75         |                                                          |
| Notation CCE             | 7.41       | 7.24       | 7.02        | 7.13    | 7.014        |                                                          |

La comparaison des moyennes des différents paramètres pomologiques, physico-chimiques, et sensoriels montre qu'à un risque d'erreur de 5%, la différence entre les huiles obtenues à partir des quatre zones étudiées n'est pas significative. Elles peuvent être donc classées dans un même groupe et elles méritent d'abriter un signe de qualité (AOC ou IP).

Sur le plan chimique, les analyses montrent que l'huile d'olive Chemlali Monastir est une huile Vierge Extra, avec une composition conforme aux normes internationales, caractérisées par:

- Une composition acide avec des taux moyens d'acide oléique (61.5-64%) et élevés d'acides palmitique (16.3-17.5%) et linoléique (14.3-16.3%),
- une richesse en tocophérols avec des teneurs qui varient entre 758 et 1030 ppm,
- une teneur en polyphénols qui varie entre 177 et 210 ppm,
- une richesse en carotènes (15.75-17) et en chlorophylles,
- un taux d'insaponifiables inférieure à 1.5% et
- une teneur élevée en stérols totaux qui varie de 1623.82 à 2169 ppm.



L'Huile d'olive "Chemlali Monastir" est un huile d'olive caractérisée par des odeurs de fleurs de champs et d'amande verte. L'intensité du fruité est moyenne à légère alors que celles de l'amertume et le piquant sont légères.

Par ailleurs et afin de garantir la conservation des composés responsables de l'arôme et du goût de l'huile ainsi que les composés mineurs qui protègent l'huile et garantissent sa valeur nutritionnelle et thérapeutique, la récolte des olives doit se faire au cours du mois de décembre et plus précisément durant la deuxième quinzaine de ce mois (indice de maturité IM= 3).

#### 4. Conclusion

Malgré l'importance socio-économique du secteur oléicole en Tunisie et la grande diversité génétique, les huiles d'olives tunisiennes ne sont pas très bien valorisées sur le marché national et international. Elles souffrent d'une déficience d'image et de notoriété. Cette étude a mis l'accent sur la haute richesse du patrimoine oléicole tunisien. En outre, elle a confirmé que le facteur génétique, le terroir et le savoir faire ont une grande influence sur les caractéristiques physico-chimiques et le profil aromatique de l'huile d'olive. Les huiles d'olive tunisiennes sont de très haute qualité et possèdent des caractéristiques chimiques et sensorielles spécifiques.

Aujourd'hui, le défi de véhiculer une image valorisante de notre produit à travers son histoire, ses variétés, ses spécificités et ses vertus s'est lancé avec le projet de création du premier signe de qualité "Huile d'olive Chemlali de Monastir" et ne doit pas s'arrêter ainsi que l'élaboration de la Tunisie doit opter rapidement pour une traçabilité déterminant les signes de qualité de l'huile d'olive, en adoptant d'autres appellations d'origine contrôlées AOC, ainsi que des indications de provenance IP.

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## **Thème 2**

**Bonnes pratiques de conduite de l'olivier : acquis et innovations**

## Alternative use of vegetable remains of olive tree orchard in a soil carbon sequestration system

O.M. Nieto<sup>1</sup>, J. Castro<sup>1</sup>, E. Fernández<sup>2</sup>

### Abstract

In traditional olive grove a large amount of vegetable remains take place and that have usually burned or rejected. The continuous addition of all the vegetable remains obtained in the pruning, harvests and cleaning of fruits, can be a real alternative for the improvement of the physical-chemical properties of a floor and to be used as mechanism of sequestration of carbon in olive orchards soils. The effect of the continuous addition of olive remains was studied in a olive orchard of Jaén (Spain). The results show like they decrease the annual emissions in  $2 \text{ t CO}_2 \text{ ha}^{-1} \text{ yr}^{-1}$  and with fixation rate of  $\text{CO}_2$  of  $0,6 \text{ t.ha}^{-1} \text{ yr}^{-1}$ , in relation to a conventional tillage olive orchard and burning the pruning remains.

**Key words:** vegetable remains, olive tree, soil carbon sequestration

## L'utilisation des débris végétaux dans l'olivieraie comme un système alternatif pour la séquestration du carbone des sols

### Résumé

Dans l'olivieraie traditionnelle une grande quantité de déchets végétaux sont produits chaque année et sont généralement brûlés ou rejetés. Le retour au champs de tous les déchets végétaux (branches obtenus de la taille des arbres, les feuilles, les grignons d'olive et le nettoyage des fruits), peut être une véritable alternative pour l'amélioration des propriétés physico-chimiques d'un sol et d'être utilisé comme un mécanisme de séquestration du carbone dans les sols des vergers d'oliviers. L'effet de l'addition continue de ces déchets du secteur oléicole a été étudié dans une olivieraie de Jaén (Espagne). Les résultats montrent qu'ils réduisent les émissions annuelles de  $\text{CO}_2$  de  $2 \text{ t ha}^{-1} \text{ an}^{-1}$  et avec les taux de fixation de  $\text{CO}_2$  de  $0,6 \text{ t.ha}^{-1} \text{ an}^{-1}$ , en comparaison avec un travail traditionnel du sol de l'olivieraie et le brûlé de branches de la taille.

**Mots clés:** Déchets végétaux, olivier, sol d'olivieraie, séquestration du carbone.

### 1. Introduction

In agricultural systems, the relationship between soils, climate change and their impact upon the science of carbon sequestration is well established (Dawson and Smith, 2007). Some authors (Jarecki and Lal, 2003; Hernández et al., 2005; Gómez et al., 2009) have reported that strategies based upon changes in land use and soil management in agricultural soils are potentially important in increasing the soil's organic-carbon (SOC) content and carbon sequestration. Olive trees constitute the most extensive woody crop in Spain, traditional olive management have favoured the constant degradation of these areas (Pastor, 2004). Nevertheless, the introduction of alternative soil-management practices to that of tilling, have decreased erosion and increased fertility (Castro et al., 2008; Gómez et al., 2009). According to the ESYRCE inquiry (MARM, 2008), 57% of olive groves continue to be tilled and only 0.5% mulching with prunings. The routine annual tasks involved in olive cultivation generate a number of residues that are amply suited for recycling as soil covering around the trees, such as the waste left from cleaning the fruit prior to extracting the oil (leaves, green twigs and superficial soil) and the twigs and small branches deriving from regular pruning. On top of this, according to traditional practice, more often than not they are burned, thus creating considerable  $\text{CO}_2$  emissions.

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Our aim was to discover the dynamics of organic carbon in the soil using the RothC model to predict the effects of changing the soil-management system from conventional tillage (T) to mulching with shredded olive pruning debris (PD) and the residues deriving from cleaning the fruit (CR).

Some authors have studied the effects of covering the ground with pruning debris. Sofo et al. (2005) underline the importance of this practice to help absorb atmospheric CO<sub>2</sub> and store it as organic matter in the soil. Ordoñez et al. (2001) described an improvement in the physical-chemical properties of the soil and a concomitant increase in fertility. The dynamics of carbon in soils under different land use and management systems have been estimated by models. One of the most widely used is the Rothamsted model for organic carbon turnover in soil (RothC) (Coleman and Jenkinson, 1996) due to its easy application and the success experienced in applying it to sites under diverse agricultural management systems throughout the world (Smith et al., 1997). No data concerning carbon storage in soils after a change in soil management are currently available and neither are there any publications concerning the effects on the soil of continuous mulching with the waste derived from olive-cleaning activities.

## 2. Materials and Methods

The study area is located in south-eastern Spain, adult olives (cv. *picual*) with a planting density of 82 trees ha<sup>-1</sup>. The initial soil-management system of the grove was T and was applied only to the open gaps between the trees (50% of the total area of the grove). The soil-management system was changed in 1997, which involves spreading shredded olive prunings and residues from the olive-fruit cleaning (leaves, fresh twigs and soil) between the trees. The ground is not tilled and all these residues remain on the surface that covered the soil between the trees (50% of total area of the grove). Soil samples were taken between trees in PD+CR soil after removing the superficial plant-residue layer and, in two neighbouring T olive groves. Three pits measuring 50 x 100 x 50 cm were dug and the samples taken 30 cm deep. Organic carbon in the soil and residues was determined by wet oxidation with dichromate according to the method of Walkley and Black. CO<sub>2</sub> emissions from burning residues were determined according to 1 g C = 3.67 g CO<sub>2</sub> (IPCC, 2000). The RothC model was used to assess SOC when changed T to PD+CR. In PD+CR carbon inputs were measured in the field as a mean of vegetable-residue inputs. For the T olive grove RothC model was run iteratively in reverse to calculate how much organic carbon needs to enter a soil annually to give the measured amount of SOC. We resorted to data available in the literature concerning tilled olive-grove soils over the last thirty years or more in similar estates in neighbouring areas.

## 3. Results and Discussion

The SOC contents under T management were slightly lower than available historical data (34.6 ± 9.1 t C ha<sup>-1</sup>), but without being significant, for this reason we assumed that T soil are in equilibrium.

**Table 1:** Measured and modelled data for the turnover of organic carbon in olive-groves soils under conventional tillage (T) and mulched with residues from pruning debris and olive-fruit cleaning (PD+CR)

| Scenario       | SOC measured<br>(t C ha <sup>-1</sup> ) | IOM<br>(t C ha <sup>-1</sup> ) | Input C modelled<br>(t C ha <sup>-1</sup> yr <sup>-1</sup> ) | Input C measured<br>(t C ha <sup>-1</sup> yr <sup>-1</sup> ) | Turnover time<br>(yr) |
|----------------|-----------------------------------------|--------------------------------|--------------------------------------------------------------|--------------------------------------------------------------|-----------------------|
| T equilibrium  | 26.4                                    | 2.0                            | 1.0                                                          | -                                                            | 26                    |
| PD+CR 10 years | 158.0                                   | 2.0                            | 25.3                                                         | 23.9                                                         | 6                     |

Table 1: Measured and modelled data for the turnover of organic carbon in olive-grove soils under conventional tillage (T) and mulched with residues from pruning debris and olive-fruit cleaning (PD+CR).

On the basis of the SOC measured and the IOM estimated using the equation of Falloon et al. (1998), the annual carbon input for T was calculated to be 1.0 t C ha<sup>-1</sup> yr<sup>-1</sup>. The RothC model estimated the carbon input into the soil of a T olive grove in equilibrium as being 1.0 t C ha<sup>-1</sup> yr<sup>-1</sup>. Romanyà et al. (2000) registered similar results for a vineyard in the Mediterranean area, with an annual carbon input of 1.4 t C ha<sup>-1</sup> yr<sup>-1</sup>. The only estimates of carbon input for olive groves were reported by Sofo et al. (2005), who registered an annual input as senescent leaves of 0.4 t C ha<sup>-1</sup> yr<sup>-1</sup>. The effectiveness of the PD+CR used in our experiment is manifest in the resulting high SOC, which were even higher than those measured in soils T and left to natural vegetation (data not shown). Other authors have also reported increases in these values on changing the soil-management system from T to cover crops (Castro et al., 2008; Gómez et al., 2009) or PD (Ordoñez et al., 2001; Sofo et al., 2005), although they obtained lower levels than the means in our experiment. When mulching with PD+CR the model predicted a continuous increase in SOC, but without reaching a state of equilibrium.

Table 2: CO<sub>2</sub> released into the atmosphere after 10 years tillage (T) and mulching with shredded pruning debris (PD)

| Scenario |                                | CO <sub>2</sub> accumulated to the atmosphere (t CO <sub>2</sub> ha <sup>-1</sup> ) |
|----------|--------------------------------|-------------------------------------------------------------------------------------|
| T        | Burn pruning debris            | 24.0                                                                                |
|          | CO <sub>2</sub> lost from soil | 12.0                                                                                |
|          | Total                          | 36.0                                                                                |
| PD       | Burn pruning debris            | -                                                                                   |
|          | CO <sub>2</sub> lost from soil | 15.9                                                                                |
|          | Total                          | 15.9                                                                                |

Table 2: CO<sub>2</sub> released into the atmosphere after 10 years tillage (T) and mulching with shredded pruning debris (PD).

During the experiment we measured a carbon input at the soil-surface between trees of  $23.9 \pm 14.3$  t C ha<sup>-1</sup> yr<sup>-1</sup>. After 10 years of change in soil-management system the annual carbon input needed to reach the SOC values between trees at the end of the experiment as estimated by the model were very similar to that measured in soils (Table 1).

CO<sub>2</sub> emissions when the PD were burned in T management and when they were shredded and spread on the ground after 10 years are given in Table 2. As a result of the addition of PD to the soil, the CO<sub>2</sub> that was hitherto released into the atmosphere during the years of tilled management was reduced by more than 55%. With regard to this decrease in CO<sub>2</sub> emission, the RothC model estimated a potential carbon sequestration of 0.5 t C ha<sup>-1</sup> yr<sup>-1</sup>. (Table 3). When PD is shredded and spread on the ground, the RothC model predicts a decrease in CO<sub>2</sub> emission of 2 t CO<sub>2</sub> ha<sup>-1</sup> yr<sup>-1</sup>. We did not model carbon sequestration after mulching with CRs because this waste matter is normally thrown away. Nevertheless, the total carbon content in the soil showed an increase during the experiment of 13.2 t C ha<sup>-1</sup> yr<sup>-1</sup>. The high values of turnover time obtained in T indicate carbon stabilization in the soil, which is to say that carbon migrates slowly from one pool to another. For PD+CR the turnover times were lower, this showed rapid migration of carbon and thus a soil that is not close to equilibrium. Abandoning tillage in favour of using organic waste to cover the ground is considered to be an efficient way of increasing carbon sequestration in agricultural soils. In our experiment we measured a carbon sequestration of 0.5 t C ha<sup>-1</sup> yr<sup>-1</sup> with PD cover (Table 3). IPCC (2000) suggested a carbon sequestration potential of 0.3 t C ha<sup>-1</sup> yr<sup>-1</sup>. Our results coincide with these values, which supports the idea that recycling PD+CR in olive groves is an effective way of storing carbon in the soil.

Table 3: SOC increase as result of changes in soil management from tillage (T) to mulching with residues (PD+CR). CO<sub>2</sub> reduction and carbon sequestration after the addition of only pruning debris (PD)

|            | SOC increase<br>(t C ha <sup>-1</sup> yr <sup>-1</sup> ) | CO <sub>2</sub> reduction<br>(t CO <sub>2</sub> ha <sup>-1</sup> yr <sup>-1</sup> ) | C sequestration<br>(t C ha <sup>-1</sup> yr <sup>-1</sup> ) |
|------------|----------------------------------------------------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------|
| T to PD+CR | 13.2                                                     | 2.0                                                                                 | 0.5                                                         |

Table 3: SOC increase as result of changes in soil management from tillage (T) to mulching with residues (PD+CR). CO<sub>2</sub> reduction and carbon sequestration after the addition of only pruning debris (PD).

#### 4. Conclusions

When the soil-management was changed from T to PD+CR, carbon storage in the soil improved considerably. Carbon turnover in PD+CR olive-grove soil was quite accurately predicted by the RothC model. A soil-management system that abandons tillage in favour of reusing both pruning debris and the residue from cleaning the olives to cover the ground, constitutes the most effective way of increasing soil quality and diminishing CO<sub>2</sub> emissions in one of the most extensive agricultural enterprises in the entire Mediterranean area.

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## Assessing possibilities of quantifying soil fertility of an olive orchard in one integrated index

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### Abstract

The soil acts physically as a water container, more or less colonized by roots. It also acts chemically as a nutrients provider to the tree. Both of these roles are performed simultaneously by intrinsic characteristic soil properties that as a whole contribute to the definition of the soil fertility. Thus, soil fertility involves a wide range of variable that make very difficult its valuation. The main objective of this work is to try to quantify soil fertility by creating a unique index namely soil fertility index. For this reason the soil quality (fertility) index was estimated by a three-step method modified from Rezai et al. (2005) and Andrews (2001). The first step is choosing of appropriate indicators and selection of the data set, the second is establishing scoring function and weighting factor for the data set indicators in order to standardize them and finally the soil fertility index (SFI) is accomplished by summing the scores for each indicator, dividing by the total number of indicators (Andrews et al., 2004).

**Key words:** Soil fertility, olive nutrition, soil quality index

## Evaluation de la fertilité d'un sol d'olivieraie à l'aide d'indicateurs de l'index de qualité des sols

### Résumé

Le sol est le support des cultures et leur réservoir d'eau et de nutriments. Le pouvoir du sol à satisfaire les besoins des cultures est défini comme étant sa fertilité. Celle-ci dépend des caractéristiques intrinsèques du sol et de ce fait d'un grand nombre de variables rendant son évaluation difficile. L'objectif de ce travail est de quantifier la fertilité du sol en créant un indice unique regroupant les principales composantes de ce terme à savoir l'Indice de Fertilité du Sol. Pour ce faire l'indice a été estimé par une méthode en trois étapes telles que proposées par Andrews (2001) et Rezai et al. (2005). La première étape est le choix des variables constituant la base des données. La deuxième est la transformation des variables par une standardisation des valeurs. Ceci est réalisé par l'établissement des fonctions de transformation (score entre 0 et 1) et du calcul du poids de chaque facteur. Enfin, l'indice de fertilité du sol est calculé par l'addition des scores de toutes les variables au point donné et la division de la somme par le nombre total des variables (Andrews et al., 2004).

**Mots clés :** Fertilité du sol, nutrition des oliviers, indice de qualité du sol.

### 1. Introduction

The general situation of the olive orchards productivity throughout the World seems to be unsatisfactory. About 70% of the olive orchards are traditional and marginal with a medium to very low productivity due, in a significant degree, to the lack of appropriate orchard management and to low soil fertility (Michelakis, 2002; Touzani, 1998, 1999). All around the Mediterranean basin the olive grove exploits marginal productivity inclining soils (Graaff and Eppink, 1999; Loumou and Giourga, 2003).

Under these conditions, the olive growing system seems to be the only one able to assure the sustainability of the natural resources through their preservation by the maintenance of the soil, the reduction of rainfall's losses, and their exploitation. Olive trees are drought resistant and because of their extensive rooting system are some of the few crops that can survive on only 200–300 mm of annual rainfall (Fresco, 1996). Thus, olive growing is the kind of cultivation that maintained the productive possibility in the barren and dry Mediterranean soils, with very high erosion levels (Loumou and Giourga, 2003). Furthermore, in the southern Mediterranean basin, the olive tree grows mainly

under arid conditions. The water shortage impact, which represents the most important limiting factor, is conditioned by soil quality, i.e. soil fertility (Braham, 1997; Fernandez-Escobar, 1997). Indeed, the soil quality determines water retention capacity and its availability to the tree. However, the trees have to uptake the nutrients during wet periods, when soil moisture is enough to assure the nutrients transfer. In reality, when these conditions are favourable, the nutrient absorption activity is boosted and the absorbed elements are stocked up in the permanent organs to be used when necessary.

These reserves may be as mineral nutrient or synthesized organic molecules, such as carbohydrates (Fernandez-Escobar, 2001). Therefore, it is necessary to study and to understand the relationship between soil fertility and olive tree nutrition. Several researches had dealt with this topic (Crescimanno, 1975; Zucconi *et al.*, 2001; Peterson and Stevens, 1994; Fernandez-Escobar, 1999; Braham, 1997; Gargouri and Mhiri, 2002; Chaves, 1975). However, none of them dealt with soil fertility as a quantifiable comprehensive parameter. More precisely, they considered this relationship for each fertility component and each nutrient alone, out of the influence of the others. This approach may lead perhaps to a wrong or approximate evaluation of the effects and the importance of each variable underestimating or overestimating its contribution to the olive tree growth and production. Soil quality (SQ) approach may be therefore conveniently used to assess this relationship between soil fertility and olive tree nutritional status. The general definitions for SQ emphasize the capacity of soil to perform services including the production of plants (Doran and Parkin, 1994; Karlen *et al.*, 1998). In addition, descriptions of SQ reflect appreciation for soils' fitness for use (Wander *et al.*, 2002; Larson and Pierce, 1994). Science-based soil quality index (SQi), including inherent and sometimes dynamic properties, provide the necessary integration of information for land managers to make informed decisions about complex issues such as site potential assessment and agroecosystem management (Andrews & Carroll 2001; Rezaei 2003; Rezaei *et al.* 2005). From an agronomic point of view the best SQi is that one assuring the best plant nutrition status leading to a satisfying level of growth and yield (Andrews *et al.*, 2002). In that case the soil quality indices should be called Soil Fertility index (SFi).

The objective of this study is to elucidate the relationship between soil fertility and olive tree nutritional status through the establishment of a Soil Fertility index (SFi).

## 2. Material and Methods

### 2.1. Study area, sampling and laboratory analysis

The olive orchard used for this study is located in central Tunisia (Sfax region - 34.37° N 10.16° E). The orchard covers 134 ha. The trees of the cv Chemlali, 80 yrs old, with very low plantation density (24x24 m apart = 17 trees/ha), are conducted under rainfed conditions. The soil is sandy (more than 90% of sand). The fertilization plan consists of 3 – 4 Kg of  $\text{NH}_4\text{NO}_3$  per tree distributed in spring and autumn (1:2). A 200x200 m grid pattern was established; each intersection point (node) represented a sampling point (figure 1). A total of 27 sampling points (Ii, i = 1 to 27) were identified. Soil samples were

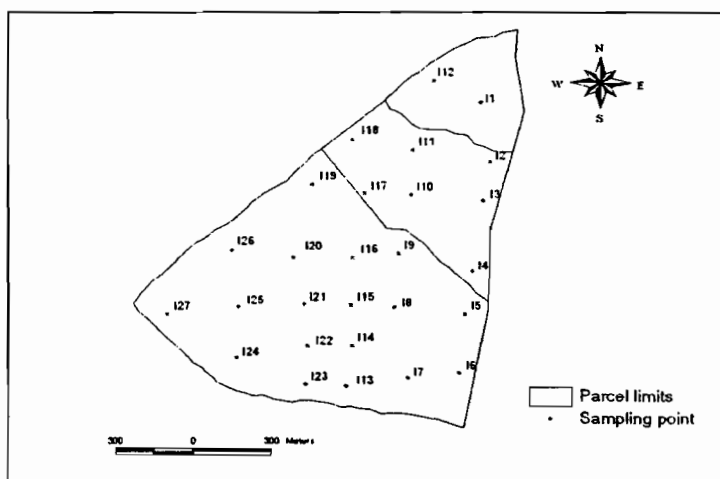


**Figure 1:** Sampling point repartition on the investigation area.

taken at 1 m depth in mid-July 2005. Each soil sample was collected as follows: four 500g soil cores were taken within 2 m radius of each grid point and one more core right at the intersection point. These 5 samples were mixed thoroughly to provide a bulked sample and to ensure its representativeness. Soil samples were air-dried overnight and passed through a 2 mm sieve. Organic matter content was determined by dichromate oxidation using Walkley and Black method (Pauwel *et al.*, 1992). Total  $\text{CaCO}_3$  content was determined after application of HCl and measurement of  $\text{CO}_2$  produced volume. Olsen method was used to determine available phosphorous concentration. Available K and Na were determined using flame photometer after extraction by ammonium acetate (Pauwels *et al.*, 1992). Soil electrical conductivity was measured on the soil extract saturated soil.



## 2.2. Determination of wood carbohydrates content

The wood carbohydrates content (WCc) was estimated by the sugars content. For this reason sugars content was determined following sulphuric acid – phenol method (Robyt and White, 1987). A sample of 300mg of fresh matter is mixed with 5ml of methanol 80% and maintained at 70°C during 30'. Then, 1 ml of the extract is mixed with 1 ml of 5% aqueous phenol and 5 ml of concentrated sulphuric acid. The solutions were allowed to stand for 20' for color development. The absorbance was measured at 640 nm, using a UV-visible spectrophotometer (Jenway 6405 UV/Vis). The total carbohydrates may be estimated using glucose as the standard.

## 2.3. Estimation of soil fertility index

The soil quality index was estimated by a three-step method modified from Rezai et al. (2005) and Andrews (2001).

Step I: Selection of appropriate indicators and selection of the data set.

The expert opinion method was adopted for the selection of the data set to be used in the generation of the soil fertility index (Andrews et al., 2002). We required a data set that would describe the basic soil functions necessary for obtaining the best olive tree mineral nutrition status. These functions are to provide sufficient nutrients and sufficient amount of water. The selected data set includes soil parameters previously described. Available phosphorus, exchangeable potassium, organic matter content, limestone, gypsum, electrical conductivity, exchangeable Na and chloride contents formed the data set.

Step II. Scoring function and weighting factor for the data set indicators.

The development of soil quality criteria for olive growing in semiarid and arid areas is difficult. An integrated procedure was derived from methods developed by Karlen and Stott, (1994), Andrews et al. (2002a, 2002b) and Rezaei et al. (2005). On the basis of both literature and the observed interactions between data set indicators and plant response variables, scoring of each observed indicator value was performed using non linear scoring curves (Karlen & Stott 1994). Non-linear scoring techniques involve the use of curvilinear scoring functions with a y-axis ranging from 0 to 1 and an x-axis representing a range of site- or function-dependent scores for that variable (Karlen and Stott, 1994; Andrews and Carroll, 2001). CurveExpert v. 1.3 shareware was used to generate the scoring functions. Dependent variable was carbohydrate content in the wood (Drossopoulos and Niavis, 1988; Ulger et al., 2004).

Step III. Calculating the soil fertility index (SFi).

This step is accomplished by summing the scores for each indicator, dividing by the total number of indicators (Andrews et al., 2004). Thus, the SFi is obtained by the following equation:

$$SFi = \frac{\sum_{i=1}^n S_i}{n}$$

Where S represents the scored indicator value and n is the number of indicators.

## 2.4. Statistical analysis

Data were analyzed statistically (SPSS 11.0 software). Classical descriptors were determined, such as mean, maximum, minimum, standard deviation and skewness of data distribution. Also, correlation matrix was calculated for all variables. On the other hand, ANOVA analysis was carried out to identify differences between experimental units for all parameters.

## 3. Results

Correlation matrix for soil characteristics showed highly significant correlation between electrical conductivity (EC), Cl, and CaSO<sub>4</sub> content (correlation coefficients of 0.596 for EC-Cl pair and -0.362



for EC-CaSO<sub>4</sub> pair). EC showed also significant correlation with Na and CaCO<sub>3</sub> (correlation coefficients 0.272 and 0.282 for the EC-Na and EC-CaCO<sub>3</sub> pair, respectively). Na content presented high significant positive correlation with chloride content, (coefficient of correlation 0.514). For this reason only the effect of Na may represent those of EC and Cl. On the other hand, available P<sub>2</sub>O<sub>5</sub> and OM showed significant correlation with a coefficient of 0.335. No significant correlation was found for all the other pairs of soil parameters (Table 1). Organic matter showed highly significant differences between sampling points (SP). The minimum values were observed in the southern eastern part of the investigation area: SP I7 and I8 with OM content of 0.21 and 0.23%, respectively. The highest OM content was observed in the southern western part of the orchard at SP I27 level, where OM content reached 0.57%. Two other zones had sampling points with OM value more than 0.5%. In the southern part of the orchard, sampling points I21, I22 and I23, had OM values of 0.53, 0.51, and 0.52%, respectively.

**Table 1:** Correlation matrix between soil traits pairs.

|                               |      | P <sub>2</sub> O <sub>5</sub> | K <sub>2</sub> O | Na      | OM     | CaCO <sub>3</sub> | CaSO <sub>4</sub> | EC      | Cl |
|-------------------------------|------|-------------------------------|------------------|---------|--------|-------------------|-------------------|---------|----|
| P <sub>2</sub> O <sub>5</sub> |      | 1                             |                  |         |        |                   |                   |         |    |
|                               | Sig. | -                             |                  |         |        |                   |                   |         |    |
| K <sub>2</sub> O              |      | 0.196                         | 1                |         |        |                   |                   |         |    |
|                               | Sig. | 0.155                         | -                |         |        |                   |                   |         |    |
| Na                            |      | -0.042                        | 0.173            | 1       |        |                   |                   |         |    |
|                               | Sig. | 0.764                         | 0.210            | -       |        |                   |                   |         |    |
| OM                            |      | 0.335*                        | -0.105           | 0.235   | 1      |                   |                   |         |    |
|                               | Sig. | 0.013                         | 0.449            | 0.087   | -      |                   |                   |         |    |
| CaCO <sub>3</sub>             |      | 0.050                         | 0.086            | 0.344*  | 0.173  | 1                 |                   |         |    |
|                               | Sig. | 0.0717                        | 0.537            | 0.011   | 0.211  | -                 |                   |         |    |
| CaSO <sub>4</sub>             |      | 0.010                         | 0.088            | -0.037  | 0.021  | -0.266            | 1                 |         |    |
|                               | Sig. | 0.941                         | 0.528            | 0.792   | 0.878  | 0.052             | -                 |         |    |
| EC                            |      | -0.014                        | -0.036           | 0.272*  | -0.131 | 0.282*            | -0.362**          | 1       |    |
|                               | Sig. | 0.921                         | 0.798            | 0.046   | 0.343  | 0.039             | 0.007             | -       |    |
| Cl                            |      | -0.126                        | 0.182            | 0.514** | 0.008  | 0.255             | -0.341*           | 0.596** | 1  |
|                               | Sig. | 0.362                         | 0.187            | 0.000   | 0.956  | 0.063             | 0.012             | 0.000   | -  |

\* Significant correlation at the level of 0.05 (bilateral);

\*\* Significant correlation at the level of 0.01 (bilateral).

The eastern part presented organic matter values of 0.56, and 0.52% for I4 and I5. On the other hand, the organic matter contents of the majority of the other SPs were near the mean value of 0.4%.

Limestone distribution in the orchard had also an important variation among the SPs. The minimum value was located in the eastern part of the toposequence, where limestone content was 0.25% at I8. In the same area, low total CaCO<sub>3</sub> content was detected: 0.75% and 1.7% for I4 and I5. Conversely, the highest value of 14.5% was observed for I11 which is located in the centre of the northern part of the parcel. The SPs with relatively high CaCO<sub>3</sub> content are localised in two zones. The first one is the mid-southern zone: I21, I22, and I23 (CaCO<sub>3</sub> content between 12.6 and 13.9%). The second is those of I11 and I10 in the centre of the upper part. Concerning calcium sulphate variation in the soil upper layer of the toposequence, a wide range was observed from 0.01 to 0.8%. The lowest values were located in the eastern part at I4 and I9 levels. In contrary, the highest values were observed in the western part in correspondence to SPs I26 (maximum), I19 (0.65%), I17 (0.65%), and I18 (0.60%).

However, the zone of low CaSO<sub>4</sub> content is adjacent to an area with high gypsum content which corresponded to SPs I3 (0.56%), I5 (0.66%), I8 (0.37%), I10 (0.45%), and I16 (0.54%). P<sub>2</sub>O<sub>5</sub> contents in the soil showed a moderate variation, with the minimum of 8.73 ppm located at I9, in the mid-eastern part of the orchard. This minimum value is somewhat above the critical level of 8 ppm proposed by Gargouri and Mhiri (2002). The maximum was located at I4, in the eastern boundary of the parcel. Consequently the areas where are located the extreme values of soil available phosphorus content are adjacent. In addition, no poor or, conversely, rich zones were determined. Indeed, the lowest values were neighboring the highest values. For example, I8 (P<sub>2</sub>O<sub>5</sub> content = 12.2 ppm) is located near I15 (21.2 ppm). Soil exchangeable potassium contents were highly variable, oscillating between 40 and 181 ppm. Moreover, 11 locations had K<sub>2</sub>O content under the critical level of 80 ppm determined for sandy soils in semi-arid regions. These locations were divided in two zones: the northern one and the east-southern one. The northern part is represented by: I2 (70.3ppm), I11 (52.6ppm), I12 (73.8ppm), and I18 (69.9ppm), the upper point of this part is above the threshold, i.e. I1

has an exchangeable potassium content of 84.7ppm. The second area included I6 (55.4ppm), I8 (43.4ppm), I9 (68.7ppm), I14 (78.6), I21 (65.4ppm), I22 (44.3ppm), and I23 (69.5ppm), the soil of the lower part of this area is relatively rich in exchangeable K counting I7 (102.4ppm) and I13 (131ppm). Available Na content variation is less important than K<sub>2</sub>O one. Indeed, the variation range is from 50 to 108 ppm. The maximum exchangeable Na concentration is located in the west southern part of the olive orchard (I24). This zone also includes several other sampling points with relatively high exchangeable Na contents. These sampling points are: I25 (93.5ppm), I26 (80.25ppm), and I27 (98ppm). A second relatively rich zone is located in the other side of the southern area of the parcel. Five sampling points formed this area: I4 (83ppm), I5 (92ppm), I7 (81ppm), I13 (85.5ppm) and I14 (97.5ppm). Between these two rich zones is located a poor zone including the significant lowest exchangeable Na values: I8 (57.5ppm), I9 (67ppm), I15 (50ppm), I20 (59ppm), I22 (66.5), and I23 (63.7ppm).

The wood carbohydrates contents showed important differences between the EUs. To understand the relationship between soil fertility and olive tree nutritional status, a Soil Fertility index (SFi) was developed. This index was designed with the aim of quantifying the intrinsic capability of the soil to assure the best nutritional status to the olive tree. For this purpose the leaf carbohydrates content was used as an indicator of olive tree nutritional status (dependent variable), together with several soil parameters such as exchangeable K<sub>2</sub>O, available P<sub>2</sub>O<sub>5</sub>, lime, organic matter, gypsum, exchangeable Na contents and electrical conductivity (EC) of the soil. The scoring method was based on the use of curvilinear scoring functions to transform indicators into dimensionless and, thus, combinable scores (Andrews *et al.* 2002) (Table 2).

**Table 2:** Scoring function for each component of the data sets\*.

| Indicator (xi)                      | Max.observ. value | Bivariate model                                            | R <sup>2</sup> | Max.predicted WCc (mg/g) | Scoring functions                                           |
|-------------------------------------|-------------------|------------------------------------------------------------|----------------|--------------------------|-------------------------------------------------------------|
| K <sub>2</sub> O (ppm)              | 180.72            | $Y = 0.5 + 0.002x_i + 1.43x_i^2$                           | 0.12           | 0.59                     | $S = 0.85 + 0.003x_i + 2.42x_i$                             |
| P <sub>2</sub> O <sub>5</sub> (ppm) | 27.95             | $Y = \frac{0.57}{1 + 128.5 \exp^{-12.67x_i}}$              | 0.83           | 0.57                     | $S = \frac{1}{1 + 128.5 \exp^{-12.67x_i}}$                  |
| OM (%)                              | 0.59              | $Y = 0.06 + 2.7x_i - 3.35x_i^2$                            | 0.73           | 0.6                      | $S = 0.1 + 4.5x_i - 5.58x_i^2$                              |
| Lime (%)                            | 14.5              | $Y = \frac{0.57}{1 + 634.04.5 \exp^{-3.16x_i}}$            | 0.82           | 0.57                     | $S = \frac{1}{1 + 634.04.5 \exp^{-3.16x_i}}$                |
| Gypsum (%)                          | 0.79              | $Y = \frac{-7.94 + 102.85x_i}{1 + 153.85x_i + 50.36x_i^2}$ | 0.85           | 0.61                     | $S = \frac{-13.02 + 167.21x_i}{1 + 153.85x_i + 50.36x_i^2}$ |
| Exchang.Na                          | 109.33            | $Y = \frac{0.57}{1 + 3.36 \exp^{-0.46x_i}}$                | 0.83           | 0.57                     | $S = \frac{1}{1 + 3.36 \exp^{-0.46x_i}}$                    |
| EC (dS/m)                           | 3.42              | $Y = \frac{-16.93 + 3.3x_i^{0.59}}{3.3 + x_i^{0.76}}$      | 0.82           | 0.58                     | $S = \frac{-29.19 + 5.69x_i^{0.59}}{3.3 + x_i^{0.76}}$      |

\*Function Y is wood carbohydrates content and xi is an ascribed indicator or variable value, and S is score from 0–1 based on measured values for xi

The potassium, despite its importance in olive tree nutrition, had very low a coefficient of determination. On the other hand, the phosphorus, sodium and lime scoring functions were based on reaching optimal value. The same trend was observed for organic matter scoring curve but an important decrease takes place after reaching optimal value. Finally, the scoring function for gypsum and electrical conductivity followed the less is better logic (Table 2), i.e. the lower is the amount of gypsum or EC the higher is the leaf carbohydrates content.

Finally, the SFi was calculated by summing the scores for each indicator and dividing the sum by the total number of indicators. The descriptive statistics showed slight high negative skewness which indicate that the SFi distribution shows a long tail on the left. Thus, this parameter is not normally

distributed. Low coefficient of variation ( $CV = 3.2$ ) was also observed. Indeed, the calculated values vary between 0.8 and 1.

#### 4. Discussion

The soil of the studied area is extremely poor with regards to organic matter content. Indeed, Soyergin *et al.* (2002) stated that the olive trees need at least 1% of OM content to have adequate growth.

Freeman and Carlson (1994) reported an even greater critical limit of 1.5%. The organic matter content is higher in the upper layer of the soil than in depth. Galvez *et al.* (2004), in an olive orchard growing on a toposequence in southern Spain, found that the OM content was relatively higher in the upper horizon than in the underlying ones decreasing from 0.8 to 0.2%, from 0.9 to 0.3% and finally from 1 to 0.5% in the three tested profiles. Nevertheless, Lopez-Granados *et al.* (2002) studied the variation of OM in the horizon 0-10 cm and 10-35 cm in two regions of southern Spain and they did not find significant variation according to depth. In our study the samples were taken from 1 m depth layer, consequently the analytical results represent an average of the contents of surface and deep layers.

Nevertheless, the obtained values are low and, in some SPs, are far under the thresholds. Thus, the studied orchard undoubtedly needs to be enriched in organic matter in order to be able to satisfy plant requirements and to retain, as high as possible, water since it represents the most limiting factor under arid climates. On the other hand,  $\text{CaCO}_3$  and  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$  contents in the studied orchard were adequate for olive tree growth. Indeed, the olive tree tolerates a wide margin of lime content up to very high values (76%). The soil of the orchard is classified as slightly gypsic (FAO, 2006) since the gypsum content is less than 5%. This condition is favorable to olive tree growth. The lime and gypsum contents showed significant correlation with soil electrical conductivity. The  $\text{CaCO}_3$ -EC correlation is positive and, the lime acts as soluble ion source (FAO, 2006). On the other hand, gypsum-EC correlation is negative which indicates that the presence of gypsum limits the amount of soluble ions.

This result is in contrast with the suggestions of the guidelines for soil description (FAO, 2006). In fact, gypsum (calcium sulphate) is a salt itself, and may increase the salt content. On the other hand, the gypsum may immobilize chloride and sodium in the soil reducing EC, as suggested by Shuman (1993). The significant correlation between EC, exchangeable Na, and chloride is evident since these ions are the main factors increasing the soil electrical conductivity (FAO, 2006). Finally, available  $\text{P}_2\text{O}_5$  was significantly correlated with OM content. The organic matter is really the principal source of phosphorus in this soil type as suggested by Freeman and Carlson (1994). The development of a model able to integrate all the involved soil parameters seems to be necessary to elucidate this relationship and to estimate the potential growth and yield permitted by soil conditions. The soil fertility index (SFI) developed in this research work tried to integrate all the studied soil parameters. The leaf carbohydrates content was chosen as plant nutrition indicator (dependent variable). However, this parameter, as reported above, did not show high variation in the studied orchard. For this reason the obtained SFI was between 0.83 and 0.98, i.e. a very narrow range. This result clearly indicates the need to widen the sample's size and to find a better indicator of the tree nutritional status plant carrying on with the research work. However, the obtained scoring equations were similar to those obtained by Andrews *et al.* (2002) who worked on annual crops in California. On the other hand, Razaei *et al.* (2005) found a highly consistent index working on annual crops and on a large sample.

In addition, it has to be recognized that the known adaptation mechanisms of trees complicate the establishment of those types of index, which needs long term studies. This establishment of a sound satisfactory soil fertility index may clarify the relationship between soil fertility and plant nutrition highlighting the potentialities of the orchards and, eventually, the need of correction. However, this index clearly needs long-term studies, good indicators and extensive sample. In addition, it seems important, also, to understand the relationship between olive tree nutritional status and olive tree growth, yield and production quality.

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## Qualitative and quantitative changes of soil microbial communities as a result of sustainable agricultural practices in an Italian olive grove

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### Abstract

The aim of the present work was to evaluate the effects of two soil management systems, so called 'sustainable' (ST) and 'conventional' (CT) on the composition, genetic diversity and carbon substrate utilization of soil microbial communities in a Mediterranean olive orchard. ST system included no-tillage, limited chemical fertilization, and organic matter inputs from drip irrigation with wastewater, spontaneous cover crops and pruning material. CT system was characterized by soil tillage, chemical fertilization, no irrigation and heavy pruning. After seven years of treatments, average olive yield was 8.4 and 3.1 t ha<sup>-1</sup> yr<sup>-1</sup> in ST and CT, respectively. CT had a significant higher number of total bacteria and actinomycetes if compared to ST, whereas fungi were significantly lower. In ST, the number of the bacteria involved in the nitrogen cycle isolated from the wetted areas under the drippers (ST-WET) were significantly higher than in inter-row areas (ST-INTER). The patterns of denaturing gradient gel electrophoresis of microbial 16S/18S rDNA showed differences between ST and CT, whereas those of 16S/18S rRNA evidenced that ST-WET clustered separately from CT and ST-INTER. Diversity indexes evaluated by Biolog<sup>®</sup> assay were significantly different between ST and CT. The results revealed qualitative and quantitative changes of soil microbial communities in response to sustainable agricultural practices that stimulate soil micro-organisms and improve olive yield and quality.

**Keywords:** sustainable olive growing, cover crops, organic matter, DGGE, Biolog<sup>®</sup>.

## Changements qualitatifs et quantitatifs des communautés microbiennes du sol à la suite de pratiques agricoles soutenables dans une oliveraie italienne

### Résumé

L'objectif de ce travail était d'évaluer les effets de deux systèmes de gestion des sols, dits «soutenable» (ST) et «conventionnel» (CT) sur la composition, la diversité génétique et l'utilisation des substrats carbonés par les communautés microbiennes du sol dans une oliveraie Méditerranéenne. ST-système comprenait le non-travail du sol, la fertilisation chimique limitée, et les apports de matière organique à partir de l'irrigation avec des eaux usées, des cultures de couverture et de taille du matériel. CT a été caractérisée par le travail du sol, la fertilisation chimique, ni d'irrigation et de forte taille. Après sept années de traitements, le rendement moyen d'olive a été de 8,4 et 3,1 t ha<sup>-1</sup> an<sup>-1</sup> dans le ST et CT, respectivement. CT a eu un nombre plus élevé de l'ensemble des bactéries et les actinomycètes, si on la compare à la ST, alors que les champignons ont été sensiblement plus faible. En ST, le nombre de bactéries du cycle de l'azote isolés du wetted les zones relevant de la goutteurs (ST-WET) étaient significativement plus élevés que dans les zones inter-rangs (ST-INTER). Les dénaturations gradient gel electrophoresis de rDNA 16S/18S ont montré des différences entre les ST et de CT, alors que ceux de 16S/18S rRNA témoigne que ST-WET regroupées séparément de CT et ST-INTER. Les indices de diversité des Biolog<sup>®</sup> tests étaient significativement différents entre les ST et CT. Les résultats ont révélé des changements qualitatifs et quantitatifs des communautés microbiennes du sol à la suite de pratiques agricoles soutenable qui stimulent les micro-organismes du sol et d'améliorer le rendement et la qualité de l'huile d'olive.

**Mots clé:** Oliveraie soutenable, couverture végétale, matière organique, DGGE, Biolog<sup>®</sup>

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## 1. Introduction

A new approach in olive orchard management is imposed by environmental emergencies, such as soil degradation, water shortage and greenhouse effect (Lal 2004, Hochstrat et al. 2006). Particularly, in semi-arid areas, the use of agronomical techniques able to conserve the natural resources is recommended (Kushwaha and Singh 2005). An integrated approach of culture-dependent and culture-independent methods has provided new tools to study the whole soil microbiota. One of the most useful molecular technique to reveal qualitative genetic (DNA) and functional (RNA) changes in the structure of soil bacterial and fungal communities is based on the characterization of soil-extracted nucleic acids by the amplification of regions of the bacterial and fungal ribosomal RNA gene (16 rRNA

and 18 rRNA, respectively) resolved by denaturing gradient gel electrophoresis (DGGE) (Crecchio et al. 2004). Metabolic microbial community diversity in the structure of soil bacteria communities can be estimated using the Biolog<sup>®</sup> metabolic assay, based on the ability of microbial isolates to oxidize different carbon and nitrogen sources (Zak et al. 1994). The community-level physiological profiles (CLPPs), obtained by the Biolog<sup>®</sup> method, are used to differentiate microbial populations from various soil environments or subjected to various treatments (Crecchio et al. 2004).

The present study was performed to explore the effect of sustainable agricultural management systems on genetic, functional and metabolic diversity of soil microbial communities, with a particular emphasis to those involved in nitrogen cycle, by using a combination of culture-dependent and independent methods. The trial was carried out during a 7-year period in an Italian olive orchard under semi-arid conditions. The effects on the productive response of the olive trees and fruit characteristics were also examined.

## 2. Materials and methods

### 2.1. Horticultural practices and fruit features

The study was carried out in a mature olive orchard (*Olea europaea* L. - cv Maiatica, a double aptitude variety) located in Southern Italy (Ferrandina - Basilicata Region, 40°29' N, 16°28' E). Olive trees were vase trained and planted at a distance of about 8 m x 8 m. The climate in the area is classified as semi-arid. The mean annual temperature ranges from 15 to 17°C. The soil of the experimental grove is a sandy loam (WRB: *Haplic Calcisol*), with a mean bulk density of 1.5 t m<sup>-3</sup>.

In 2000, the olive orchard was splitted into two plots managed according to sustainable agronomical techniques (Sustainable Treatment - ST) and conventional ones (Conventional Treatment - CT). The ST was irrigated with municipal wastewater treated by a pilot unit and distributed daily from May to October by drip irrigation (6 self-compensating drippers per plant delivering 8 L h<sup>-1</sup>). Irrigation volume applied over the annual growth season averaged 293 mm (2000-2006). The average annual amounts of N, P, and K distributed by the treated wastewater (293 mm yr<sup>-1</sup>) were 54, 3 and 50 kg ha<sup>-1</sup> yr<sup>-1</sup>, respectively.

The ST soil surface was covered by spontaneous weeds and grasses and mowed at least twice a year. Irrigated trees were lightly pruned each year, in order to improve fruiting potential by controlling the amount of fruiting wood and enhancing flower bud differentiation. Crop residues and pruning material (8.5 t ha<sup>-1</sup> yr<sup>-1</sup> dry matter, mean 2000-2006) were left on the ground as mulch. Fertilisers were applied along the growing seasons by a guided fertirrigation, taking into account wastewater and soil chemical composition, and mineral element balance in the orchard system. The CT was grown under rainfed conditions and managed according to the traditional horticultural practices of the area (Xiloyannis et al. 2008), that is by tillage performed 2-3 times per year and mineral fertilization carried out once per year, in early spring, using ternary compounds (NPK 20-10-10 fertilizer at doses ranging from 300 to 500 kg ha<sup>-1</sup>). In the CT, heavy pruning was performed every two years and pruning residues were burned out of the field.

Fruits were harvested by a trunk shaker and nets. Yield was measured on 12 trees per treatment. Fruit, pulp and stone were dried to a constant weight at 65°C in a forced-draft oven. Pulp percentage and flesh to stone ratio were also determined on fresh weight basis.



## 2.2. Soil sampling, microbial counts and microbial community metabolic profiles (Biolog®)

In February 2007, three composite samples of bulk soil (20 seven-cm-diameter cores pooled on site per treatment) were randomly collected and immediately stored in sterilized plastic pots at 4°C after removing visible crop residues. Samples were collected from the top soil layer (0-10 cm) of both treatments, ST and CT. Particularly, in ST soil sampling was performed in the wetted area under the drippers (ST-WET) and in the non irrigated inter-row area (ST-INTER).

Three replicates of 5 g-sub-samples (dry weight equivalent) of each soil sample were suspended in 45 ml sterile 0.1% sodium pyrophosphate-one quarter strength Ringer solution and sonicated for 2 min to disperse microbial cells. Aliquots of ten-fold serial dilutions were spread plated in triplicate on 1/10 strength TSA (Tryptic Soy Agar) medium amended with cycloheximide 0.1 mg ml<sup>-1</sup> for bacterial counting, and inoculated in MEA (Malt Extract Agar) medium implemented with streptomycin 0.03 mg ml<sup>-1</sup> and tetracycline 0.02 mg ml<sup>-1</sup> in triplicate for fungal counting. Counting took place after suitable incubation period (72 h for bacteria and 120 h for fungi) at 28°C. Actinomycetes were isolated by using Casein Starch Agar modified supplemented with 0.12 mg ml<sup>-1</sup> of cycloheximide (Sigma, NY, USA). The isolation of *Azotobacter* was carried out with Brown's substrate modified, whereas the identification of proteolytic bacteria were identified by MPN method in a cultural medium containing gelatine (Oxoid Lim., Hampshire, UK). Ammonifying bacteria were isolated in a liquid cultural medium containing asparagine and incubated at 28°C for 15 days. *Pseudomonas* were cultured on *Pseudomonas* Agar Base medium (Oxoid) with the addition of *Pseudomonas* C-N Supplement (Oxoid).

Sole carbon source utilization patterns of soil microbial communities, also called community-level physiological profiles (CLPPs), were assessed using the Biolog® 96-well Eco-Microplates (AES Laboratoire, France), containing 31 different carbon sources, three times replicated. Data were analysed to determine metabolic diversity indices, including average well colour development (AWCD, the mean of the blanked absorbance values for all the substrates, that provides a measure of total cultural bacterial activity), Shannon's substrate diversity index (H'), substrate evenness (E, equitability of activities across all utilized substrates) and substrate richness (S, the number of utilized substrates), according to Zak et al. (1994). The microplates were incubated at 25°C in the dark and colour development was measured as optical density (OD) every 24 h over a 144 h period using a Microplate E-Max Reader (Bio-Rad) with a E590-nm wavelength filter, and the data were collected by the Microlog 4.01 software (Biolog, CA, USA).

## 2.3. Denaturing gradient gel electrophoresis (DGGE)

A direct method was used for DNA and RNA extraction from soil samples by a bead beater system. Samples of 500 mg of soil were processed by FastDNA® Spin Kit for Soil (MP Biomedicals, OH, USA) and RNA Power Soil Isolation Kit (MoBio, CA, USA). Nucleic acids quantity and quality were assayed on 0.7% agarose gel containing 0.5 µg ml<sup>-1</sup> of ethidium bromide. Extracted RNA was retro-transcribed to c-DNA by RETROscript™ First Strand Synthesis Kit for RT-PCR (Ambion, TX, USA). DNA and c-DNA were amplified in a PCR thermocycler (Bio-Rad Laboratories, CA, USA) with the following primer pairs (MWG-Biotech AG, Germany): i) 968F-1401R for the 16S rDNA gene and ii) FR1-GC and FF390 for the 18S rDNA gene. PCR amplifications were performed according to Crecchio et al. (2004). DGGE was performed by the Bio-Rad DCode™ Universal Mutation detection System (Bio-Rad Laboratories, Hercules, CA, USA). PCR products (10 µl) were loaded into 6% (16S rDNA amplicons) or 8% (18S rDNA amplicons) polyacrylamide gel (37.5:1 acrylamide: bisacrylamide) with an urea-formamide parallel gradient (45-60% for 16S rDNA and 30-60% for 18S rDNA amplicons). Sybr Green I stained gels were photographed with Bio-Rad Gel Doc 2000 documentation system (Bio-Rad Laboratories).

## 2.4. Fingerprints and statistical analyses

Genetic fingerprints were analysed by the Bionumerics software version 4.5 (Applied Maths, Belgium). The normalization of the profiles in each lane was carried out by loading a standard reference pattern in three different points of the denaturing gel. Profiles comparison and clustering were performed by applying the unweighed pair-group method using arithmetic average (UPGMA) algorithm, based on the Pearson correlation coefficient (Boon et al., 2002). The values of total and specific microbial groups and Biolog® metabolic indices (AWCD, H', E and S) were treated by analysis of variance (ANOVA).



### 3. Results

#### 3.1. Tree productive responses, olive characteristics and microbial counts

The olive trees belonging to ST produced almost constantly every year with an average yield of  $8.4 \text{ t ha}^{-1} \text{ yr}^{-1}$  (mean 2001-2006) while the CT plants showed a significant ( $P < 0.05$ ) lesser productive level ( $3.1 \text{ t ha}^{-1} \text{ yr}^{-1}$ ) and a strong biennial bearing behaviour with low or no production in 2002, 2004, and 2006 (the so-called "off" years). The starting year of the trial, 2000, was an "off" year for both the examined treatments. Drupes picked from the ST showed a significant amelioration of their commercial characteristics such as fresh weight, drupe size, pulp percentage, and pulp to stone ratio, which are important parameters for table olives increasing their market value (Table 1).

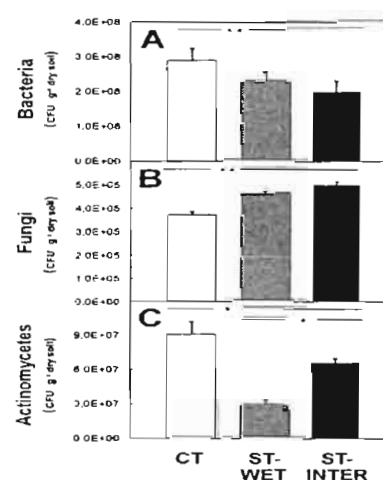
**Table 1:** Fruit characteristics and pulp to stone ratio (mean 2001-2006  $\pm$  SD) in sustainable (ST) and conventional (CT) treatments. Values with asterisks are significantly different at  $P < 0.05$ .

| Parameter                   | Unit of measure           | ST             |   | CT             |
|-----------------------------|---------------------------|----------------|---|----------------|
| Fruit fresh weight          | (g)                       | $3.8 \pm 0.92$ | * | $2.3 \pm 0.78$ |
| Longitudinal fruit diameter | (mm)                      | $23 \pm 2.17$  | * | $20 \pm 2.88$  |
| Equatorial fruit diameter   | (mm)                      | $17 \pm 1.66$  | * | $14 \pm 1.79$  |
| Pulp                        | (% on fresh weight basis) | $85 \pm 3.89$  | * | $78 \pm 5.03$  |
| Pulp/stone ratio            | (on fresh weight basis)   | $5.8 \pm 1.54$ | * | $3.8 \pm 1.20$ |

The different soil treatments significantly affected both total cultivable bacteria, significantly lower in ST-WET and ST-INTER ( $P < 0.01$ ), and total fungal counts, significantly lower in CT if compared to the two ST treatments ( $P < 0.01$ ) (Fig. 1a, b). The number of actinomycetes was significantly higher in CT if compared to the two ST treatments ( $P < 0.05$ ), and significantly higher in ST-INTER than in ST-WET ( $P < 0.05$ ) (Fig. 1c).

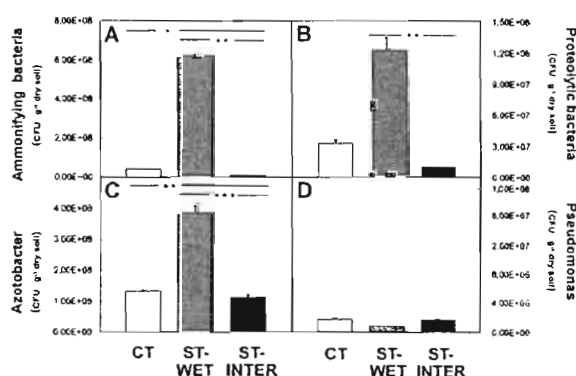
**Figure 1:** (a) Total bacterial, (b) fungal, and (c) actinomycetes counts in the three treatments: conventional (CT; white bars), sustainable under the drippers (ST-WET; grey bars), sustainable in the inter-row area (ST-INTER; black bars). The values represent the average ( $\pm$  SD) of three independent replicates for each soil treatment. Significance levels:

\*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ .



The number of ammonifying bacteria, proteolytic bacteria and *Azotobacter* isolated from ST-WET treatment was significantly higher than in ST-INTER ( $P < 0.01$ ,  $P < 0.01$  and  $P < 0.001$ , respectively) (Fig. 2a, b, c). Moreover, CT significantly differ from the two ST soils both for ammonifying bacteria ( $P < 0.05$ ) and *Azotobacter* ( $P < 0.01$ ) (Fig. 2a, c) being the number of microorganisms in both cases intermediate between ST-WET and ST-INTER. *Pseudomonas* counts were not significantly different between CT and ST neither between ST-WET and ST-INTER (Fig. 2d).

**Figure 2:** Ammonifying bacteria (a), proteolytic bacteria (b), *Azotobacter* (c) and *Pseudomonas* (d) in the three treatments: conventional (CT; white bars), sustainable under the drippers (ST-WET; grey bars), sustainable in the inter-row area (ST-INTER; black bars). Statistics as in Figure 1.



### 3.2. Genetic, functional and metabolic fingerprinting

The genetic dendrograms of bacterial 16S rDNA and fungal 18S rDNA showed that molecular patterns of CT were discriminated from patterns of the sustainable treatments (ST-INTER and ST-WET) (Fig. 3a, b). Anyway, Pearson similarity coefficients for 16S rDNA, ranging from 95.1 to 98.5, indicate that electrophoretic profiles relative to bacterial community, were quite similar (Fig. 3a). On the other hand, functional DGGE patterns of rRNA evidenced that irrigated sites under drip emitters (ST-WET) clustered separately from CT and ST-INTER both for bacteria and fungi ribosomal genes (Pearson coefficient = 88.4 and 33.7 respectively) (Fig. 3c, d). The values of Biolog<sup>®</sup> metabolic indices showed that AWCD and  $H'$  were significantly affected ( $P < 0.01$  and  $P < 0.05$ , respectively) by soil treatment (ST vs. CT) (Fig. 4a, b). Moreover, ST-WET significantly differs from ST-INTER soils both for AWCD ( $P < 0.01$ ) and  $H'$  ( $P < 0.05$ ) (Fig. 4A, B). The values of E and S showed no significant differences between CT and ST neither between ST-WET and ST-INTER (Fig. 4c, d).

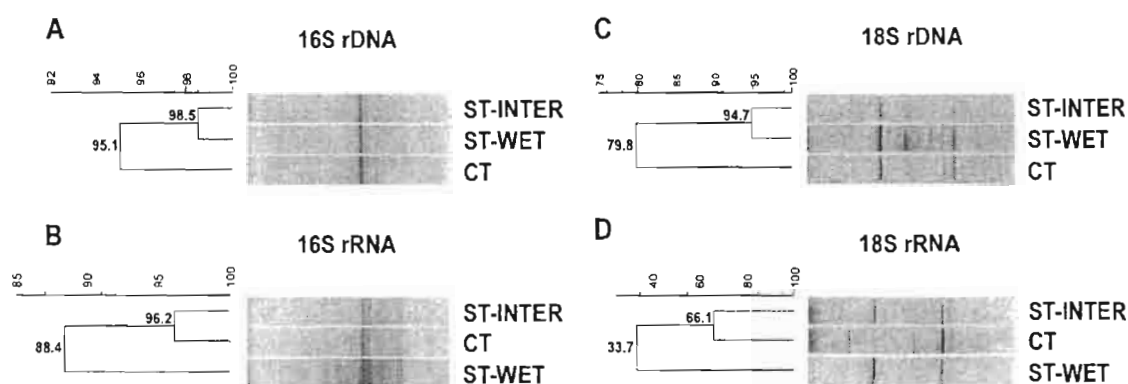


**Figure 3:** Genetic 16S DGGE fingerprints of soil bacterial communities (a, b) and functional 18S DGGE fingerprints of soil fungal communities (c, d) in the three treatments: conventional (CT), sustainable under the drippers (ST-WET), sustainable in the inter-row area (ST-INTER).

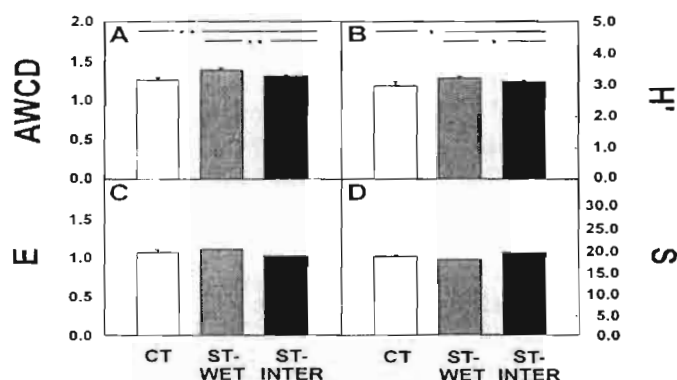


**Figure 4:** (A) Average well colour development (AWCD), (B) Shannon's substrate diversity index ( $H'$ ), (C) substrate evenness (E), and (D) substrate richness (S) in the three treatments: conventional (CT; white bars), sustainable under the drippers (ST-WET; grey bars), sustainable in the inter-row area (ST-INTER; black bars). Statistics as in Figure 1.

### 4. Discussion

The better yield level and fruit quality showed by the olive trees subjected to the ST (Table 1) can be clearly attributed to the adopted orchard management system. First of all irrigation that provided both water and mineral elements supply. In fact, the reclaimed wastewater used in this trial was rich in nutrients (especially N, P, and K) (Table 1), which were distributed by water along the irrigation season and integrated, when it was necessary, by fertirrigations based on orchard requirements.

Total fungal number was significantly higher in ST sites (Fig. 1b), likely because soil fungi rely on external available nutrients (Govaerts et al. 2008) and so respond promptly to changes in organic nutrient matter deriving from cover crops and wastewater irrigation/fertirrigation. The three different orchard management systems also caused significant differences in total bacteria (Fig. 1a).

Particularly interesting are the effects of agricultural management on the number of some of the bacteria involved in nitrogen cycle (Fig. 2). In fact, the results showed that ST-WET site has a higher number of *Azotobacter*, proteolytic and ammonifying bacteria when compared to ST-INTER (Fig. 2a, b, c). The number of *Pseudomonas*, denitrifying bacteria that transform anaerobically nitrates to nitrogen gas or nitrous oxides, was not significantly different between treatments (Fig. 2d). The particular conditions of ST-INTER soils with respect to ST-WET were reflected by the significantly higher actinomycetes number (Fig. 1c). In fact, actinomycetes (e.g., *Streptomyces*) produce a number of enzymes that help degrade organic plant material, such as lignin and chitin, and so are abundant in soils rich of organic inputs, as ST-INTER soils.

In our study, this effect is clear in both 16S and 18S rDNA genetic DGGE dendrograms, that revealed a discrimination between CT and ST systems (Fig. 3a, c). As for the fungal counts, the effects on bacterial community structures were very likely due to the input of organic matter deriving from cover crops (ST-WET and ST-INTER) and wastewater irrigation/fertirrigation (ST-WET). The observed differences between CT and ST soils were likely due also to the two soil management regimes. In fact, zero tillage with residues recycling and mulching result in a soil with good physical and chemical qualities, and high, stable yields, compared to conventional tillage (Govaerts et al. 2008). In contrary, differences in functional DGGE dendrograms of both 16S and 18S rRNA, that reflect the status of metabolically active microorganisms, indicated that the irrigation regime is the main factor inducing changes in bacterial and fungal communities of sites under the emitters (ST-WET) (Fig. 3b, d).

Soil bacterial metabolic diversity indices estimated by Biolog® CLPP are usually higher in sustainable than in conventional soils (Govaerts et al. 2008). Our results show that the values of AWCD and H' were significantly increased by the ST (Fig. 4a, b). In addition, comparing the two sustainable treatments, AWCD and H' were higher in ST-INTER soils (Fig. 4a, b), where cover crops could be an important discriminating element for microbial substrate utilization. Indices of metabolic diversity do not necessarily reflect the composition of the bacterial communities as two communities can have the same H' value but utilize different substrates.

Our results demonstrated that soil microorganisms respond to the application of a sustainable orchard management with evident benefits for olive yield and quality (Table 1). Sustainable orchards showed a higher microbial complexity and diversity. The study of the response of soil microbiota to different agricultural management systems and the quantitative and qualitative analysis of soil microbial communities could lead to identify agricultural practices that support and stimulate soil microorganisms in order to improve orchard production.

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## Soil management and soil water balance in a mature rainfed olive orchard

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### Abstract

An experiment was performed for a 3-year period in a rainfed olive grove located in Southern Italy to evaluate the effect of different soil management techniques on Soil Water Content (SWC). The compared treatments were Sustainable System SS (non-tillage, spontaneous vegetation cover, annual recycling of pruning material) versus Conventional System CS (tillage, no pruning material recycling). Surveys on soil structure and hydrological behaviour were performed. SWC was measured by Bouyoucos blocks placed at different soil depths. Ten years of sustainable soil management increased the storage of rainfall water particularly in the deeper layers. In SS soil macroporosity was higher than CS system and homogeneously distributed along the profile, favouring the vertical water movement down to deeper horizons. In CS the occurrence of soil crusting and compacted layers hindered infiltration and percolation of rainfall water.

**Keywords:** sustainable olive growing, cover crops, organic matter recycling, soil macroporosity, soil water holding capacity

### Gestion comparée de matière organique et de l'eau dans le sol des oliveraies soutenables ou conventionnelles

### Résumé

La recherche a été effectuée pendant 3 ans dans une oliveraie non irriguée, située au Sud de l'Italie, afin d'évaluer l'effet des différentes techniques de gestion sur l'humidité du sol (SWC). On a évalué les effets sur la structure et sur les caractéristiques hydrologiques des sols d'un Système Soutenable SS (organique) et d'un Système Conventionnel SC (travail du sol) de gestion du sol. Dix ans de gestion soutenable du sol ont augmenté la capacité de stockage de l'eau des précipitations pour la plupart des couches en profondeur. Dans le SS la valeur de la macroporosité du sol n'est pas élevée, mais elle est répartie de façon homogène en direction du profil, de telles façons à favoriser le mouvement vertical d'eau en profondeur. D'autre part, dans le SC, l'encroûtement et le compactage de la couche superficielle du sol ont empêché l'infiltration et la percolation des eaux de pluie.

**Mots clés:** Oliveraie soutenable, couverture végétal, recyclage matière organique macroporosité du sol, capacité de rétention d'eau du sol.

### 1. Introduction

Olive tree (*Olea europaea* L.) is the most widespread crop of the Mediterranean Sea Basin where it grows in semi-arid climates. Since ancient times olive tree is grown under rainfed conditions due to its ability to tolerate drought stress (Xiloyannis et al. 1999; Sofo et al. 2007).

Mechanical tillage is the most common technique used for soil management in olive orchards. Such practice is essentially performed to hinder weed competition for water and nutrients and to reduce soil evaporation by interrupting water capillary rise and augmenting soil surface roughness (Ozpinar and Cay 2006). On the other hand, continuous tillage induces oxidative soil metabolism (high organic matter mineralization) negatively affecting soil physical and hydrological features, such as porosity, soil structure and aggregation. This condition reduces water infiltration rate and triggers runoff and erosion processes (Abid and Lal 2009). Such phenomena are really serious in olive orchards particularly when they are placed on steep lands (Kosmas et al. 1997; Raglione et al. 1999; Francia Martínez et al. 2006; Flesskens and Stroosnijder 2007; Gómez et al. 2009).

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A spontaneous vegetation cover in the olive orchards represents an alternative soil management able to overcome the cited disadvantages linked to the mechanical tillage. Particularly, many researchers reported an improvement of soil water moisture in cover cropped olive groves (Hernandez et al. 2005; Durán-Zuazo et al. 2009). Nevertheless, such environment-friendly practice is not common in semi-arid areas where soil water availability represents the limiting factor for crop production. Instead, under such limiting climatic conditions, more efforts should be addressed to store rainfall water in the soil and to reduce runoff losses.

An experiment, lasted 3 years, was performed in a mature rainfed olive grove located in Southern Italy to evaluate the effect of two different soil management systems, Sustainable (cover cropped with spontaneous species) and Conventional (continuously tilled), on Soil Water Content (SWC). In the Sustainable System, cover cropping was combined with the recycling of olive pruning material, an important source of organic matter internal to the olive grove.

## 2. Materials and methods

Experimentation was carried out for a 3-year period (2007-2009) in a rainfed olive grove located in Southern Italy (Ferrandina - Basilicata Region, 40°29' N, 16°28' E). Mature trees (*Olea europaea* L. - cv Maiatica) were vase trained and planted at a distance of about 8 m x 8 m. The climate in the area is classified as semi-arid with an annual rainfall of 561 mm (mean 1976-2006). The mean annual temperature ranges from 15 to 17°C.

The soil of the experimental grove is a sandy loam classified as a Haplic Calcisol (FAO WRB 1998). In 2000, the top 60 cm soil layer had a low content of organic matter (12 g kg<sup>-1</sup>). The key meteorological parameters (air temperature, rainfall, humidity, etc.), were measured daily in 2006, 2007, 2008, and 2009 by a standard weather station close to the trial area. The reference evapotranspiration (ET<sub>o</sub>), provided by SAL service of the Extension Regional Service ([www.alsia.it](http://www.alsia.it)), was estimated using Blaney-Criddle, radiation, and Hargreaves methods (mean value).

Since 2000, the olive orchard was split into two parts subjected to different soil management system: Sustainable System - SS, and Conventional System - CS. In the SS, the soil surface was covered by spontaneous weeds and grasses which were mowed at least twice a year. Olive trees belonging to the SS were lightly pruned each year. Cover crops and pruning were cut and their residues were left on the ground surface as mulch. The natural vegetation together with pruned material and senescent leaves ensured an annual C distribution of about 5 t ha<sup>-1</sup>.

The soil of the CS was managed by tillage (milling at 10 cm soil depth) performed 2-3 times per year in order to keep the soil bare. Heavy pruning was carried out every two years but pruned residues were burned out of the olive grove.

With the aim to characterize soil structure, vertically orientated thin sections (5.5 x 8.5 cm) were obtained from undisturbed soil samples collected in November 2007 at different depths (0-10, 10-20, 20-30, 30-40, and 40-50 cm) along the profile in the two different management systems (60 soil thin sections in total). The images were analyzed using the Image-Pro Plus software produced by Media Cybernetics (Silver Spring, MD, USA). Total porosity and pore distribution were measured according to pore shape and size, the instrument being set up to measure pores larger than 50 µm. Pore shape was expressed by a shape factor [ $\text{perimeter}^2 / (4\pi \cdot \text{area})$ ] so that pores could be divided into regular (more or less rounded) (shape factor 1-2), irregular (shape factor 2-5), and elongated (shape factor >5). These classes correspond approximately to those used by Bouma et al. (1977). Pores of each shape group can be further subdivided into size classes according to either their equivalent pore diameter (regular and irregular pores), or their width (elongated pores) (Pagliai et al. 1984). According to this procedure, the elongated transmission pores (50-500 µm) (Greenland 1977) were quantified. Thin sections were also examined using a Zeiss 'R POL' microscope at 25X magnification to observe soil structure.

Saturated hydraulic conductivity was measured *in situ* using a constant head well permeameter (Model 2800 Guelph Permeameter - Soilmoisture Equipment Corp., Santa Barbara, USA). The measurements were made in May 2007 at 12 cm depth with at least three replicates. In order to evaluate only the vertical water flux, measurements were carried out in confined well by a plastic tube (diameter = 5.5 cm). As the well radius changes respect to the standard condition (radius was 2.25 cm instead of 3.0 cm), the well shape factor C (Reynolds et al., 1986), used in the calculation, was modified according to Zhang et al. (1998).

Volumetric Soil Water Content (SWC -  $\text{mm mm}^{-1}$ ) was measured by means of electrical resistance blocks (Eijkelkamp) installed in November 2006 at different soil depth (25, 50, 75, 100, 150, 200 cm) using a pneumatic driller opening holes of 40 mm. A portable, battery-operated ohmmeter was used to measure resistance. Field measurements were carried out from January 2007 at 10/15-day intervals. Previously, the electrical resistance blocks were calibrated in the laboratory in order to find the best relationship between the measured resistance values and the volumetric soil moisture content of the studied soils.

### 3. Results and discussion

#### 3.1. The pluviometric pattern of the experimental years

The pluviometric pattern, recorded in the studied area, was quite different among the experimental years. Assuming that the soil water reserve was restored by the precipitation falling from October to March ("recharge period"), the rainfall values recorded in such period were the following: 243.4 mm from October 2006 to March 2007; 288.6 mm from October 2007 till March 2008; 599.6 mm from October 2008 to March 2009. Precipitation felt during the vegetative season (April-September) was 287.8, 248.8, and 376.0 mm in 2007, 2008, and 2009, respectively.

#### 3.2. Soil structure and hydrological characterization

Soil structure characterization by image analysis of thin sections showed similar macroporosity values between the SS and CS treatments at 0-10 cm depth (Figure 1). Conversely, below the 10 cm depth, the macroporosity of the CS drastically decreased to values smaller than 5%, so identifying a very compact soil according to Pagliai (1988). Such condition is a clear effect of the continuous tillage. In addition, the reduction of macroporosity in the CS essentially affected the transmission elongated pores (size class 50-500  $\mu\text{m}$ ) which are directly involved in water fluxes within the soil (Figure 1). On the contrary, macroporosity in the SS was homogeneously distributed along the profile, affecting positively water movement in the soil (Figure 1).

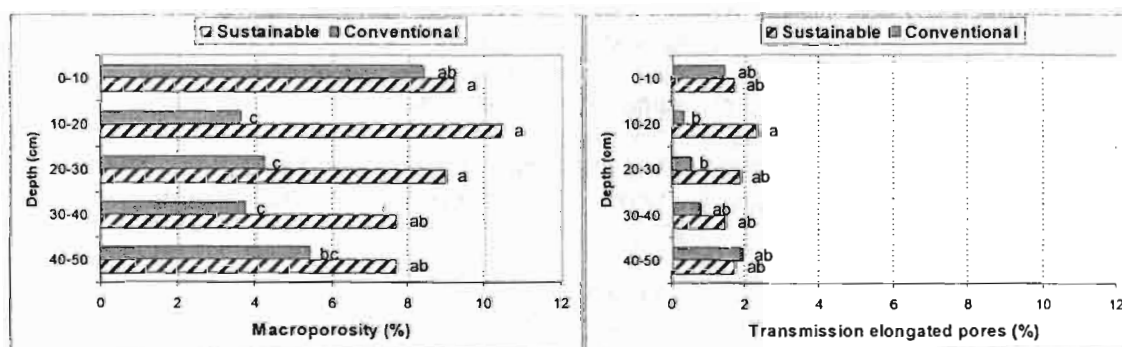


**Figure 1:** Macroporosity (pore size class:  $>50 \mu\text{m}$ ) and transmission elongated pores (pore size class: 50-500  $\mu\text{m}$ ) at different soil depths in the SS and CS. Bars marked with the same letter are not different ( $P < 0.05$ ) by Duncan's multiple range test.

Differences between the soil management systems were found for  $K_{\text{sat}}$  measured at 12 cm depth according to the standard procedure for Guelph Permeameter.  $K_{\text{sat}}$  value was  $0.31 \pm 0.16 \text{ m d}^{-1}$  (mean  $\pm$  standard deviation) in the SS and  $1.08 \pm 0.77 \text{ m d}^{-1}$  in the CS setting the former in the medium-low  $K_{\text{sat}}$  class, the latter in the medium class (Rossi Pisa 1997). The highest  $K_{\text{sat}}$  values measured in the CS were probably due to the recent tillage practice that increased macroporosity of surface horizon. Nevertheless,  $K_{\text{sat}}$  measurements performed to evaluate the vertical water flux (confined) showed a significant reduction in the CS being equal to  $0.013 \pm 0.007 \text{ m d}^{-1}$  ( $K_{\text{sat}}$  class: very low) confirming the presence of a ploughpan, as demonstrated by the drastic reduction of the soil macroporosity below the 10 cm layer (Figure 1). In SS,  $K_{\text{sat}}$  confined was, instead,  $0.16 \pm 0.07 \text{ m d}^{-1}$  lower than the value measured adopting the standard procedure but belonging to the same saturated conductivity class ( $K_{\text{sat}}$  class: medium-low) (Rossi Pisa 1997).



### 3.3. Volumetric Soil Water Content (SWC)

SWC was affected by the soil management systems as well as by the different pluviometric pattern recorded in the studied years. As mentioned before, the climatic pattern recorded in 2007 and 2009 was substantially different so providing the occasion to study the response of the two examined soil management systems under diverse pluviometric conditions.

SWC ( $\text{mm mm}^{-1}$ ), measured at different depths in two successive survey dates of the "recharge period" in 2007 and 2009, was reported in Table 1 and 2, respectively.

Generally, SWC was higher in the SS than in CS. Differences between the soil management systems were more evident below the 0-50 cm layer in both the considered years (Table 1 and 2).

At the end of the previous vegetative seasons, November 2006 and October 2008, SWC in both the SS and the CS was the same along the soil profile and at the lowest level (around the wilting point measured at  $-1.5$  MPa). On January 9<sup>th</sup> 2007, the recharge of rainfall water, fallen from October 2006 (70.0 mm), was evident in the CS soil only in the upper 50 cm (Table 1). Conversely, the soil of the SS not only showed a good moisture level at the 0-50 cm depth but also a gradual water enrichment along its profile. At the end of March 2007, SWC in the CS soil slightly increased especially in the 0-100 layers (31 mm) while in the deepest layers the increase was not important (2.6 mm). In the SS, a moisture increase of 89.6 mm was recorded in the 0-200 cm layer. About the 94% of such increase occurred in the layers below 50 cm (Table 1). The SS was able to retain an amount of water greater of about 45% than that stored by the CS.

**Table 1:** Soil Water Content (SWC, mean  $\pm$  standard error) measured 09-01-2007 (t0) and 29-03-2007 (t1) at different soil depths in the SS and CS. Rainfall and ETo of the considered period: 149.0 and 142.5 mm, respectively.

| Soil layer (cm) | SS              |                 |               | CS             |                |               |
|-----------------|-----------------|-----------------|---------------|----------------|----------------|---------------|
|                 | SWC (t0)        | SWC (t1)        | SWC (t1 - t0) | SWC (t0)       | SWC (t1)       | SWC (t1 - t0) |
|                 | mm              |                 |               | Mm             |                |               |
| 0 - 50          | 103.6 $\pm$ 7.0 | 108.6 $\pm$ 5.3 | 5.0           | 75.1 $\pm$ 5.1 | 85.6 $\pm$ 5.4 | 10.5          |
| 50 - 100        | 84.7 $\pm$ 13.5 | 115.7 $\pm$ 1.5 | 31.0          | 38.7 $\pm$ 1.0 | 59.2 $\pm$ 7.2 | 20.5          |
| 100 - 150       | 70.0 $\pm$ 6.0  | 104.3 $\pm$ 8.6 | 34.3          | 37.7 $\pm$ 0.0 | 39.0 $\pm$ 1.3 | 1.3           |
| 150 - 200       | 60.8 $\pm$ 5.8  | 80.1 $\pm$ 10.0 | 19.3          | 37.7 $\pm$ 0.0 | 39.0 $\pm$ 1.3 | 1.3           |
| Total 0-200     | 319.1           | 408.7           | 89.6          | 189.2          | 222.8          | 33.6          |

Rainfall recorded from October 2008 till January 4<sup>th</sup> 2009 was equal to 342.0 mm. Unlike in the previous case, such amount of rainfall was able to wet the soil of the CS at the 0-100 cm depth. At the end of the "recharge period" (March 2009) the soil moisture in the CS was greater than that recorded in 2007 showing a total moisture increase equal to 127.9 mm (Table 2). In the CS a water infiltration was also observed along the soil profile till the deepest layers. On the other hand, the soil of SS reached an optimum water level in all the soil depths already at the beginning of 2009. As a matter of fact, the water increase recorded at the end of March 2009 was of only 7.3 mm (Table 2).

**Table 2:** Soil Water Content (SWC, mean  $\pm$  standard error) measured 04-01-2009 (t0) and 31-03-2009 (t1) at different soil depths in the SS and CS. Rainfall and ETo of the considered period: 260.2 and 139.9 mm, respectively.

| Soil layer (cm) | SS              |                 |               | CS             |                 |               |
|-----------------|-----------------|-----------------|---------------|----------------|-----------------|---------------|
|                 | SWC (t0)        | SWC (t1)        | SWC (t1 - t0) | SWC (t0)       | SWC (t1)        | SWC (t1 - t0) |
|                 | mm              |                 |               | Mm             |                 |               |
| 0 - 50          | 109.7 $\pm$ 1.0 | 110.9 $\pm$ 1.4 | 1.2           | 94.8 $\pm$ 4.2 | 102.1 $\pm$ 2.8 | 7.3           |
| 50 - 100        | 110.0 $\pm$ 1.6 | 110.0 $\pm$ 1.2 | -0.1          | 66.5 $\pm$ 7.0 | 91.2 $\pm$ 2.8  | 24.7          |
| 100 - 150       | 106.0 $\pm$ 0.4 | 111.1 $\pm$ 2.2 | 5.1           | 37.7 $\pm$ 0.0 | 90.3 $\pm$ 1.2  | 52.6          |
| 150 - 200       | 109.0 $\pm$ 4.3 | 110.1 $\pm$ 1.5 | 1.0           | 37.7 $\pm$ 0.0 | 80.9 $\pm$ 11.8 | 43.2          |
| Total 0-200     | 434.7           | 442.0           | 7.3           | 236.6          | 364.5           | 127.9         |

#### 4. Conclusions

Under these experimental conditions, the soil management systems and the annual pluviometric pattern strongly influenced the SWC.

From the obtained data, it is evident that the SS had a higher capacity to store autumn-winter rainfall water than the CS even when the precipitation fallen in this period was scarce. The best structural features found in the SS soil improved the water infiltration so facilitating and speeding the recharge also of the deeper layers which, mostly, supply water to the olive roots under rainfed conditions. In addition, the natural vegetation, while consuming water from the surface layers during the autumn-winter period, increased the water recharge in the soil profile.

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S1-C5

## Occurrence of *Clostridium perfringens* in olive tree cultivated soils, edible olives and olive oils

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### Abstract

Olive tree culture is of important economic significance for Mediterranean countries. The increased knowledge on the soil microflora has raised the question of their role in soils dedicated to the culture of olive tree (*Olea europaea*) and their subsequent presence in olive tree products due to the particular harvesting practice. Among the anaerobes, our interest is focused on *C. perfringens* which is widely distributed in nature and is present in large numbers in the soil, sewage, and in the human and animal intestinal tract and could be served as an indicator of olive products hygienic quality. Thus, 100 samples from olive tree cultivated soils, 50 samples of edible olives and 50 samples of olive oil were collected and examined for the presence *C. perfringens* vegetative and spore forms as well as for other bacterial species. Additionally, all isolated *C. perfringens* strains were tested for their antimicrobial activities against eight antibacterial agents. In soils, *C. perfringens* spore forms were found more frequently (65%), comparing to *C. perfringens* vegetative forms (25%). *C. perfringens* was recovered from 20% of the edible olives samples and from 3% of oil samples, indicating that a systematic monitoring required in order to obtain adequate information for assessing overall quality from public health point of view.

**Key words:** *C. perfringens*, olive harvesting practice, soil microflora, table olive.

## Fréquence de *Clostridium perfringens* au sol de culture d'olivier, aux olives comestibles et à l'huile d'olive

### Résumé

La culture de l'arbre d'olivier est d'une importance économique significative pour les pays de la Méditerranéenne. Le rôle des microorganismes au niveau du sol de culture de l'arbre d'olivier (*Olea europaea*) et leur présence aux produits recueillis semble être en connexion avec la technique de récolte. Notre intérêt a été porté sur la bactérie anaérobie *C. perfringens* qui est un germe tellurique, isolé aussi bien du sol et des égouts, ainsi qu'à partir de la flore intestinale humaine et animale. Ce microorganisme pourrait servir comme un indicateur excellent de la qualité hygiénique des produits oliviers. Dans ce but, 100 échantillons du sol de culture d'olivier, 50 échantillons d'olives comestibles et 50 échantillons d'huile d'olive ont été recueillis et étudiés pour la présence des formes végétales et sporulées de *C. perfringens*, ainsi que la présence d'autres espèces bactériennes. De plus, nous avons recherché l'activité antimicrobienne des souches *C. perfringens* sur 8 agents antibactériens. La fréquence de formes sporulées de *C. perfringens* aux sols de culture d'olivier semble être supérieure (65%) par rapport aux formes végétales isolées (25%). *C. perfringens* a été isolé dans 20% des olives comestibles et seulement dans 3% des échantillons d'huile d'olive. Il est évident que la technique de récolte ainsi que le suivi systématique de la qualité hygiénique jouent un rôle crucial pour l'arbre d'olivier et ses produits.

**Keywords:** *Olea europea*, soil, *Clostridium perfringens*, olives, olive oil.

### 1. Introduction

Olive tree (*Olea europaea*) cultures are of important economic significance for Mediterranean countries, as both agricultural and industrial activities are promoted. In Greece, olive tree plantation keeps a capital role as extended areas of the country are reserved to this culture, which is used both for edible olive and table oil production. Moreover, edible olive as well as table oil consists of the basic exportation product of the country in all over the world.

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Albeit its importance in exportation item, olive and oil production, remains traditional in most areas. Olives are harvested by beating the tree braches with wooden poles to drop them to the ground from where they are collected. Storage of olives before any other processing is the usual practice in countryside. If during storage, water activity and temperature are modified, mould spores could grow in olives were be able to produce toxinogenic substances, as aflatoxins or ochratoxins (Bezirtzoglou E, 2002; Roussos S, 2006). Presence of toxinogenic substances not only decreases the oil olive quality, but in elevated concentrations could threatened the consumers health.

Another important tool for production of quality olive products, is the soil nature where olive trees are cultured. Soil microflora represents a high diversity and seems to be influenced by the use of pesticides, fertilizers and other techniques applied for the culture as the use of treated wastewater (Bezirtzoglou E, 2006). The recent increased knowledge on the anaerobic soil microflora has raised the question of their role in soils dedicated to the culture of olive tree and their subsequent present in olive products.

Among the anaerobes species our interest is focused on *Clostridium perfringens*, which is widely distributed in nature and is naturally present in large numbers in the soil (Matches JR, 1974; Bezirtzoglou E, 2006) sewage and in the human and animal intestinal tract (Bezirtzoglou E, 1996; 1997), where it occurs in both the vegetative and resistant spore forms.

## 2. Material and Methods.

A 100 samples of soil were collected, coming from the North-West part of Greece, including the geographical areas of Arta (n:25), Preveza (n:25), Ioannina (n:25) and Thesprotia (n:25) (Figure 1). Our samples consist of cultivated soil dedicated entirely to the cultivation of the *Olea europaea*.

All 100 samples from cultivated soil were collected aseptically, in a plastic bag during the harvesting season. Triplicate samples of 500g were collected by the aid of a sterilized spoon instrument at a depth of 10 to 30 cm below the soil surface, in a way that the sample soil include also the cultivated bulb. The soil pH is reported between 4.5-6.5. Subsequently 50 g of thoroughly mixed soil specimen was brought aseptically in a flask. In this same flask, the surface of the cultivated product was scraped from soil and washed out by 450 ml of PBS. Samples were mixed well by vigorous manual shaking before performing any analysis. Samples for physicochemical analysis were placed in 500 ml bottles. At the laboratory, all samples were stored at  $5 \pm 2^{\circ}\text{C}$  until analyses were completed. All analyses were completed within 24 h of sample collection. All samples were alternatively passed through two sterile membrane filters. The first



**Figure 1:** Geographical Departments of SW Greece included in our study.

(20  $\mu\text{m}$  pore size) was used for retention of the soil impurities and the second (0.45  $\mu\text{m}$ ) for *C. perfringens*. The growth medium used was lactose-sulfite (L.S. broth) (Bezirtzoglou and Romond, 1990).

Initially, the membranes were placed into the first tube of a ten-fold dilution series and further diluted to  $10^4$ , and incubated aerobically in a water bath at  $46^{\circ}\text{C}$  for 24 h (Figure 2). A portion of each sample was heated for 15 min at  $80^{\circ}\text{C}$  and seeded into lactose – sulfite broth to detect spores. Interpretation of results was based on the clouding of the medium from lactose fermentation, presence of a black precipitate (iron sulphate) and gas visible in the inverted Durham tubes within 24 h at  $46^{\circ}\text{C}$ .

All isolated *C. perfringens* strains were tested for their antimicrobial activities in Mueller –Hinton agar by applying the following antibiotic discs: amoxicillin (25 $\mu\text{g}$ ), penicillin G (10 units), kanamycin (30  $\mu\text{g}$ ), tetracycline (30 $\mu\text{g}$ ), streptomycin (10 $\mu\text{g}$ ), erythromycin (15  $\mu\text{g}$ ), chloramphenicol (30  $\mu\text{g}$ ) and metronidazole (15 $\mu\text{g}$ ).

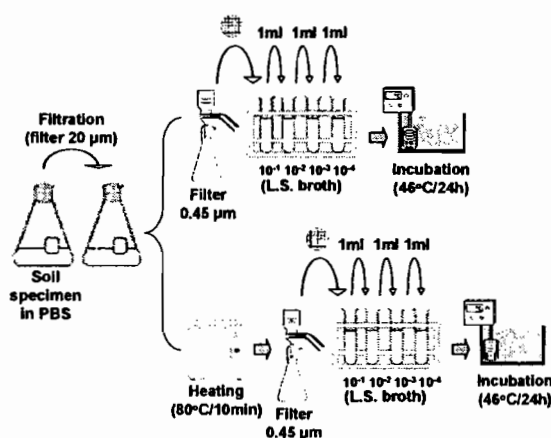


**Figure 2:** Schematical representation of experimental protocol.

Olive samples (25 g) were collected aseptically in sterilized stomacher plastic bags and transferred to laboratory. After the addition of 225 ml sterile Ringer's solution the samples were homogenized for 5 min by the aid of a stomacher. The rest of the procedure is similar to the one already described for soil.

Olive oil samples were collected from the oil mills in sterile falcon tubes and transferred to the laboratory, diluted (1:5) with warm (35°C) Ringer's solution and homogenized for 5 min before heating and seeding in L-S broth as above.

### 3. Results

In soil samples, a 65% mean presence of *C. perfringens* spore forms was observed, comparing to the significantly lower presence (25%) of vegetative forms. In Table 1, similar positivity pattern was observed for the area of Arta (72%) and Preveza (68%) in contrast to the those of Thesprotia (60%) and Ioannina (60%) where the positivity percentage of *C. perfringens* were lower. In Table 2 we report the percentage of resistance for *C. perfringens* against the eight antibiotics.

Ten olive samples (20%) and one olive oil sample (2%) were positive for the presence of *C. perfringens* spore forms while no vegetative forms were detected.

**Table 1:** Occurrence of *C. perfringens* in all sampling areas.

| Area           | Number of Samples (n) | Vegetative forms | Spores | Total |
|----------------|-----------------------|------------------|--------|-------|
| Arta           | 25                    | 24%              | 60%    | 72%   |
| Preveza        | 25                    | 16%              | 56%    | 68%   |
| Ioannina       | 25                    | 16%              | 52%    | 60%   |
| Thesprotia     | 25                    | 12%              | 60%    | 60%   |
| Mean frequency |                       | 17%              | 75%    | 65%   |

**Table 2:** Percentage of resistant strains of *C. perfringens*.

| Agent           | Resistant (%) |
|-----------------|---------------|
| Amoxicillin     | 80            |
| Penicillin      | 70            |
| Kanamycin       | 53            |
| Tetracyclin     | 54            |
| Streptomycin    | 25            |
| Erythromycin    | 29            |
| Metronidazole   | 65            |
| Chloramphenicol | 54            |

#### 4. Discussion

The extraction and use of olive oil in the Mediterranean area exists for more than 6000 years and it is linked tightly to the Mediterranean culture, history and habits (Aragon JM and Palancar MC, 2000; Niaounakis M and Halvadakis CP, 2004). Greece is sharing a major position as one of the largest olive producers in Mediterranean area together with Spain, Italy, Turkey, France and Tunisia. The Mediterranean area alone provides 98% of the total surface area for olive tree culture and total productive trees, as well as 97% of the total olive production (Niaounakis M and Halvadakis CP, 2004). Constructively, olive products consist of an important economic sector for the European Community and there is a need for extensive knowledge on the olive tree cultivation, with the microbiological aspects included (Boskou D, 1996).

The soil surface consists of an aerobic environment, which comes in disagreement with *C. perfringens* anaerobic nature. However, *C. perfringens* was found in noticeable levels in this study (Mean frequency: 65%). Cultivated soils are often associated with animal pasture, manure fertilization and also irrigation with treated wastewater. Thus, these are strong indications of faecal contamination and subsequently occurrence of *C. perfringens* since this organism is a natural intestinal inhabitant (Simon MTP, 1985). Other investigators have shown that this organism can also exist and multiply in the soil as nearly vegetative forms without the necessity of faecal contamination. Moreover, *C. perfringens* possess a specialized enzymatic equipment (Jean, 2004) which may explain how the germ can survive occasionally oxidative stress (Pan et al., 2001; Imlay, 2002). Our results showed that *C. perfringens* vegetative cell recoveries, were lower than spore densities in all cultivated areas, and this was attributed to the strict anaerobic nature of the germ. Furthermore, the use of the L.S. broth for *C. perfringens* identification offers considerable advantages as it can detect small numbers of this microbe in samples that also containing a large density of other bacteria, including other clostridia species (Bezirtzoglou et al., 1990). Therefore L-S consists of a rapid and simple technique to differentiate *C. perfringens* bacterial communities of special interest to public health.

All strains of *C. perfringens* were tested for their susceptibility to the following antibiotics; amoxicillin (25µg), penicillin G (10 units), kanamycin (30 µg), tetracycline (30µg), streptomycin (10 µg), erythromycin (15 µg), chloramphenicol (30 µg) and metronidazol (15 µg).

Most of our strains showed resistance (Table 2) to the above antibiotics applied usually for human and veterinary care. Amoxicillin (80%) and penicillin (70%) exhibited the highest percentages of resistance followed by metronidazol (65%), tetracycline (54%), chloramphenicol (54%), kanamycin (53%), erythromycin (29%), and finally streptomycin (25%).

Fermented foods have generally been considered less likely to be vehicles for foodborne infection or intoxication than fresh foods because of the competitive activity and metabolites of the functional microbiota (Panagou EZ, 2006). According to the standard of the IOOC (IOOC, 2004), olive products, when tested by appropriate methods of sampling must be free from pathogenic and spoilage microorganisms as well as toxins that may impose a risk to human health. As our results showed, a minimum percentage of end-product (i.e. edible olives and olive oil) were found positive to spores of *C. perfringens* indicating that under improper handling, storage and distribution there is a risk for consumers.

Organic pollution of the cultivated soil is mainly associated with pesticides, herbicides and fertilizers used in the agriculture. It is then conceivable that the presence of *C. perfringens* as bacterial indicator depend not only on physicochemical conditions, but it is mainly associated with the nature of the organic pollution. Systematic monitoring of the cultivated soil ecosystems must include bacteriological parameters together with chemical indices of organic pollution in order to obtain information adequate for assessing their overall quality. Furthermore, as previous investigators suggested (Panagou EZ, 2006), proper harvesting techniques, standardization and packing in hermetically sealed containers, followed by thermal treatment must be favoured over marketing the end-product in bulk, in order to ensure the food safety and the public health.

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## Effet de la solarisation sur la croissance et la longueur de la période juvénile dans des plantes de semis d'olivier

El Riachy Milad<sup>(1)</sup>; Rallo Romero, Luis<sup>(1)</sup>; De La Rosa Navarro, Raúl<sup>(2)</sup> and León Moreno, Lorenzo<sup>(2)</sup>

### Résumé

L'effet de la solarisation sur la croissance et la durée de la période juvénile (PJ) de plantes de semis d'olivier a été étudié. Des plantes obtenues par pollinisation libre de 'Manzanilla de Sevilla' et par croisements entre 'Arbequina' x 'Arbosana' et 'Picual' x 'Koroneiki' ont été expérimentées. La solarisation consistait en couvrir le sol par un film plastique noir selon un dispositif Split-plot. La croissance des plantes, la température et l'humidité du sol ont été mesurées. Les résultats indiquent que la solarisation augmenta la croissance. En plus, la solarisation augmenta la température du sol, conserva l'eau d'irrigation et élimina les mauvaises herbes. Les observations préliminaires indiquent un possible raccourcissement de la durée de la PJ par effet de la solarisation.

**Mots clés:** Amélioration génétique, solarisation, période juvénile, plantes de semis, croissance.

## Effect of soil solarization on growth and length of the juvenile period in olive seedlings

### Abstract

The effect of soil solarization on growth and on the length of the juvenile period (JP) of olive seedlings has been studied. Seedlings obtained from free pollination of 'Manzanilla de Sevilla' and from crosses between 'Arbequina' x 'Arbosana' and 'Picual' x 'Koroneiki' were tested. Solarization treatment consisted on covering the soil of the trees by a black plastic film in a Split-plot design. Growth, soil temperature and soil humidity were measured. The results indicated that solarization increased clearly the growth of the seedlings. Moreover, solarization resulted in higher soil temperature, conservation of irrigation water; elimination of weeds and smaller number of died seedlings in solarized soils with respect to not solarized. Preliminary data showed that solarization may shorten the JP.

**Keywords:** breeding, solarization, juvenile period, olive seedlings, growth.

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## Photosynthetic performances, osmolytes accumulation and antioxydative activities of three olive-tree varieties subjected to drought

Bechir Ben Rouina, Chedlia Ben Ahmed, Saïd Jilani & Anouar Jribi

### Abstract

The recognized tolerance of olive tree to drought and its capacity to grow in shallow, poor quality soils makes the species among the most interesting for cultivation in arid and semi-arid areas. This agronomic interest is enhanced by the fact that, despite the severe rain-fed conditions, olive tree shows a remarkable response to any improvement in the cropping conditions. The effects of water stress on tissue water content, vegetative growth and biomass production, gas exchange, osmolyte accumulation and the antioxydative activities were investigated on fully developed plants of Chemlali Sfax cultivar submitted to severe drought. Low water availability (LW) affected growth and biomass accumulation. Under LW conditions, total leaf area was sharply reduced (26%) due to a combination of leaf growth reduction and shedding of older leaves, minimizing water losses by transpiration. Water stress also caused a marked decline on photosynthetic capacity, and stomatal conductance was the major factor affecting photosynthesis. Drought affected negatively the net photosynthetic rate ( $5.3 \mu\text{mol}/\text{m}^2.\text{s}$  for LW v.  $10.3 \mu\text{mol}/\text{m}^2.\text{s}$  for WW) and stomatal conductance ( $69.3 \text{ mmol}/\text{m}^2.\text{s}$  v.  $98.5 \text{ mmol}/\text{m}^2.\text{s}$ ). Leaves grown under LW conditions showed decreases in starch, chlorophyll and carotenoid concentrations and plants developed some defense mechanisms against oxidative stress, like the increase in phenolic compounds, proline and soluble sugar concentrations which can improve its antioxidant responses. Furthermore, the activity of catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX) increased as leaf water potential decreased.

**Keywords:** *Olea europaea* L., drought, Pn, gs, proline, antioxidant enzymes.

## Performances photosynthétiques, accumulation des osmolytes et activités antioxydatives chez trois variétés d'olivier soumis au stress hydrique

### Résumé

L'assimilation nette du  $\text{CO}_2$ , l'accumulation de sucres et de proline, ainsi que l'activité enzymatique anti-oxydative ont été étudiées sur l'olivier, variétés Chemlali Sfax, planté au plein champs dans la région du Chaâl, soumis à un stress hydrique sévère (LW) en comparaison à des oliviers bien irrigués (WW). Chez le traitement stressé, l'accroissement des pousses végétatives et l'activité photosynthétique sont statistiquement réduits par rapport au témoin bien alimenté en eau. Les résultats obtenus montrent que la variété Chemlali Sfax présente une meilleure résistance au stress hydrique, grâce à une régulation stomatique édifante permettant un meilleur statut hydrique de la plante et une utilisation efficiente de l'eau d'une part et à une activité enzymatique anti-oxydative supérieure (SOD, APX, CAT, PPO). L'accumulation de la proline, de l'amidon et des sucres solubles se trouve majorée ; alors que les concentrations tissulaires en chlorophylles et en caroténoïdes sont réduites. Dans des conditions de précarité des ressources hydriques (LW), la surface totale des feuilles a été réduite de 26% par rapport aux arbres bien alimentés en eau (WW) diminuant au minimum les pertes d'eau par transpiration. La sécheresse a affecté négativement la photosynthèse nette ( $5.3 \mu\text{mol}/\text{m}^2.\text{s}$  pour LW vs.  $10.3 \mu\text{mol}/\text{m}^2.\text{s}$  pour WW) et la conductance stomatique ( $69.3 \text{ mmol}/\text{m}^2.\text{s}$  vs.  $98.5 \text{ mmol}/\text{m}^2.\text{s}$ ). En outre, chez les arbres stressés, les feuilles sont moins riches en amidon, en chlorophylles (a+b) et en caroténoïdes.

**Mots clés:** *Olea europaea* L., sécheresse, Pn, gs, proline, antioxydants.

## 1. Introduction

Olive (*Olea europaea* L.) is an evergreen tree traditionally cultivated in the Mediterranean basin where long periods of soil water deficit are usually present during the dry seasons, for oil and table fruit consumption (Ben Rouina et al., 2007). 95% of world olive orchards area is located in 10 countries of the Mediterranean basin (COI, 1998; Anon, 2001). During the summer months, olive, like other Mediterranean xerophytes, is usually subjected to high solar irradiances, high air temperatures, high vapor pressure deficit and limited water availability in the soil. The reduction of moisture availability anticipated in the climate change scenarios would inevitably add to the problem of water scarcity throughout the Mediterranean region. A survey of the scientific literature revealed that olive tree has important attributes that enable survival and production in drought-prone environments (Ben Rouina et al., 2002a; 2002b; 2007; Ben Ahmed et al, 2009a; 2009b).

In Tunisia, olive tree is the most extended crop not only for its socioeconomic role, nor for the health benefits of olive oil, but also for its great importance in the preservation of green area landscape in semi-arid and arid areas, its limitation of desertification and the prevention of soil erosion and land degradation, and so its involvement in the maintenance of ecosystem durability. Moreover, this species is characterized by its tolerance to contrasting environmental conditions distinguishing the arid climate of Mediterranean type of Tunisia. Olive crop occupies 35% of cultivated lands in Tunisia with a total of 70 millions trees distributed all over the area from the North to the South (1.685 million hectares). Depending mostly on the climatic conditions, the biannual alternate bearing phenomenon characterizing this species leads to the irregularity of production (Prista and Voyiatzis, 2006; Ben Rouina et al., 2007; Ben Ahmed et al, 2009a; 2009b). The intensity of this phenomenon is reinforced under the contrasting environmental conditions of arid climate in Tunisia (Ben Rouina et al., 2007; Ben Ahmed et al, 2009a; 2009b) and that affect the biological cycle of olive tree.

The aim of this study was to identify the drought resistance mechanisms of olive tree and the limitations and damages imposed by water shortage. It was also our goal to investigate how irrigation assists the olive tree to withstand Mediterranean field conditions.

## 2. Material and methods

The experiment was located in two commercial olive orchards in Chaâl, Tunisia (34°30'54 N, 10°20'17 E; altitude 118 ft) between 1999 and 2009. This area is characterized by an arid Mediterranean climate with an annual average rainfall of 184.4 mm (i.e. long term average for the period 1924-2008), mean  $ET_0$  (using the potential evapo transpiration equation of FAO Penman-Monteith) of 1525 mm/year and mean temperatures of 11.1°C in January and 27.8 °C in August. The two adjacent olive orchards 'cv Chemlali Sfax' were managed using the same pruning, fertilizers, weeds control and pest management practices of regional growers. In the first experiment, we investigated the morpho-anatomical, physiological and biochemical adaptations of field-grown olive Chemlali Sfax cultivar with different water availability regimes: drought (LW) and well watered (WW) treatments and we presents data on the leaf-level morphological and structural adaptations to reduce water loss. Leaf measurements included leaf tissue thickness, stomatal density, leaf area, leaf mass per unit area, relative water content, water content at saturation and cuticle transpiration rate. In the second experiment, the effect of watering regime in the vegetative growth, gas exchange and water use efficiency of biomass production were studied. Finally, plant water relations, total soluble sugars, starch, and proline concentrations were investigated.

The water management treatments were:

(i) olive trees in rain-fed orchard under severe drought conditions (LW), were grown on a layer of sand of more than 3 m in depth (sand 92.3%, clay 5.5% and loam 2.2%). The orchard covered an area of 41.3 ha and contained 702 18-year-old olive trees.

The LW orchard received during 2002, 184.4 mm of annual rainfall (Table 1) following two years of abnormal low rainfall (127 mm in 2000 and 87.7 mm in 2001). In order to characterize the effects of water deficit on the plants physiological responses the results recorded in LW orchard were compared to those recorded in a well irrigated one (WW) which received cumulative seasonal water of 415 mm corresponding to 40% of  $ET_c$ .

(ii) Olive trees in orchard 2, called WW, were well watered. Olive trees were grown on a layer of sand of more than 3 m in depth (sand 89.1%, clay 6.3% and silt 4.6%). This orchard contained 625, 18-year-old olive trees. The WW orchard was drip irrigated (i.e. two lateral drippers per row of olives) at a continuous rate of 2.4 L/h. plant at a distance of 1 m from the trunk. The average of climatic data recorded in 2002 and the irrigation period was 1 February to 30 October, resulting in a total of 35 irrigations was consigned in Tab. 1.

**Table 1:** Monthly  $ET_0$  (mm), rainfall (mm), maximum and minimum temperatures ( $^{\circ}C$ ) for Chaâl site and irrigation volume (mm) provided in the irrigated plantation.

| Month      | Jan  | Feb  | Mar  | Apr  | May  | Jun  | July | Aug  | Sep  | Oct  | Nov  | Dec  | Total |
|------------|------|------|------|------|------|------|------|------|------|------|------|------|-------|
| $T_x$      | 18.1 | 18.6 | 21.8 | 23.9 | 28.7 | 32.6 | 34.4 | 35.2 | 28.2 | 23.7 | 19.6 | 18.2 | -     |
| $T_n$      | 3.5  | 6.0  | 6.8  | 9.2  | 14.3 | 16.8 | 24.3 | 20.3 | 18.3 | 14.7 | 8.4  | 5.4  | -     |
| $ET_0$     | 43   | 59   | 102  | 135  | 177  | 192  | 229  | 214  | 156  | 109  | 66   | 43   | 1525  |
| Rainfall   | 3.1  | 6.5  | 10.2 | 13.4 | 27.2 | 0    | 0.7  | 0    | 11.6 | 18   | 45.2 | 48.2 | 184.4 |
| Irrigation | 0    | 11   | 16   | 44   | 61   | 72   | 81   | 73   | 34   | 23   | 0    | 0    | 415   |

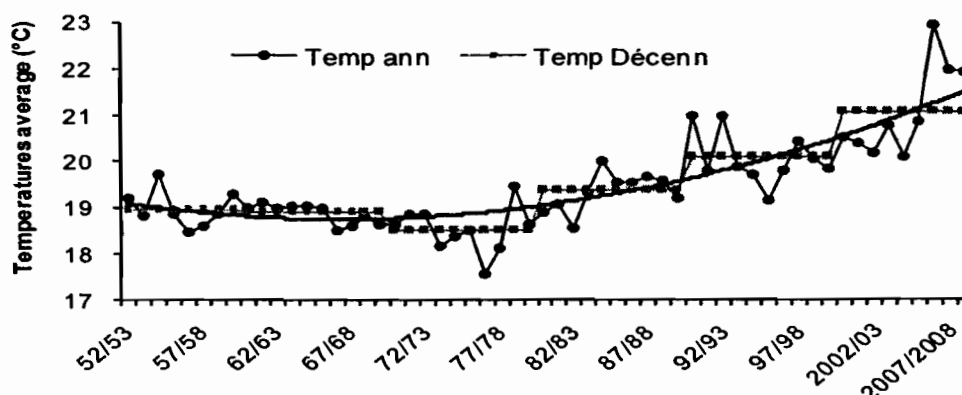
$ET_0$  = evapo transpiration Penman-Monteith;  $T_x$  = maximal temperature and  $T_n$  = minimal temperature.

### 3. Results and discussion

#### 3.1. Environmental effects on olive tree growth

The olive tree development depends on the interaction of major ambient environmental conditions (rainfall pattern, air temperature, solar radiations intensity, atmospheric humidity, air  $CO_2$  concentrations, etc...). Several researches have studied the relationship between olive tree activity and environmental conditions; they have showed that drought and heat are the most important environmental stress factors that disrupt the plant activity (Giorio et al., 1999; Moriana et al., 2002; d'Andria et al., 2004; Tognetti et al., 2004; Ben Ahmed et al., 2009a; 2009b).

Water availability is the principal cause conditioning crops growth. It interacts directly in the plant activity via photosynthesis and transpiration process and affects its growth and fruit set up (Ben Ahmed et al., 2009a; 2009b; Giorio et al., 1999; Moriana et al., 2002) stress factors that disrupt the plant activity (Giorio et al., 1999; Tognetti et al., 2004; Ben Ahmed et al., 2010). In arid area, climate change affects average temperatures and temperature extremes (fig. 1a), timing and spatial patterns of precipitation (Fig. 1b), soil moisture, runoff, and the frequency of disturbances, such as drought. Between 1981 and 2009 global warming is twice more important in arid area of Tunisia than the world average ( $1.5 - 2^{\circ}C$  against  $0.75^{\circ}C$ ). This climate warming is accompanied by a regression between 10 and 20 % of average annual precipitation.



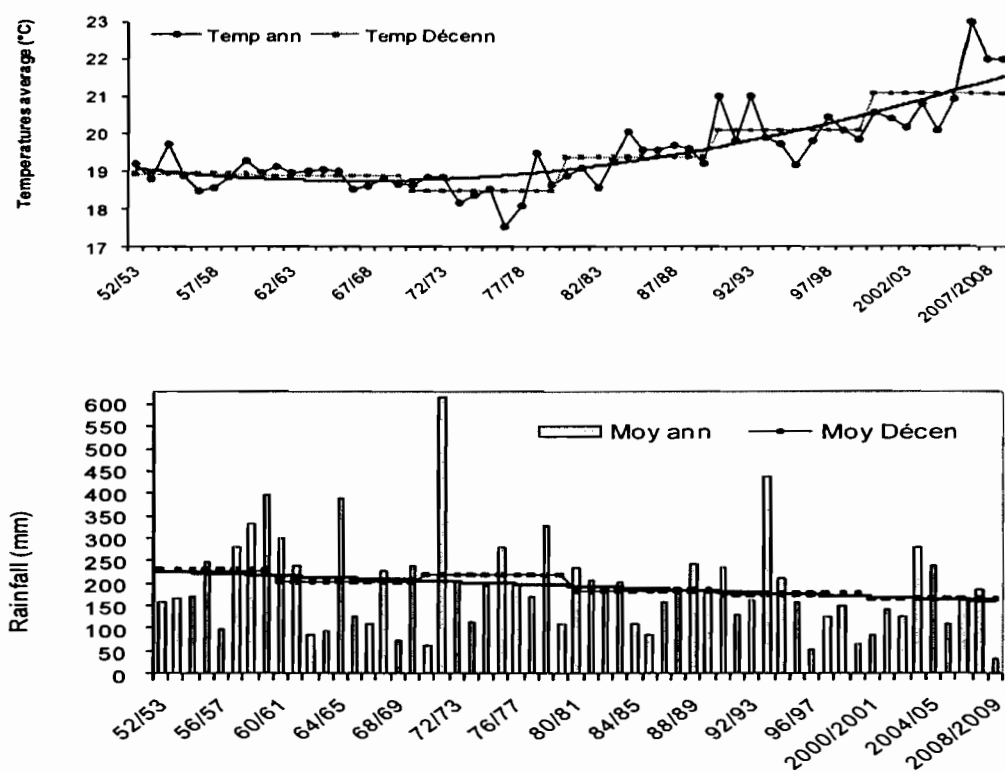


**Figure 1:** Yearly average of temperature (a) and rainfall (b) at the Chaâl farm between November 1952 and August 2009.

Low water availability (LW) affected growth and biomass accumulation of Chemlali Sfax (Tab. 2). Under drought stress, total leaf area was sharply reduced due to a combination of leaf growth reduction and shedding of older leaves, minimizing water losses by transpiration. Water stress and high temperatures also caused a marked decline on photosynthetic capacity and stomatal control was the major factor affecting photosynthesis (Fig. 2).

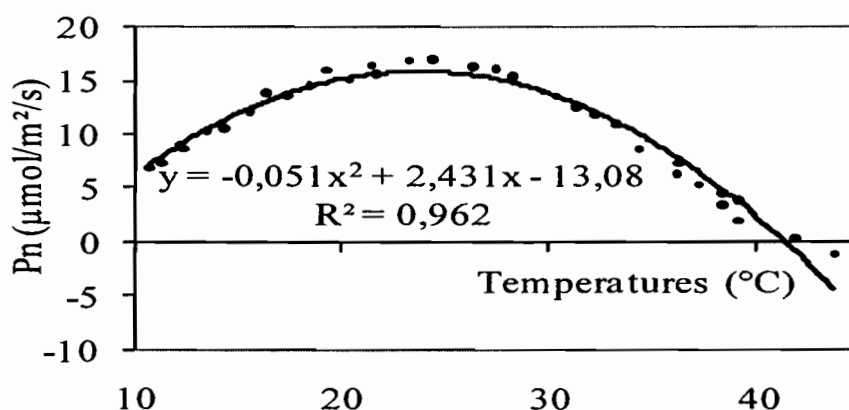


**Figure 2:** Relationship between net CO<sub>2</sub> assimilation and air temperature on Chemlali Sfax olive tree.

Under drought stress, water use efficiency of biomass production was improved in LW plants (4.5 mg of H<sub>2</sub>O cm<sup>-2</sup> h<sup>-1</sup>), whereas it decreased in irrigated trees (3.29 mg of H<sub>2</sub>O cm<sup>-2</sup> h<sup>-1</sup>). Hence, water deficit influenced significantly leaf tissue thickness, stomatal density of mature olive plants, and specific leaf area has often been observed to be reduced under drought conditions and variations in it may be due to variations in leaf thickness and/or variations in leaf density (Tab. 2).

**Table 2:** Mean values of leaf tissue thickness ( $\mu\text{m}$ ), stomatal density (stomata  $\text{mm}^{-2}$ ) and leaf characteristics of Chemlali Sfax olive trees subjected to two water regimes.

| Treatment | Leaf thickness ( $\mu\text{m}$ ) |                  |                | Stomatal density (unit $\text{mm}^{-2}$ ) | Leaf area ( $\text{mm}^2$ ) | Leaf mass area ( $\text{g m}^{-2}$ ) |
|-----------|----------------------------------|------------------|----------------|-------------------------------------------|-----------------------------|--------------------------------------|
|           | Total lamina                     | Spongy mesophyll | Trichome layer |                                           |                             |                                      |
| WW        | 426.2                            | 239.3            | 41.5           | 369                                       | 458                         | 248.3                                |
| LW        | 455.5                            | 252.7            | 43.7           | 396                                       | 401                         | 234.2                                |

### 3.2. Effects on olive tree water status and $\text{CO}_2$ assimilation

In the study zone, the aridity of the climate and the soil dryness resulted in a noticeable water deficit during a long period of the year. This last deficit was revealed by the recorded levels of RWC (Tab. 3). According to the RWC values, LW plants reached a severe level of water stress (least amount of 46% in August). Therefore, water shortage mechanisms are important for tissue desiccation avoidance, especially when they are coupled with surface reduction and high transpiration resistance of the epidermis (Ben Rouina et al., 2007; Ben Ahmed et al., 2009a,; 2009b). A special form of water conservation is the binding of water to mucilage in cells, ducts and intercellular cavities. Water reserves of this kind can protect the plant from rapid wilting and severe leaf shrinkage.

So, our results revealed that olive cultivars native to dry regions, such as Chemlali Sfax, have more capability to acclimate to drought conditions and climate. This cultivar avoids water loss with high density of foliar tissue and the presence of the thick cuticle and trichome layers. It enhanced their sclerophylly by building parenchymatous tissues and increasing protective structures like both the upper and lower epidermis (tab. 2).

**Table 3:** Net  $\text{CO}_2$  assimilation rate ( $A$ ), stomatal conductance ( $g_s$ ), transpiration rate ( $E$ ) and leaf water potential at predawn ( $\Psi_{PD}$ ) and midday ( $\Psi_{MD}$ ) of Chemlali Sfax olive tree under contrasting water availability regimes ( $n = 10$ ).

| Watering regime | $A$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) | $G_s$ ( $\text{mmol m}^{-2} \text{s}^{-1}$ ) | $E$ ( $\text{mmol m}^{-2} \text{s}^{-1}$ ) | RWC % |
|-----------------|----------------------------------------------|----------------------------------------------|--------------------------------------------|-------|
| WW              | 14.75a                                       | 138.31a                                      | 4.05a                                      | 92.5a |
| LW              | 5.31b                                        | 93.10a                                       | 3.19b                                      | 72.8b |
| % reduction     | 36.9                                         | 32.7                                         | 21.0                                       | 21.3  |

Means within a column flanked by the same letter are significantly not different at  $P < 0.05$  (LSD test).

Stomatal control of water loss was identified as an early response of Chemlali Sfax olive tree to water deficit, leading to limitation of carbon uptake by the leaves (Tab. 3). Non-stomatal factors also play an important role in limiting photosynthesis when olive trees are submitted to prolonged drought under field conditions ( $P_n = 5.31 \mu\text{mol m}^{-2} \text{s}^{-1}$  for the LW trees v.  $14.75 \mu\text{mol m}^{-2} \text{s}^{-1}$  WW trees). Among such responses, gas exchange is of particular importance in determining the efficiency of water use in response to the limited water resources.

In order to preserve photosynthesis behavior the olive tree, like some plants grown in arid environments, have evolved physiological processes to maintain to some extent tissue turgor and thus stomatal opening [6, 7]. Lowering of leaf potential due to net accumulation of compatible solutes in the cytoplasm such as proline, soluble sugars and phenols, is a well established biochemical mechanism whereby many plants adjust to low soil water availability. The close relationship observed between  $P_n$  and proline content points to an important role of this osmolyte in the maintenance of photosynthetic activity and therefore in drought tolerance.

### 3.3. Effects on osmolyte accumulation and the antioxidative activities

Leaves grown under LW conditions revealed signs of oxidative stress. One of the signs was the large reductions in leaf Chl (a+b) and Carotenoids concentration (Table 4). According to [13], the decrease of chlorophyll content is a typical symptom of oxidative stress and may be the result of chlorophyll degradation or be due to chlorophyll synthesis deficiency. Ben Rouina et al., (2007); Ben Ahmed et al., (2009a and 2009b) indicated that expanded leaves exposed to water deficit started to degrade their photosynthetic apparatus, possibly to mobilize resources for the production of new acclimated leaves.

The Chl/Car ratio was significantly lower in olive plants under LW conditions. This result can be used as an early indicator for chlorosis of LW plants and reveals an increased need for photo protection of chlorophylls by carotenoids. It is now well documented that carotenoids are involved in the protection of the photosynthetic apparatus against photo inhibitory damage by singlet oxygen, which is produced by the excited triplet state of chlorophyll.

**Table 4:** Effects of water regimes on photosynthetic pigments, carotenoids, soluble sugars, starch, proline and total phenols in leaves of Chemlali Sfax olive tree under contrasting water availability regimes (LW = dry weight, FW = fresh weight; n = 3).

| Watering regime | Chl a+b<br>mg g <sup>-1</sup> LW | Carotenoids<br>mg g <sup>-1</sup> LW | Soluble sugar<br>mg g <sup>-1</sup> LW | Starch<br>mg g <sup>-1</sup> LW | Proline<br>μg mg <sup>-1</sup> FW | Total phenols<br>μg g <sup>-1</sup> LW |
|-----------------|----------------------------------|--------------------------------------|----------------------------------------|---------------------------------|-----------------------------------|----------------------------------------|
| WW              | 15.87a                           | 3.47a                                | 89.86a                                 | 83.12a                          | 73.45a                            | 43.61a                                 |
| LW              | 8.65b                            | 2.08b                                | 126.65b                                | 58.76b                          | 225.41b                           | 69.96b                                 |

Means within a column flanked by the different letters are significantly different at  $P < 0.05$  (LSD test).

For all tissues tested in this experiment, SOD, APX, and CAT activities increased significantly in LW plants, compared to their respective WW ones. In leaves, this increase was 2.56, 3.36, and 2.07 times, respectively for SOD, APX, and CAT enzymes in comparison to their respective activities in WW-treated plants (Table 5).

**Table 5:** Anti oxidative enzymes activities of leaves and roots from well watered (WW) and low watered (LW) field-grown Chemlali olive plants.

| Treatments | Enzyme activity (units mg <sup>-1</sup> dw) |                         |                         |                         |                         |                         |                         |                         |
|------------|---------------------------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
|            | Leaves                                      |                         |                         |                         | Roots                   |                         |                         |                         |
|            | SOD                                         | APX                     | CAT                     | PPO                     | SOD                     | APX                     | CAT                     | PPO                     |
| WW         | 13.4 ± 1.1 <sup>b</sup>                     | 2.7 ± 0.3 <sup>b</sup>  | 6.5 ± 0.5 <sup>b</sup>  | 31.0 ± 1.3 <sup>b</sup> | 10.7 ± 2.1 <sup>b</sup> | 0.7 ± 0.05 <sup>a</sup> | 3.1 ± 0.04 <sup>a</sup> | 34.7 ± 1.2 <sup>b</sup> |
| LW         | 32.4 ± 2.1 <sup>a</sup>                     | 10.8 ± 0.8 <sup>a</sup> | 12.2 ± 0.6 <sup>a</sup> | 19.3 ± 1.3 <sup>a</sup> | 16.3 ± 2.3 <sup>a</sup> | 0.9 ± 0.06 <sup>a</sup> | 4.3 ± 0.9 <sup>a</sup>  | 14.4 ± 1.0 <sup>a</sup> |

Values represent the means of three samples ± standard deviations. <sup>a</sup> significant differences at the 5% level between enzymes activities levels obtained under well watered and low watered treatments ( $P \leq 0.05$ , according to Duncan's multiple range test).

#### 4. Conclusion

The recognized tolerance of olive tree to drought and its capacity to grow in shallow, poor quality soils makes the species among the most interesting for cultivation in arid and semi-arid areas. This agronomic interest is enhanced by the fact that, despite the severe rain-fed conditions, olive tree shows a remarkable response throughout the region where during the summer and drought event, plants were usually subjected to high solar irradiances, high air temperatures, high air vapor pressure deficits and limited water availability.

In water stressed olive trees differences were observed in leaf water status, photosynthetic performance, and pigments content, proline and phenols accumulation. Differences in photosynthesis could be related to differences in soil water apparatus. This tendency suggests an interaction between proline and the antioxidative defense system.

The leaves of Chemlali Sfax olive trees also had a higher succulence index than the other cultivars, which affords protection against sudden wilting and severe shrinkage. It is well adapted to drought conditions as a result of the high density of the foliar tissue and the presence of thick cuticle and trichome layers. However, the development of small leaves may reduce water loss at the whole-plant level. We identified several mechanisms at the morpho-structural level by which Chemlali olive tree cope with drought stress.

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## Développement de jeunes plants d'oliviers (*Olea europaea L.*) et apparition de la première floraison

M. Aïachi Mezghani <sup>1\*</sup>, M. Mars <sup>2</sup> et A. Jebari <sup>3</sup>

### Résumé

Le développement caulinaire de jeunes plants issus de semis d'oliviers est suivi afin de proposer des critères morphologiques capables de prédire la sortie de juvénilité et l'entrée prochaine en floraison. Le nombre de fleurs apparues à l'échelle de l'arbre est fortement corrélé avec le nombre de fleurs apparues à l'ordre de ramification 4 et 5. Le coefficient de corrélation est respectivement égal à 0,90 et 0,79. Par ailleurs, il est apparu que le nombre de fleurs total est fortement lié avec la hauteur de l'arbre, avec la vigueur observée et avec l'état de ramification atteint (ordre de ramification, nombre de ramifications...). Une analyse en composantes principales portant sur 14 critères morphologiques mesurés sur la totalité de la descendance montrent que les plants ayant développé un nombre de ramifications d'ordre 4 important ainsi qu'un nombre élevé de métamères édifiés par ces derniers et montrant un axe principal important, présentent une forte floraison. Ces paramètres constituent des critères morphologiques intéressants pour prédire l'entrée en floraison chez ces hybrides et plus généralement chez l'olivier.

**Mots clés:** Olivier, juvénilité, passage à la floraison, critères morphologiques, analyse en composantes principales.

## Development of young olive trees (*Olea europaea L.*) and occurrence of the first flowering

### Abstract

The patterns of vegetative growth were followed on seedling olive trees. The objective of this research was to propose a set of morphological variables as predictors of tree capability to enter into reproductive phase. The number of flowers was strongly correlated with the number of flowers appeared in fourth and fifth botanical order. The correlation coefficient was respectively equal to 0.90 and 0.79. It appeared that the number of flowers was strongly dependent with the height of the tree, with tree vigour and finally the ramification order reached and the number of branches. A set of 14 variables was proposed and relations between variables were studied by component analysis. The plant which developed an important number of branches on the fourth order, high metameres number on the same order and a long axis, presented an intense flowering. These morphological variables constituted interesting morphological criteria to predict the flowering occurrence at these seedlings and more generally on the olive tree.

**Keys words:** Olive tree, juvenility, flowering occurrence, morphological criteria, component analysis.

### 1. Introduction

La phase juvénile est un stade propre de la plupart des systèmes biologiques et notamment de toutes les formes de vie supérieure. Elle pourrait être définie généralement comme une phase au cours de laquelle l'organisme est dépourvu de la capacité de se reproduire (Wareing, 1959; Zimmerman, 1972; Borchert, 1976a). La durée de la période juvénile est un des problèmes rencontrés lors de l'amélioration génétique de la plupart des espèces connues comme c'est le cas du pommier et du poirier (Visser, 1970; Zimmerman, 1977; Costes et al., 2004) et de l'olivier (Lavée et al., 1996; Garcia et al., 2000).

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La juvénilité est définie comme un ensemble de caractéristiques physiologiques et morphologiques manifestées par les semis durant la phase étalée depuis la germination jusqu'à la production des premières fleurs (Hackett, 1985; Monteuis, 1988). Lors de la transition de la phase juvénile à la phase adulte, l'apparition de la floraison est davantage liée à la formation d'un nombre minimum de nœuds, à l'acquisition d'une taille minimale (Wareing, 1959; Kozlowski, 1971; Borchert, 1976 a et b; Wesley, 1976) ou à l'acquisition par la plante d'un degré de différenciation minimum qu'en nombre de jours, de mois ou d'années (Barthélémy, 1988, 1991). La vigueur semble avoir une relation sur l'entrée en floraison des jeunes plants (Visser, 1970; Santos- Antunes et al., 2005). Le passage à la floraison semble s'inscrire aussi dans une organisation spatio-temporelle de la plante (Barthélémy, 1991, 1997; Crabbé, 1991).

Le présent travail a pour objectif (i) d'étudier les relations simples (corrélations) pouvant exister entre les différents paramètres de végétation et de la floraison (ii) de proposer une série de paramètres morphologiques capables d'évaluer la sortie de la juvénilité et l'entrée prochaine en floraison de jeunes plants issus d'oliviers à l'aide d'une analyse en composante principale portant sur des paramètres phéno-morphologiques et 81 individus (plants).

## 2. Matériel et méthodes

### 2.1. Matériel végétal

Nous avons disposé au début de ce travail d'une descendance d'hybrides de 1086 plants. Les plants sont issus de croisements réalisés sur plusieurs années de semis réalisés entre 1994, 1995 et 1996. Ces croisements sont regroupés selon l'origine des croisements : soit *Chemlali* en porte graines (parent femelle) avec différents génotypiques (pollinisateurs), ou encore des croisements réciproques de la *Chemlali* en pollinisateur (parent mâle) avec d'autres génotypes porte graines (parent femelle), des croisements de différents génotypes ou autres cultivars autres que la *Chemlali*, des autofécondations et des croisements libres issus de la *Chemlali*.

Après élevage en serre dans des conditions normales (ne permettant pas de hâter l'allongement des plants faute de moyen), les 1086 plants sont plantés en Décembre 1997 et en Avril 1998 dans un verger de comportement dans la région de Sfax située à 240 km au sud de Tunis (étage bioclimatique aride supérieur) Les arbres sont menés en forme libre sans aucune intervention de taille.

### 2.2. Paramètres mesurés et analyse statistique

Nous avons réalisé une description complète de 81 plants et nous avons repéré les 41 arbres qui sont sortis de la floraison. Les sites de floraison sont localisés au niveau de l'arbre entier à travers les différents ordres de ramification. Nous avons établi une quarantaine de variables relatives au développement aérien de l'arbre, à l'architecture élémentaire développée et la quantification de la floraison à l'échelle de l'arbre.

Les variables retenues sont les suivantes:  $V_a$ : âge de l'arbre;  $V_p$ : port de l'arbre;  $V_0$ : Hauteur première charpente;  $V_1$ : Hauteur de l'arbre;  $V_4$ : périmètre du tronc;  $V_6$ : Nombre de ramifications total;  $V_8$ : Nombre de métamères total;  $V_{15}$ : nombre de ramifications longues d'ordre 3;  $V_{17}$ : Longueur moyenne de ramifications longues d'ordre 3;  $V_{18}$ : angle que font les ramifications d'ordre 3;  $V_{19}$ : somme des métamères édifiés par l'ordre 3;  $V_{20}$ : nombre de ramifications longues d'ordre 4;  $V_{22}$ : longueur moyenne des ramifications d'ordre 4;  $V_{23}$ : angle que font les ramifications d'ordre 4;  $V_{24}$ : somme des métamères édifiés par l'ordre 4;  $V_{25}$ : nombre de ramifications longues d'ordre 5;  $V_{27}$ : longueur des ramifications d'ordre 5;  $V_{28}$ : angle que font les ramifications d'ordre 5;  $V_{29}$ : somme des métamères édifiés par l'ordre 5;  $V_{30}$ : Nombre de fleurs total;  $V_{39}$ : Nombre de métamères édifiés par le chemin le plus court jusqu'à la première fleur;  $V_{40}$ : Ordre de ramification maximal.

Des corrélations entre les variables ont été établies par le test de Pearson à l'aide du logiciel SPSS (version 10.0). Compte tenu des fortes corrélations obtenues entre les différents paramètres, nous avons réalisé une analyse en composantes principales sur les mêmes individus mais avec seulement 22 variables à l'aide du logiciel Xlstat (version 2007.6).

### 3. Résultats

#### 3.1. Localisation de la floraison selon les différents ordres de ramification

A l'apparition de la floraison sur les différents arbres, les sites sont localisés au niveau de l'arbre entier à travers les différents ordres de ramification. Le nombre de fleurs total développées à l'échelle de l'arbre, est en étroite relation avec le nombre de fleurs développées à l'ordre botanique 4 ( $R=0,90^{**}$ ), le nombre de fleurs développées à l'ordre botanique 5 ( $R=0,79^{**}$ ) et en second lieu avec le nombre de fleurs développées à l'ordre botanique 3 ( $R=0,55^{**}$ ), à l'ordre 2 (soit  $R=0,54^{**}$ ) et enfin à l'ordre 6 soit avec un coefficient de corrélation égal à 0,44.

#### 3.2. Relations entre la floraison et les critères morphologiques

##### 3.2.1. Relations linéaires simples

Selon le Tableau 1, il apparaît que le nombre de fleurs est en relation avec la hauteur de l'arbre ( $R=0,25^*$ ) et avec la vigueur de l'arbre c'est à dire le périmètre du tronc ( $R=0,30^{**}$ ). Le nombre de fleurs montre une relation hautement significative avec le nombre de ramifications longues développées ( $R=0,34^{**}$ ) et avec le nombre total d'entre-nœuds édifiés par ces derniers ( $R=0,47^{**}$ ). Le nombre de ramifications longues développées à l'ordre botanique 3, 4 et 5 ainsi que le nombre total d'entre-nœuds édifiés par ces derniers et leur direction, notent une relation très étroite avec le nombre de fleurs développées.

**Tableau 1 :** Corrélation entre le nombre de fleurs total moyen par port et les différentes variables.

| Variables                                                             | Coefficient de corrélation (R) |
|-----------------------------------------------------------------------|--------------------------------|
| Hauteur arbre                                                         | 0,25*                          |
| Périmètre du tronc                                                    | 0,30 **                        |
| Nombre de ramifications longues                                       | 0,34 **                        |
| Somme des entre-nœuds édifiés                                         | 0,47 **                        |
| Nombre d'entre-nœuds formés jusqu'à l'apparition de la première fleur | 0,44 **                        |
| Ordre de ramification où est apparu la première fleur                 | 0,44**                         |
| Nombre de ramifications longues d'ordre botanique 3                   | 0,35**                         |
| Somme des entre-nœuds édifiés par les ramifications d'ordre3          | 0,26*                          |
| Nombre de ramifications longues d'ordre botanique 4                   | 0,60 **                        |
| Angle que fait les rameaux d'ordre 4 avec le porteur                  | 0,32**                         |
| Somme des entre-nœuds édifiés par les ramifications d'ordre4          | 0,55 **                        |
| Nombre de ramifications longues d'ordre botanique 5                   | 0,31**                         |
| Longueur des ramifications d'ordres 5                                 | 0,48 **                        |
| Somme des entre-nœuds édifiés par les ramifications d'ordre 5         | 0,28 *                         |
| Angle que fait les rameaux d'ordre 5 avec le porteur                  | 0,56 **                        |

\* : Corrélation significative au seuil de 5 %. \*\* : Corrélation hautement significative au seuil de 1%.

##### 3.2.2. Analyse des paramètres morphologiques à l'aide d'une analyse en composantes principales

L'analyse en composantes principales conduit à diagonaliser la matrice de corrélations donnant les valeurs propres avec le pourcentage d'inertie expliquée pour chacune. Les quatre premières valeurs propres expliquent près de 69,5% de l'inertie totale. La première valeur explique près de 35% de l'inertie totale, la seconde 16% de l'inertie, la troisième de 11,5% et enfin la quatrième 7%. Les variables utilisées pour comparer les différents individus sont exprimées dans la nouvelle base de vecteurs propres en quatre facteurs, ce qui permet d'établir les coefficients de corrélation entre les variables et les facteurs principaux des axes (Tableau 2).

**Tableau 2:** Corrélations des variables (R) avec les quatre premiers axes.

| Variables       | Axe 1 | Axe2  | Axe3  | Axe4  |
|-----------------|-------|-------|-------|-------|
| V <sub>a</sub>  | 0,51  | -0,30 | -0,43 | 0,31  |
| V <sub>p</sub>  | -0,28 | 0,69  | 0,50  | 0,17  |
| V <sub>0</sub>  | 0,41  | -0,59 | -0,39 | -0,08 |
| V <sub>1</sub>  | 0,42  | -0,61 | -0,46 | 0,03  |
| V <sub>4</sub>  | 0,60  | -0,21 | -0,12 | 0,41  |
| V <sub>6</sub>  | 0,70  | -0,27 | 0,28  | -0,30 |
| V <sub>8</sub>  | 0,77  | 0,36  | 0,36  | -0,24 |
| V <sub>15</sub> | 0,48  | -0,40 | 0,65  | -0,25 |
| V <sub>17</sub> | 0,29  | -0,07 | 0,05  | 0,58  |
| V <sub>18</sub> | 0,12  | -0,41 | 0,17  | 0,02  |
| V <sub>19</sub> | 0,48  | -0,38 | 0,70  | -0,12 |
| V <sub>20</sub> | 0,81  | 0,27  | 0,00  | 0,01  |
| V <sub>22</sub> | 0,58  | 0,15  | 0,25  | 0,50  |
| V <sub>23</sub> | 0,53  | 0,14  | 0,37  | 0,32  |
| V <sub>24</sub> | 0,81  | 0,27  | 0,01  | 0,04  |
| V <sub>25</sub> | 0,60  | 0,62  | -0,24 | -0,25 |
| V <sub>27</sub> | 0,67  | 0,45  | -0,27 | -0,21 |
| V <sub>28</sub> | 0,67  | 0,44  | -0,21 | -0,16 |
| V <sub>29</sub> | 0,60  | 0,60  | -0,23 | -0,26 |
| V <sub>30</sub> | 0,66  | 0,03  | -0,03 | -0,15 |
| V <sub>39</sub> | 0,61  | -0,38 | -0,29 | 0,08  |
| V <sub>40</sub> | 0,70  | 0,40  | 0,12  | 0,23  |

La Figure 1a représente la projection des points variables selon le plan P<sub>1</sub> factoriel formé par le premier et le second facteur expliquant 51 % de la variabilité.

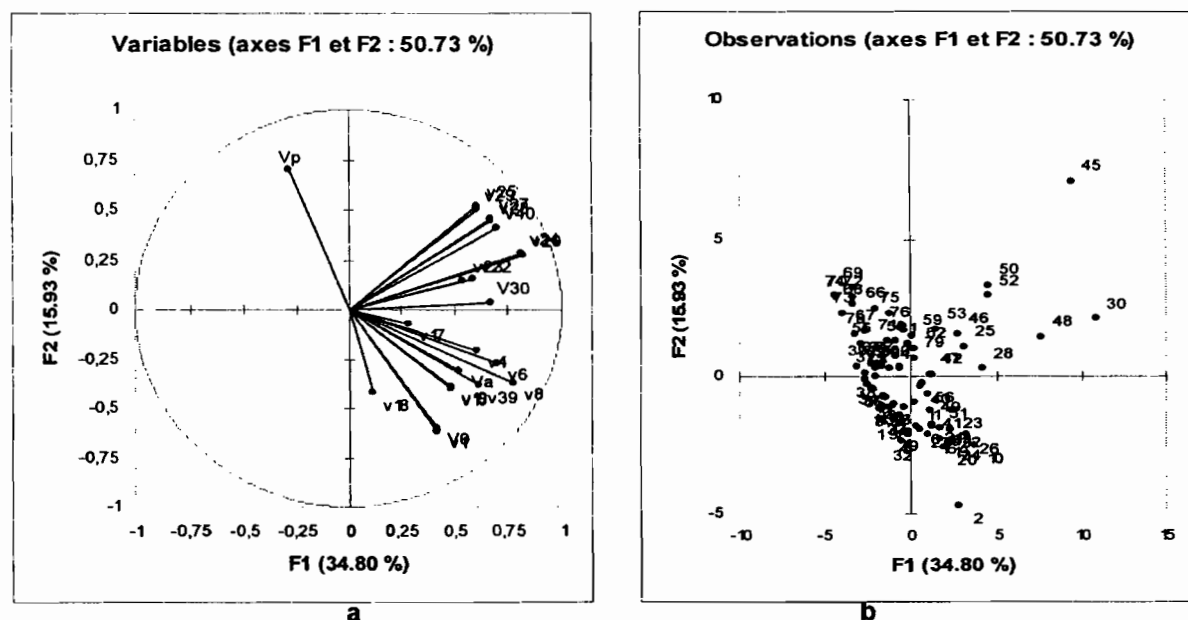


**Figure 1:** Projection des variables selon le plan factoriel P<sub>1</sub> formé par l'axe 1 et 2 (a). et représentation des individus ayant une forte contribution au plan factoriel P<sub>1</sub> (b).

Le premier facteur expliquant à lui seul 35 % de la variabilité, montre que toutes les variables évoluent dans le sens positif de ce dernier à l'exception de la variable  $V_p$  évoluant dans le sens contraire. Les variables montrant une forte contribution à l'axe1 sont: le périmètre du tronc ( $V_4$ ), le nombre de ramifications total ( $V_6$ ) et la somme des entre-nœuds édifiés par les différentes ramifications ( $V_8$ ), le nombre de ramifications à l'ordre 4 ( $V_{20}$ ), la somme des métamères édifiés par ces derniers ( $V_{24}$ ) ainsi que le nombre total de fleurs apparues à l'échelle de l'arbre ( $V_{30}$ ). Le nombre de ramifications à l'ordre 5 ainsi que les caractéristiques de croissance des ces ramifications ( $V_{25}$ ,  $V_{26}$ ,  $V_{27}$ ,  $V_{28}$  et  $V_{29}$ ), l'ordre de ramification maximum atteint ( $V_{40}$ ) et le nombre de métamères édifiés jusqu'à l'apparition de la première fleur ( $V_{39}$ ), augmentent dans le sens positif de l'axe. Cet axe caractérise la relation pouvant exister entre la floraison et l'état général de l'arbre aussi bien au niveau de la vigueur de l'arbre que la différenciation morphologique atteinte par les structures raméales de l'arbre. Il apparaît aussi que le port général ne montre pas une influence directe sur le passage à floraison des arbres.

Le second facteur (expliquant 16 % de la variabilité) oppose d'une part le port de l'arbre ( $V_p$ ) et d'autre part les variables de la hauteur de l'arbre ( $V_1$ ) et de sa première charpente ( $V_0$ ). Cet axe semble caractériser le port de l'arbre et de sa hauteur.

La représentation des individus sur le plan factoriel  $P_1$  fait ressortir des groupes distincts (figure 1b). Ainsi, les arbres se trouvant sur la droite de l'axe 1, se caractérisent par un nombre important de fleurs ( $V_{30}$ ), une ramification importante ( $V_6$ ), un nombre de ramification d'ordre 4 et 5 ( $V_{20}$ ,  $V_{25}$ ) et un nombre important de métamères édifiés par ces derniers ( $V_{24}$ ,  $V_{29}$ ) ainsi qu'une vigueur importante ( $V_4$ ). D'autre part, les individus se trouvant à gauche de l'axe 1, indiquent les arbres peu fleuris ou encore juvéniles ( $V_{30}$  faible) et dont la ramification est peu développée et l'ordre de ramification ne dépasse pas 3.

L'axe 2 sépare d'une part les plants dont la hauteur ( $V_1$ ) et la hauteur de la première charpente ( $V_0$ ) sont importants vers le bas de l'axe et d'autre part les individus dont la hauteur est peu importante vers le haut de l'axe. La variable port de l'arbre ( $V_p$ ) augmente dans le sens positif de l'axe indiquant les individus à port buissonnant ou mixte vers le haut et les arbres à port arborescent vers le bas.

### 3. Discussions & Conclusions

Le but de cette recherche est d'analyser le phénomène de l'apparition de la floraison qui se caractérise par l'omniprésence d'une double question: Quand et où une structure florale va t-elle s'initier dans le système d'axe ramifié que constitue le végétal? Des critères phéno-morphologiques sont recherchés comme indicateurs de l'entrée prochaine en floraison de jeunes oliviers.

Ainsi, le nombre de fleurs apparues à l'échelle de l'arbre, est en étroite relation avec la hauteur de l'arbre ou encore l'importance de l'axe principal de l'arbre. Ces résultats confirment les recherches antérieures réalisées par différentes équipes de recherches ayant travaillé sur les aspects morphologiques accompagnant la sortie de la juvénilité étudiée chez différentes espèces (Borchert, 1976 a et b; Koslowski, 1971; Wesley, 1976; Zimmerman, 1972, 1977). Il est aussi apparu que la floraison est aussi en relation avec le périmètre du tronc c'est à dire la vigueur de l'arbre. Ces résultats corroborent ceux de Visser (1970) sur pommier et ceux de Msallem (2002) et Santos-Autunes et al. (2005) sur olivier. Une recherche de Nesme et al. (2005), a porté sur la mise au point de paramètres morphologiques permettant d'estimer la vigueur chez le pommier qui est à son tour un excellent indicateur de la mise à fruit.

Le nombre de fleurs apparues est fortement corrélé avec celui des fleurs apparues à l'ordre 4 et 5. Au niveau de l'architecture de l'arbre et lors de la transition de la phase juvénile à la phase adulte, la formation des structures reproductrices s'inscrit dans une séquence précise et ordonnée de différenciation végétative dans une organisation spatio-temporelle donnée de la plante. La floraison est une étape obligatoire du programme morphogénétique de l'espèce. Ces résultats corroborent les observations de Barthélémy (1988, 1991) sur les espèces tropicales et de Crabbé (1991) sur les espèces ligneuses plus généralement.

Il apparaît ainsi que les arbres entrent plus rapidement dans la phase reproductive quand le maximum d'axes est maintenu sur l'arbre d'où la nécessité d'un nombre minimum d'interventions d'opérations de taille pendant le jeune âge. Costes et al. (2004) étudiant le développement de jeunes plants de poirier jusqu'au passage à la floraison, constatent que les arbres ayant développé une augmentation des ramifications sylleptiques durant les deux premières années de leur développement, c'est à dire une augmentation de la ramure, montrent une entrée en floraison plus précoce. Ce paramètre peut être utilisé dans les programmes d'amélioration pour sélectionner les génotypes les plus précoces au niveau de l'entrée en floraison (Sansavini et Musacchi, 1994).

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## Phenology and olive (*Olea europaea* L. cv Frantoio) fruit maturation monitoring in Tuscany

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### Abstract

Olive (*Olea europaea* L.) is an evergreen tree and one of the most important tree crops in Mediterranean agro-ecosystems. Maximization of olive productivity at the end of the growing season requires detailed knowledge on fruit growth and oil accumulation processes. To achieve this objective, in the last decade, regional agricultural services have adopted crop management strategies based on field monitoring networks, physical and chemical analyses, meteorological data sets, and internet-based services. In this work we describe the Decision Support System (DSS) implemented by our research group on the web site of ARSIA – Extension Service for Agriculture in Tuscany, Italy – for phenology and olive fruit maturation monitoring.

**Keywords:** DSS, internet-based-services, meteorological data, maturity index.

## Phénologie et pilotage de la maturation du fruit d'olive (*Olea europaea* L. cv Frantoio) en Toscane

### Resumé

D'olive (*Olea europaea* L.) est un arbre à feuilles persistantes et l'une des cultures d'arbres les plus importants dans les agro-écosystèmes méditerranéens. La maximisation de la productivité d'olive à la fin de la saison de croissance requiert des connaissances approfondies sur la croissance des fruits et des processus d'accumulation d'huile. Pour atteindre cet objectif, dans la dernière décennie, des services régionaux de l'agriculture ont adopté des stratégies de gestion des cultures basées sur les réseaux de surveillance sur le terrain, des analyses physiques et chimiques, météorologiques ensembles de données et services basés sur Internet. Dans ce travail, nous décrivons la Decision Support System (DSS) mis en œuvre par notre groupe de recherche sur le site web de ARSIA - Prolongation du service de l'agriculture en Toscane, Italie - pour leur phénologie et de suivi de maturation des fruits d'olive.

**Mots-clés:** DSS, internet-based-services, des données météorologiques, l'indice de maturité, Toscane.

### 1. Introduction

Tuscany is among the major regions in the world devoted to the cultivation and production of high quality olive oil. However, the olive culture sector in Tuscany, as in many others Italian regions, very often suffers for the small farms dimension that makes difficult to introduce innovations aimed to improve olive oil quality. In order to cope with these needs, the public administrations are involved in the developing of services that enable farmers and technicians to plan more accurately their orchard management strategies. It is in this scenario that since 2005 ARSIA – Extension Service for Agriculture in Tuscany started the implementation of a Decision Support System (DSS) for assessing olive phenology and the degree of ripeness of the olive fruit. The system has been created using the knowledge gained over the years through the web site [agroambiente.info](http://agroambiente.info) (Ricciolini and Guidotti 2004) and general software architecture of the DSS for grape ripening (Sebastiani et al., 2004). Olive DSS is based on a monitoring network of olive farms and an agro-climatic network of meteorological stations. The final objective is to provide real-time and on-line information on the olive phenology and fruit ripening to the various actors of the olive oil sector. Another objective is to create a large database that can be used for the development of agro-climatic models useful in determining the dynamics of the phenology and fruit ripening in olive.

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## 2. Material and Methods

The DSS on the ripening of the olives was developed using a single variety, Frantoio that is the most common olive oil cultivar in Tuscany. The monitoring network was activated in 2005 selecting a group of olive farms spread across a homogenous climatic area of Tuscany, which was also characterized by the cultivation and production of high quality olive oil. The DSS is freely available by technicians, farmers and researchers and is integrated with the other services provided by ARSIA (<http://agroambiente.info.arsia.toscana.it/arsia/arsia/>) (Figure 1).

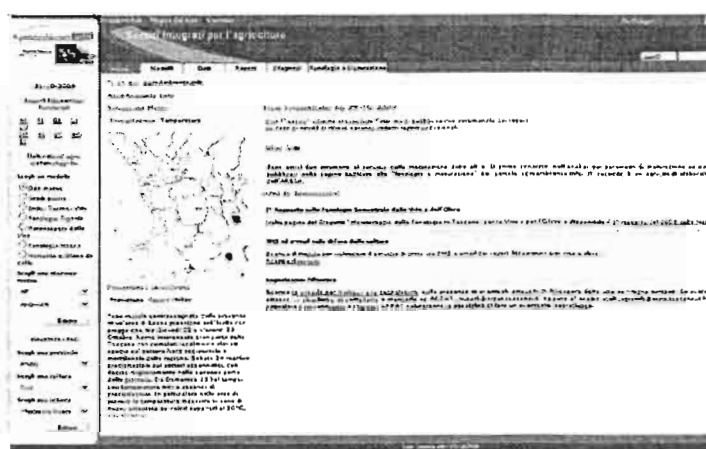


**Figure 1:** the website of ARSIA <http://agroambiente.info.arsia.toscana.it/arsia/arsia/>

In each farm a group of olive trees (10-20 plants) is selected on the basis of their representativeness and proximity to the meteorological stations. On these plants, phenological measurements are performed using a BCCH simplified phenological scale during the year at variable time intervals (monthly, biweekly and weekly according to the predicted speeds of the phenological changes). In the period of olive fruit ripening (mid September – early December) a sample of 500-600 grams of olives is collected weekly for specific physical-chemical analysis in laboratory. The parameters evaluated are: pulp-stone ratio, average weight of fruits (g), relative humidity (%), oil content (% on dry weight) content in polyphenols (as gallic acid, g kg<sup>-1</sup> dry matter), maturity index according to Uceda and Frias (1975). All the phenological and physical-chemical analysis are loaded in the DSS database that is also updated daily with data from the network of weather stations.

## 3. Results

The DSS is accessible through internet and from the main page you can activate several links having the following functionality: a) display pages containing the analytical (phenological and physical-chemical) data of the farm monitored b) processing meteorological data c) obtain weekly reports on olive fruit ripening process (Figure 2, 3 and 4).



**Figure 2:** the main page of the olive and grape DSS with the links to the phenology and fruit ripening web pages.

For every new connection the olive DSS executes a query to the database by creating Web pages: those reporting the phenological and physical-chemical data on site (farms) (Figure 5) or those elaborating the meteorological patterns. Using some computing functions integrated into the DSS the users can calculate for each weather station the actual value of the Degree Day. The calculations are based on data collected by the agro-meteorological network ARSIA at individual weather stations (Figure 6).





**Figure 4:** the main page of the olive DSS for olive fruit ripening monitoring. From this page is possible to access to the analytical data (left bottom) and to the reports (right).

The results of these years of DSS testing on phenology and olive fruit ripening have demonstrated the possibilities of the system for delivering and updating this information in real time, plus the ability to make this service accessible and easily usable by other entities engaged in territorial analysis.

Research institutions, regional government, consortiums and associations of producers may thus have a common tool for sharing and analyzing data, and for the development of agro-climatic models useful in determining the dynamics of phenology and olive ripening in Tuscany.



**Figure 5:** the page reporting the physical-chemical data for olive fruit ripening on each site monitored. In this example the data for the week 12-18 October are shown.



**Figure 6:** the page reporting the meteorological elaborations: Degree Day (DD) at different thresholds (10 and 7.5 degrees) or period for 2009. In the other columns the differences in day (plus or minus) to attain the same degree days are also reported.

Finally, in table 1, we report the numbers visited DSS pages in the year 2008 and 2009 (still in progress). It is clear the improvement in the number of visited pages in 2009 in respect to 2008 and the distribution along the different months with a clear peak in October for ripening web pages.

In conclusion the field monitoring networks, providing the physical and chemical data on olive ripening and phenology, together with meteorological data sets, and internet-based services proven to be a useful and appreciated system for real time monitoring and support decisions at olive orchard, research and administration level.

**Table 1:** Number of visited pages in 2008 and 2009 dived per months.

| Months    | 2009      |          | 2008      |          |
|-----------|-----------|----------|-----------|----------|
|           | Phenology | Ripening | Phenology | Ripening |
| January   | 3805      | 395      |           |          |
| February  | 7316      | 98       |           |          |
| March     | 8683      | 145      |           |          |
| April     | 5711      | 133      | 39        | 56       |
| May       | 4699      | 166      | 189       | 397      |
| June      | 5782      | 290      | 223       | 134      |
| July      | 18321     | 167      | 5537      | 306      |
| August    | 21287     | 217      | 6934      | 391      |
| September | 19311     | 499      | 6088      | 646      |
| October   | 12085     | 1965     | 4423      | 1822     |
| November  |           |          | 2706      | 611      |
| December  |           |          | 2862      | 232      |

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**Growth, chemical composition of vegetative tissues, total per plant uptake and utilization efficiency of Mn, Fe, Zn, Ca, Mg, K and P by the olive cultivar 'Chondrolia Chalkidikis', when cultivated in three soils with different physicochemical properties**

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**Abstract**

Three-month-old rooted olive cuttings (*Olea europaea* L., cv. 'Chondrolia Chalkidikis', about 20-25 cm in height) were grown outdoors for 140 days (from the 30<sup>th</sup> of May until the 17<sup>th</sup> of October), in three soils (parent material marl, gneiss schist. and peridotite), to find out possible differences between them concerning growth, total per plant uptake and utilization efficiency of Mn, Fe, Zn, Ca, Mg, K and P. Plant growth was not affected by the soil type. Total per plant Mn, Fe and Mg content was significantly greater in the peridotite soil, compared to the gneiss schist and marl soils. Finally, the utilization efficiency of Mn, Fe and Mg was significantly lower in the peridotite soil, compared to the other two soils.

**Key words:** Nutrient uptake; nutrient use efficiency; olive cultivar; 'Chondrolia Chalkidikis'; parent material.

**Croissance, composition chimique de tissus végétaux, absorption et efficacité d' utilisation de Mn, Fe, Zn, Ca, Mg, K et P par la variété d'oliviers 'Chondrolia Chalkidikis', cultivés sur trois sols différents**

**Résumé**

Des plants d' oliviers (*Olea europaea* L., variété 'Chondrolia Chalkidikis', de haut 20-25 cm), âgés de trois mois, ont été cultivés pendant 140 jours (du 30 mai jusque au 17 octobre), à la campagne, sur trois sols (matériau d'origine marl, gneiss schist, et peridotite), pour investiguer si des différences statistiquement significatives existaient entre eux, en ce qui concerne la croissance, l' absorption et l' efficacité d' utilisation des éléments Mn, Fe, Zn, Ca, Mg, K et P. La croissance des plantes n'a pas été influée par le type de sol. La quantité totale des plants aux Mn, Fe et Mg a été significativement plus grande au sol de peridotite, qu'aux autres deux sols. Finalement, l'efficacité d'utilisation des Mn, Fe et Mg a été significativement plus basse au sol de peridotite, qu'aux autres deux sols.

**Mots clés:** Absorption ; efficacité d'utilisation des éléments ; variété d'oliviers ; 'Chondrolia Chalkidikis' ; maternel génétique parentale.

**1. Introduction**

Differential uptake and utilization efficiency of several macro- and micronutrients by different plant species, or cultivars have been observed by many researchers (Damon and Rengel, 2007; Jiang, 2008; Rengel and Damon, 2008). Furthermore, in the cases of micronutrient deficiency the chosen genotypes should have high uptake and transport capacity to the leaves of the particular element (Rengel, 2001). In addition to that, high internal nutrient utilization efficiency is also another property that one genotype should have under those conditions (Jiang and Ireland, 2005; Jiang, 2006). However, no attention has been paid until now concerning the influence of soil type when the same genotype/cultivar is cultivated, on its' nutrient uptake and utilization efficiency. On the other hand, although olive tree is considered as a species having great capacity to survive and produce in low-fertility soils, almost nothing has been reported so far concerning nutrients' (especially micronutrients') absorption, distribution and utilization efficiency.

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The aim of the present investigation was to study the influence of the soil type when olive trees are cultivated, on their growth, absorption and utilization efficiency of seven (micro- and macro-) nutrients. For that purpose, the olive cultivar 'Chondrolia Chalkidikis' was chosen for investigation, since it is very resistant to cold and widely cultivated for table olives production in the region of central Macedonia, of northern Greece. Three soils, from different parent material (Marl, Gneiss schist and Peridotite) and with different physicochemical properties, were chosen as a medium for planting, as they are the three basic soil types on which 'Chondrolia Chalkidikis' is cultivated in the area of Thessaloniki, central Macedonia, Greece.

## **2. Materials and Methods**

### **2.1. Plant material and soil sampling**

Three-month-old rooted olive cuttings (*Olea europaea* L., cv. 'Chondrolia Chalkidikis'), about 20-25 cm in height were grown outdoors for 140 days (from the 30<sup>th</sup> of May to the 17<sup>th</sup> of October) under ambient conditions, in black plastic bags, containing three kg of soil. The plants were randomized and separated, based on their height and initial total fresh weight, in three similar groups (corresponding to the three soils used) and six replicates per soil were included. The total number of plants was 18 (3 soils X 6 replicates). Three soils, derived from different parent material (Marl, Gneiss schist. and Peridotite) and having different properties, were included in the study. The soils were collected from the area around the city of Thessaloniki, i.e. Epanomi (Marl), Souroti (Gneiss schist.) and Galatista (Peridotite) and represent the three basic soil types on which olive tree is growing in the region of Thessaloniki, Macedonia, northern Greece. The soil samples were collected from the upper 60 cm of each soil, where most of the olive root system is usually growing. During the period of experimentation, the plants were irrigated, three times a week until the end of August and two times a week from the beginning of September to the 17<sup>th</sup> of October, with 200 ml of distilled water.

### **2.2. Soil sample analysis**

After collection, soil samples were dried at room temperature, separated from stones and sieved to pass a 10 mesh screen before analyses. General chemical analyses, as well as extraction of micronutrients, were conducted in each one of the three soils. General chemical analyses of the soils included the pH measurement, the % content of organic matter, the exchangeable cations Ca, Mg and K, the particle size analysis and the % CaCO<sub>3</sub>. The pH was measured in soil with distilled water (solution 1:1) (Bates, 1964), the organic matter with the K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> method (Allison, 1965), the exchangeable cations according to the ammonium acetate method of pH 9 (pH 9 was chosen in order to reduce the solubilization of CaCO<sub>3</sub> as the pH values of the three soils were above 7) (Bower et al., 1952; Alifragis and Papamichos, 1995), the particle size analysis according to the 'Bouyoucos' method. Finally, the % CaCO<sub>3</sub> content was determined with the calcium meter method, while the extraction of Mn, Fe, Zn in the three soils was conducted with the DTPA method (Alifragis and Papamichos, 1995).

### **2.3. Plant growth parameters**

Once the plants were harvested (at the 140<sup>th</sup> day), shoot length, as well as fresh and dry weight of leaves, stems, roots and total plant fresh and dry weights were measured. Samples were initially weighed (fresh weight), then washed with tap and afterwards with distilled water, dried at 75°C for 24 hours, and weighed again (dry weight).

### **2.4. Mineral concentrations of plant tissues and total plant uptake of Mn, Fe, Zn, Ca, Mg, K and P**

After the plants were divided into stems, roots and leaves, washed once with tap and twice with distilled water, dried at 75°C for 24 hours and milled to a fine powder to pass a 30 mesh screen. A portion of 0.5 g of the fine powder of each sample was dry-ashed in a muffle furnace at 515 °C for 5h. Then, the ash was dissolved in 3 mL of 6 N hydrochloric acid (HCL) and diluted with double distilled water up to 50 mL and the concentrations of the elements Ca, Mg, K, Mn, Fe and Zn were determined by atomic absorption spectroscopy (Perkin-Elmer 2340, Waltham, MA, USA). The concentrations of the microelements were expressed in µg/g. D.W., while those of macronutrients in % D.W. Multiplying the concentration of each nutrient (µg or mg/g. D.W.) found in each plant part by its dry weight, the content (absolute quantity) of each nutrient per plant part at the end (at the 140<sup>th</sup> day) was calculated.

By addition of the nutrient contents of different plant parts, total nutrient content ( $\mu\text{g}$  or  $\text{mg}$ ) per plant, and thus total nutrient uptake per plant was computed. Finally, the nutrient utilization efficiency of each nutrient (NUE), which is defined as the amount of biomass produced per unit of nutrient, was further calculated (Chapin and Van Cleve, 1991).

## 2.5. Statistics

All data were statistically analyzed by using the SPSS software package (SPSS 16.0.1. for Windows, Chicago, IL) and, particularly, for comparison of means of all the variables measured in each one of the three soils, Duncan's test was performed, for  $P \leq 0.05$ .

## 3. Results

### 3.1. Physicochemical properties of the three soils

The physicochemical properties of the three soils are shown in Table 1.

**Table 1:** Physicochemical properties of the three soils.

| Soil          | Sand | Clay | Loam | Texture | Organic matter (%) | pH   | CaCO <sub>3</sub> (%) | P/Olsen (p.p.m.) | Ca              | Mg   | K    | Mn          | Fe  | Zn  |
|---------------|------|------|------|---------|--------------------|------|-----------------------|------------------|-----------------|------|------|-------------|-----|-----|
|               | %    |      |      |         |                    |      |                       |                  | meq /100g. soil |      |      | mg/ kg soil |     |     |
| Marl          | 62.4 | 14.8 | 22.8 | SCL     | 2.88               | 7.63 | 3.5                   | 28.0             | 36.90           | 2.25 | 1.40 | 7.0         | 3.0 | 1.5 |
| Gneiss schist | 68.4 | 20.8 | 10.8 | SL      | 1.68               | 7.15 | 1.3                   | 15.0             | 10.68           | 2.17 | 1.20 | 6.0         | 5.0 | 1.5 |
| Peridotite    | 52.4 | 26.8 | 20.8 | SCL     | 4.44               | 7.97 | 15.4                  | 17.5             | 36.21           | 2.46 | 6.70 | 12.0        | 4.0 | 1.0 |

As it is clear from the above table, the soils from parent material Marl and Peridotite were sandy-clay-loam (SCL), while that from Gneiss schist was sandy-loam (SL). The organic matter content (%) of the Gneiss schist. soil was relatively low, while that of the other two soils was sufficient and ranges between 2.88 and 4.44%. The CaCO<sub>3</sub> (%) content was medium (15.4%) in the Peridotite soil, while in the other two soils was very low. Among the exchangeable cations, Ca dominates in the Marl and Peridotite soils (which were saturated in Ca), while in Gneiss schist was approximately 27% of that in the other two soils. The greatest concentration of the DTPA extractable Mn was recorded in the Peridotite soil, while in the other two soils it was only about 50% of that in the previous one.

### 3.2. Plant growth

All the growth parameters studied (shoot length, fresh and dry weights of all tissues, total plant fresh and dry weight) did not differ significantly between the three soils, in which plants of 'Chondrolia Chalkidikis' were grown (data non-shown).

### 3.3. Mineral concentrations of plant tissues and total plant uptake of Mn, Fe, Zn, Ca, Mg, K and P

The concentrations of seven nutrients and the total per plant uptake of them are shown in Table 2. It is clear from that table that the total plant content of Mn, Fe and Mg was significantly greater in the Peridotite soil, than in the other two soils. In contrast to that, the concentrations of Mn, Fe and Mg in leaves did not differ between the three soils (Table 2).

**Table 2:** Mineral concentrations of roots and leaves and total per plant content of seven nutrient elements in the olive plants, grown in the three soils studied.

| Vegetative tissue          | Soil          | Mn         | Fe    | Zn    | Ca     | Mg     | K     | P     |
|----------------------------|---------------|------------|-------|-------|--------|--------|-------|-------|
|                            |               | mg/kg d.w. |       |       | % d.w. |        |       |       |
| <b>Roots</b>               |               |            |       |       |        |        |       |       |
|                            | Marl          | 41b        | 1790b | 20b   | 0.93a  | 0.22b  | 0.76a | 0.11a |
|                            | Gneiss schist | 42b        | 1810b | 26a   | 0.64b  | 0.27ab | 0.74a | 0.14a |
|                            | Peridotite    | 60a        | 3930a | 18b   | 0.81ab | 0.33a  | 0.71a | 0.07b |
| <b>Leaves</b>              |               |            |       |       |        |        |       |       |
|                            | Marl          | 22a        | 54a   | 11a   | 0.95a  | 0.08a  | 0.81a | 0.15a |
|                            | Gneiss schist | 17a        | 48a   | 11a   | 0.94a  | 0.08a  | 0.89a | 0.21a |
|                            | Peridotite    | 21a        | 62a   | 14a   | 0.99a  | 0.10a  | 0.97a | 0.16a |
| <b>Total plant content</b> |               | mg         |       |       | mg     |        |       |       |
|                            | Marl          | 0.70ab     | 17b   | 0.43a | 248a   | 34b    | 231a  | 38a   |
|                            | Gneiss schist | 0.60b      | 17b   | 0.43a | 190a   | 35b    | 195a  | 41a   |
|                            | Peridotite    | 0.90a      | 42a   | 0.42a | 232a   | 49a    | 231a  | 34a   |

The different letters in each column symbolize significant differences between the three soils, for  $P \leq 0.05$  (SPSS; Duncan's test). The same also applies in Table 3.

### 3.4. Utilization efficiency of Mn, Fe, Zn, Ca, Mg, K and P

Table 3 shows the utilization efficiency of Mn, Fe, Zn, Ca, Mg, K and P in the three soils. As it is clear from that table, the utilization efficiency of Mn, Fe and Mg by the olive cultivar 'Chondrolia Chalkidikis' was significantly lower in the Peridotite soil, than in the other two soils.

**Table 3:** Nutrient utilization efficiency of seven nutrient elements in the three soils when 'Chondrolia Chalkidikis' was grown.

| Soil          | MnUE                                                               | FeUE  | ZnUE   | CaUE                                                    | MgUE    | KUE     | PUE     |
|---------------|--------------------------------------------------------------------|-------|--------|---------------------------------------------------------|---------|---------|---------|
|               | mg d.w. of the plant/ total micronutrient content in $\mu\text{g}$ |       |        | mg d.w. of the plant/ total macronutrient content in mg |         |         |         |
| Marl          | 44.61a                                                             | 1.87a | 75.75a | 134.58a                                                 | 984.20a | 144.02a | 885.25a |
| Gneiss schist | 45.22a                                                             | 1.59a | 63.72a | 147.36a                                                 | 790.37b | 143.80a | 683.33a |
| Peridotite    | 33.65b                                                             | 0.76b | 75.13a | 139.20a                                                 | 658.25c | 139.58a | 945.28a |

## 4. Discussion

According to Therios (2005), the most appropriate soils for growth and fruiting of olive trees are the sandy-loam (SL), after sufficient fertilization with N, K and P and with adequate water content. However, the low C.E.C. capacity of the Gneiss schist soil (only 17%, compared to those of 43% and 48% of the Marl and Peridotite soils, respectively, data non-shown), together with the low organic matter content (Table 1) were possibly the basic reasons why plant growth (as expressed by shoot length and total plant weight) was not greater in the Gneiss schist soil, compared to the other two sandy-clay-loam soils (data non-shown).

The DTPA extractable Mn concentration in the Peridotite soil was two times greater, than in the Gneiss schist soil (Table 1). This is possibly the reason why total per plant Mn content was significantly greater in the Peridotite soil, compared to the Gneiss schist soil. Nevertheless, the concentrations of Mn in leaves did not differ between the three soils. This happened because significantly greater concentrations of Mn were recorded in the root system of the olive plants when they were grown in the Peridotite, than in the Marl and Gneiss schist soils (Table 2). Therefore, in the case when 'Chondrolia Chalkidikis' was grown in the Peridotite soil, although absorbed significantly greater quantity of Mn, limited transport of Mn from the root system to the leaves took place, than in the case when it was grown in the Marl soil (29.6% of the total plant Mn was distributed in leaves and 57% was 'retained' in the root system, compared to 19.9% in leaves and 68.6% in the root system and 19.5% and 69.7% when it was grown in the Gneiss schist and Peridotite soils, respectively, data non-shown). Based on the above remark, it could be concluded that 'Chondrolia Chalkidikis' was more Mn-efficient (greater percentage of the total plant Mn distributed in leaves) when was grown on the Marl soil, than on the Peridotite one. According to Jiang and Ireland (2005) and Jiang (2006), Mn efficient wheat cultivars owned this ability to a better internal utilization of Mn, rather than to a higher plant Mn accumulation. In our case, significantly lower utilization efficiency of Mn (expressed as mg total plant d.w. / $\mu\text{g}$  of the total Mn content) took place in the Peridotite soil, compared to the other two soils (Table 3). However, between Gneiss schist and Marl soils, the olive plants grown on the Marl soil had better internal utilization efficiency (greater percentage of the total Mn content distributed in leaves).

The concentrations of Fe in the three soils were approximately at the same level (Table 1); however, the olive plants accumulated significantly greater amount of Fe when they were grown in the Peridotite soil. According to Maruyama et al. (2005), who made a comparison of iron availability in leaves of barley and rice, the difference in the Fe acquisition ability between these two species was affected by the differential mugineic acid secretion. Maybe a similar mechanism (i.e. greater or different kind of organic secretions from the root system of the olive plants in the Peridotite soil and formation of Fe-organic complexes), is true for 'Chondrolia Chalkidikis' in the three soils. Although in the Peridotite soil olive plants accumulated significantly greater amount of Fe, compared to the other two soils, the greatest part of it was accumulated in the root system (Table 2), and limited transport from root system to leaves took place (96.7% of the total plant Fe quantity was retained in the root system and only 1.3% of it was distributed in leaves, while for example in the Marl and Gneiss schist soils the corresponding percentages were 92.5% and 3.0% and 95.7% and 2.1%, respectively, data non-



shown). Furthermore, the concentrations of Fe in leaves did not differ between the three soils (Table 2). From all the above data, it could be concluded that 'Chondrolia Chalkidikis' was more Fe-efficient (as it was also more Mn-efficient) when was cultivated in the Marl soil (due to better internal utilization), than in the Peridotite and Gneiss schist soils. From Table 3 it is clear that Fe utilization efficiency (expressed as mg total plant d.w. / $\mu$ g of the total Fe content) was significantly greater in the Marl and Gneiss schist soils, than in the Peridotite one. However, between Marl and Gneiss schist soils, the olive plants grown in the Marl soil had better internal utilization efficiency (greater percentage of the total plant Fe content distributed in leaves, as also happened with Mn).

The concentrations of all the macronutrients in leaves did not significantly differ between the three soils, as well as the total per plant quantities of them, with the exception of Mg (significantly more Mg accumulated by the olive plants in the Peridotite soil); however, the greatest part of it accumulated in the root system. It is of great importance that although the concentrations of Mg in the three soils were approximately at the same level, olive plants accumulated significantly greater quantity of it when they were grown in the Peridotite soil, than in the other two soils. This finding could be ascribed to a possible differential colonization of the root system by mycorrhiza between the three soils (colonization by different fungus genus/ species, or/and different percentage of root system colonization by fungus). In other experiments we did with 'Chondrolia Chalkidikis' and another two olive cultivars, we found two different arbuscular mycorrhiza fungus genus (*Glomus* and *Gigaspora*) and different colonization of the root system by fungus between these three soils (Chatzistathis, unpublished data). Better mineral nutrition with P and Mg of different plant species, inoculated with different mycorrhiza fungus genus, than when they were inoculated, has been found by Arines and Vilarino (1989), Liu et al. (2000) and Nogueira and Cordoso (2002). Furthermore, according to Satter et al. (2006), the uptake and utilization efficiency of N, P, K, Ca and Mg by *Acacia mangium* seedlings was significantly influenced by arbuscular mycorrhiza inoculation. It is possible in our case that the significantly lower utilization efficiency of Fe and Mg in the Peridotite, compared to the other two soils, could be also owned to a different colonization of the root system by mycorrhiza fungus between the three soils, as that described previously. In contrast to that, the significantly lower utilization efficiency of Mn in the Peridotite soil was probably due to almost twice greater concentration of it in that soil, compared to the other two (Table 1), as no significant difference in total plant biomass existed between the three soils (data non-shown).

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## Thrips Infestation as a New Record in Egyptian Olive Groves

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### Abstract

New damage symptoms on leaves of olive (*Olea europaea*) were noticed for the first time in Egypt by M. El-Kholy in March 2008 identified as sand blasting effect as the discovery was accidentally preceded by a sandstorm. In December 2008 and April 2009 the persisting infestation on sampled leaves were identified by an investigating institution as *Alternaria alternata*. Treatment with fungicides was not effective. Following the flowering and fruit set season of 2009 other damage symptoms (which were never seen before) have been observed on fruitlets and developing fruits. Therefore M. El-Kholy inspected different olive growing regions, ascertaining that the infestation is widespread in two regions; El-Arish on the northeastern coast (31° 07' 32" N – 33° 48' 44" E) and inland Ismailia (30° 28' 28" N – 32° 07' 30" E). Some growers perceived this new damage as caused by Peacock Spot (*Spilocaea oleaginea*) which is confined in certain areas in the country and others contributed it to malnutrition. Close-up images of the damage symptoms on both leaves and fruits were emailed to olive agronomists and entomologists in all producing countries around the Mediterranean, Australia and the USA. Neither the symptoms nor the probability of the causative agent were identified except by Dr. Mohamed Ali Triki, Institut de l'Olivier of Tunisia and Dr. Leandro Ravetti, Modern Olives, Australia that it might be caused by a piercing sucking insect, mentioning that the symptoms were not seen before. The unknown insect was isolated and with the assistance of Dr. Essam Agamy, and Dr. Monir El-Husseini the insect was identified as thrips. For identification, images of microscopic slides were emailed to specialists in almost all olive producing countries. In September 2009 the thrips was classified as *Dendrothrips ornatus* by Dr. Enrique Quesada Moraga, Associate Professor, Agricultural and Forestry Engineering School, University of Cordoba. Later it was classified by Dr. Monir El-Husseini as *Dendrothrips eremicola* Priesner which belongs to the desert fauna of Egypt and was never mentioned previously as pest on any crop in the country.

The slashing and feeding activities by the mouthparts cause silvery bronze streaks on the attacked leaves with closely visible yellow lesions, mostly round-shaped with bleached centers at the spots where sap has been sucked. The vein skeleton of olive leaves resists the thrips attack due to denser parenchyma tissue; therefore the lesions exist around the central vein in an elliptical shape. The more damage to the leaves the more they give a leathery texture feeling. Feeding on the olive leaf tips has been rarely observed. Severely affected leaves have shown premature defoliation. Highly infested trees appear very unhealthy due to defective photosynthesis and transpiration, exhibiting a significant decline in yields. It has been also noted that the thrips attack flower ovaries when in full bloom and also the small fruitlets immediately after setting causing either abortion or malformed fruits. The damaged surviving fruitlets from this initial attack will develop further but will have scabby silvery scars, deformation and/or splitting. As a result, damaged table fruits must be discarded and the oil produced will be of inferior quality.

Since the insect is not registered in Egypt, M. El-Kholy carried out 14 different trials on trial blocks in his grove with different commercially approved insecticides. Some were not effective at all and the most effective biological ones in suppressing population were based on Abamectin and Spinosad. Other controlling measures are being applied which involve monitoring with sticky blue traps and sanitation of grove surface from leaves, litters, and pruning leftovers. Scratching the soil under the trees with a garden fork helps in exposing the prepupal and pupal stages thus interrupting the life cycle.

The emergence of this pest is attributed to different possibilities which are being under investigation, among which is disturbing the natural fauna and flora balance in the desert ecosystem by reclamation activities and growing monoculture on large areas. Current activities to resolve the problem involve:

1- Collecting information to construct a trusted data base on the status of this pest in Egyptian olive plantations concerning distribution, host plants, susceptibility of olive varieties, biology and generations.

2- Selecting a proper and safe biopesticide for rapid suppression of thrips population on olive trees.

3- Searching for effective natural enemies of *D. eremicola* in the virgin desert ecosystem on its original host plants for developing as biological control agents on commercial level.

4- Fostering close cooperation between scientists and olive growers to transfer knowledge and technology through both field days and farmer-to-farmer extension to understand the relationship between the different ecological aspects of the desert ecosystem and the new pest problem to benefit from and avoid emergence of future new infestations by pests.

The case indicates clearly the importance of regional and interregional cooperation on common crops.

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## La mycorhization : une méthode biotique pour améliorer la croissance et contourner le stress abiotique chez l'olivier (*Olea europaea* L.)

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### Résumé

L'inoculation en pépinière par une mycorhyze à vésicules et arbuscule (MVA) *Glomus intraradices* de jeunes plantes d'oliviers a montré une meilleure installation de la symbiose mycorhizienne chez Chemlali et Meski soldée par une amélioration de la croissance, de la ramification du système racinaire et de la biomasse végétative et racinaire. A l'exception de Zarrazi, les racines des plantes mycorhizées contiennent plus de sodium que les témoins. Alors que dans les feuilles des plantes mycorhizées, la teneur en Na reste inférieure à celui des plantes non mycorhizées. Ceci nous a permis de conclure que la souche mycorhizienne a amélioré le mécanisme d'exclusion du sodium au niveau des racines chez certains cultivars.

En terme de ce travail, les résultats obtenus ont montré que l'inoculation des plantules en pépinière a permis la réduction du stress salin, chez les cultivars étudiés, quand les doses Na Cl de l'eau d'irrigation sont inférieures à 75 mM.

**Mots clés :** Olivier, mycorhize, *Glomus intraradice*, Croissance, , salinité, Exclusion sodium

## Mycorrhization: a biotic method to improve olive tree (*Olea europaea* L.) growth and to mitigate abiotic stress

### Abstract

The inoculation in nursery by arbuscular mycorrhizal fungus (AMF) (*Glomus intraradices*) of young plants of olive trees showed a better installation of the of symbiosis at Chemlali and Meski varieties balanced by an improvement of the growth, root system ramification and the vegetative and root biomass.

With the exception of Zarrazi cv., the roots of inoculated plants contain more sodium than the witnesses. While in the leaves of the mycorrhized plants, Na rate remains less than non-inoculated plants. This allowed us to conclude that the mycorrhizal stump improved the mechanism sodium root exclusion of the tested cultivars. In terms of this work, the obtained results showed that the AMF *Glomus intraradices* inoculation of the plants in nursery allowed the attenuation of the salt stress in studied cultivar when the Na Cl water rate's less than 75 mM.

**Key words:** Olive tree, mycorrhize, *Glomus intraradices*, growth, salinity, Na exclusion,

### 1. Introduction

L'utilisation des mycorhizes revêt une importance particulière sous des climats arides et semi arides où la salinité des sols réduit l'apport par la plante des fertilisants phosphaté et azoté. Ainsi, certains travaux ont pu montrer que la tolérance des plantes à la salinité peut être améliorée par les symbiotes (Dixon et al., 1993). L'efficacité de ces symbioses entre le champignon et la hôte reste toutefois liée, en plus des conditions pédoclimatiques, aux espèces symbiotiques mises en jeu.

En dépit de leur rareté, les études ont montré que l'olivier est parmi les espèces qui peuvent être infectées par les endomycorhizes (MVA). Inoculée par *Glomus mosseae* les racines des jeunes plantes d'oliviers des cultivars Moraïolo, Leccino et Frantoï sont devenues plus ramifiées permettant aux plantules une meilleure croissance (Citernesi et al., 1998). L'inoculation des plantules de différents cultivars avec des champignons mycorhiziens a montré une efficacité importante à surmonter les contraintes de culture (Hayman et al., 1976; Roldan – Fajero et Barea, 1986). Des études plus récentes (Porcel et al., 2006) ont montré que les endophytes engendrent chez les plantes des transformations morphologiques et physiologiques pour tolérer les contraintes du milieu. La colonisation mycorhizienne diminue les effets osmotiques chez les plantes occasionnés par la toxicité de Na<sup>+</sup> (Ruiz-Lozano and Azcon, 2000 ; Rabie and Almadini, 2005)

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## 2. Matériels et condition d'inoculation et de stress salin

Les boutures semi ligneuses enracinées et ayant le même âge et relativement le même nombre de racines adventives, des variétés Chemlali, Zalmati Meski et Zarrazi, sont repiquées dans des pots de 1.5 l remplis de substrat préalablement stérilisé (pour éliminer autre organisme vivant). Le substrat est mélangé à 2:1 constitué de sable grossier et de tourbe.

Pour chaque cultivar deux lots de 10 plantules sont choisis, l'un mycorhizé (M) au niveau de chaque plantule par 3 g de substrat infecté par une souche améliorée de *Glomus intraradices* (Premier Tec, Québec, Canada). L'autre lot non mycorhizé (NM) est pris comme témoin. Les pots sont placés en serre d'acclimatation durant deux mois. Puis ils ont été transférés sous ombrière à l'abri des pluies. Les plantules reçoivent périodiquement et équitablement de l'eau courante stérilisée. Chaque mois la longueur totale des pousses végétatives est mesurée.

Après 9 mois, des plantules des deux lots sont séparées de leurs substrats par un lavage soigneux. La biométrie des différents types de racine (racines adventives, ordre 1 R1), ordre 2(R2) et ordre 3 (R3)) ainsi que les poids frais et secs de la partie végétative et racinaire de chaque plantule sont procédés. Le rendement en matières sèches (MS%) dans les feuilles, le bois et les racines a été obtenue après dessiccation des organes dans une étuve à 74°C pendant 48 heures.

Le pourcentage de colonisation des racines par *Glomus intraradices* est déterminé par la méthode de colorisation et d'observation de Phillips and Hayman (1970).

Pour étudier l'effet du stress salin sur les plantules mycorhisées comparées à un témoin (plantules non mycorhisées), des plantules âgées de 6 mois des 4 cultivars sont repiquées dans les mêmes conditions citées précédemment. Durant un mois toutes les plantules (inoculées et témoins) sont irriguées avec l'eau ordinaire de robinet stérilisée. Un mois après l'inoculation, un traitement salin est réalisé en irriguant les plantules avec de l'eau contenant les concentrations suivantes (25, 50, 75 et 100 mM de NaCl) durant 11 mois. Pour éviter le choc de l'accumulation excessive de NaCl, Les doses NaCl sont fractionnées en les répartissant entre les irrigations.

Les deux essais sont conduits en dispositif expérimental aléatoire avec 2 traitements (mycorhizé:M et non mycorhizé: NM) et 10 répétitions pour chaque traitement. La signification entre les résultats est réalisée avec une analyse de la variance (ANOVA) et la comparaison entre les moyennes est faite moyennant le test Duncun.

## 3. Résultats et interprétations

### 3.1. Effet de *Glomus intraradices* sur la ramification racinaire

Les résultats obtenus (Fig.1) montrent que le champignon a provoqué chez leurs hôtes une amélioration de la ramification du système racinaire. En effet, si le nombre des racines adventives n'a pas beaucoup changé, celui des racines latérales du 1er, 2ème et 3ème ordre a augmenté, de même pour les longueurs totales. Ayant des diamètres variant entre 1 et 2 mm ces racines sont les sites préférentiels des endomycorhizes. Concernant le taux de répartition des racines, la figure (1) montre qu'une dominance des racines d'ordre 3 (85% du système racinaire) chez les plantes M de Chemlali et Meski (70%). Cette répartition est au profit des racines d'ordre 1 et 2 de Zalmati M (30% et 60%) et de Zarrazi M (35% et 50%).

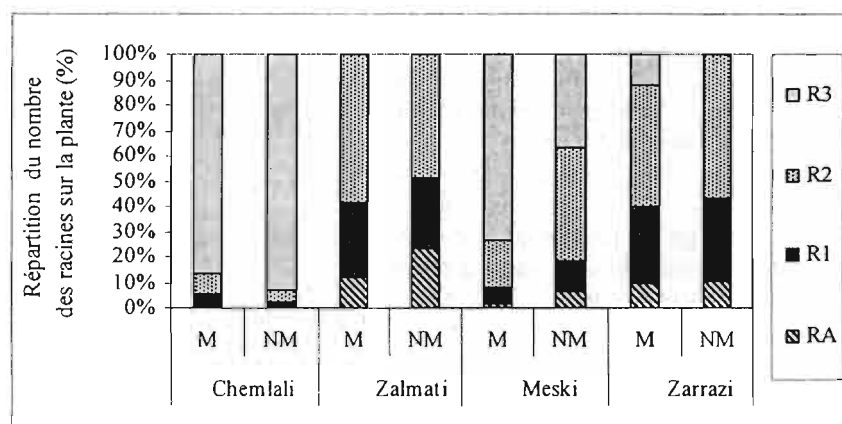


**Figure 1:** Répartition du nombre des racines RA, R1, R2 et R3 des plantes M et NM avec RA : racines adventives ; R1, R2 et R3 : racines d'ordre 1, 2 et 3.

Les chevelures racinaires les plus intenses sont observées chez les plantules mycorhizées de Chemlali, Meski (fig.1). L'analyse statistique montre des différences hautement significatives au seuil de 1% pour le même cultivars entre les plantes mycorhizées et non mycorhizées. Des résultats identiques sont obtenus sur le cultivar Frantoio et Leccino inoculée avec *Glomus mosseae* (Gm). Chez ces cultivars la symbiose a augmenté la longueur et le nombre du 1<sup>er</sup>, 2<sup>ème</sup> et 3<sup>ème</sup> ordre. Les racines adventives ne sont pas affectées par Gm (Citernes et al. 1998). D'autres études ont montré aussi que les mycorhizes, par le développement de leur mycélium, modifie l'architecture des racines (Smith et Read, 1997; Berta et al. 1995). L'analyse morphométrique du système racinaire chez *Vitis vinifera* (Schellenbaum et al. 1991), *populus* sp. (Hooker et al. 1992), *Platanus acerifolia* (Tisserant et al., 1992) et *Prunus ceracifera* (Fortuna et al., 1998) a montré une amélioration de la ramification du système racinaire des plantes mycorhizées.

### 3.2. Effet de *Glomus intraradices* sur la biomasse végétative et racinaire

Dans le but de quantifier l'effet de *Glomus intraradices* (Gi) sur la croissance des plantes nous avons déterminé le rendement en matières sèches (MS%) =  $\text{poids sec/poids frais} \times 100$  au niveau de tous les organes de la plante. L'examen du Tableau (1) montre que les biomasses racinaires et végétatives sont plus importantes chez les plantes mycorhizées. Positivement Corréllés avec le poids frais et sec du matériel végétal, les rendements en matière sèche (MS) sont nettement améliorés avec la mycorhization. Chez le cultivar Chemlali la MS des feuilles, du bois et des racines passe de 26,78; 43,77 et 44,45% dans le témoin à 37,83; 50,5 et 51,24% chez les hôtes. L'analyse statistique révèle une différence significative au seuil 5% entre les taux des plantes mycorhizées et non mycorhizées. De même pour Meski ou le taux en MS est plus important chez les plantes mycorhizées. La diminution dans les biomasses végétatives et racinaires chez les plantes mycorhizées de Zalmati et Zarrazi est probablement liée à l'absence de racines tertiaires chez celles-ci.

**Tableau 1:** Effet de *Glomus intraradices* sur pendement en matières sèches (%).

| Cultivars | Mycorhization | Matière sèche (%) |       |         |
|-----------|---------------|-------------------|-------|---------|
|           |               | Racine            | Bois  | Feuille |
| Chemlali  | M             | 37,83             | 50,5  | 51,24   |
|           | NM            | 26,78             | 43,77 | 44,45   |
| Zalmati   | M             | 28,91             | 44,36 | 41,21   |
|           | NM            | 37,66             | 44,59 | 41,72   |
| Meski     | M             | 47,24             | 55,71 | 45,231  |
|           | NM            | 34,35             | 45,94 | 42,88   |
| Zarrazi   | M             | 33,33             | 42,87 | 40,6    |
|           | NM            | 36,99             | 43,64 | 41,64   |

M : plante mycorhizée ; NM : plante non mycorhizée



### 3.3. Colonisation mycélienne des racines

Les observations microscopiques périodiques des racines chez les plantes mycorhisées ont montré que la colonisation mycélienne a concerné exclusivement les racines d'ordre 2 et 3 ayant des diamètres variant entre 0,5 et 1 mm.

En se basant sur le nombre de racines colonisées par rapport aux racines observées un taux de colonisation mycorhizienne du système racinaire a été calculé pour chaque cultivar. Les résultats consignés dans le tableau 2 montre que les systèmes racinaires des cultivars Chemlali et Meski détiennent les taux les plus importants de colonisation mycorhizienne avec respectivement 62% et 49%. Les résultats obtenus révèlent que les taux de colonisation sont en étroite relation avec la morphologie racinaire des cultivars (Tab.2). En effet, les cultivars Chemlali et Meski ont le système racinaire le plus envahi par le mycélium du champignon et le plus développé par la présence de racines fines d'ordre 3 dont le diamètre est inférieur ou égale à 0.5 mm.

**Tableau 2:** Taux de colonisation mycorhizienne du système racinaire des cultivars avant le traitement salin.

| Cultivar | Nombre de racines observées | Nombre de racines colonisées | Taux de colonisation (%) |
|----------|-----------------------------|------------------------------|--------------------------|
| Chemlali | 157                         | 97                           | 62                       |
| Zalmati  | 120                         | 35                           | 29                       |
| Meski    | 135                         | 66                           | 49                       |
| Zarrazi  | 125                         | 28                           | 22,4                     |

Des résultats similaires ont été notés par d'autres auteurs sur d'autres espèces. En effet, sur blé, Sanders et Sheikh (1983) ont constaté une corrélation positive entre le développement du système racinaire de quelques lignées et celui du taux de mycorhization. Sur 4 espèces agro forestières, Guissou et al. (2001) ont signalé une amélioration de l'absorption minérale chez les plantes mycorhisées ayant un bon développement racinaire.

### 3.4. Réponse des plantes mycorhisées à la salinité

#### 3.4.1. La croissance végétative

Généralement, chez les plantes mycorhisées (M) et non mycorhisées (NM), la croissance végétative évaluée par la longueur cumulée des pousses végétatives tend vers la baisse progressivement sous l'effet de la salinité (Fig.2). Cette diminution est plus prononcée chez les témoins (NM). La salinité a diminué, en fonction du temps, l'infection mycorhizienne de *Glomus intraradices* (Gi). Toutefois, l'effet de la salinité a été légèrement atténué avec *Glomus intraradices* chez tous les cultivars (Fig.2). En effet, la croissance des plantules mycorhisées des 4 cultivars, s'est maintenue stable après 10 mois avec la dose 75mM NaCl en comparaison avec le témoin (NM) dont une forte chute de la croissance y est observée avec la même dose. Gi a relativement retardé l'effet du stress salin chez les plantes mycorhisées. Les résultats ont montré aussi que, l'effet de Gi diminue quand NaCl augmente dans le substrat d'élevage et que la dose 100 mM exerce un effet inhibiteur voire létale sur Gi dès son installation (Fig. 2).

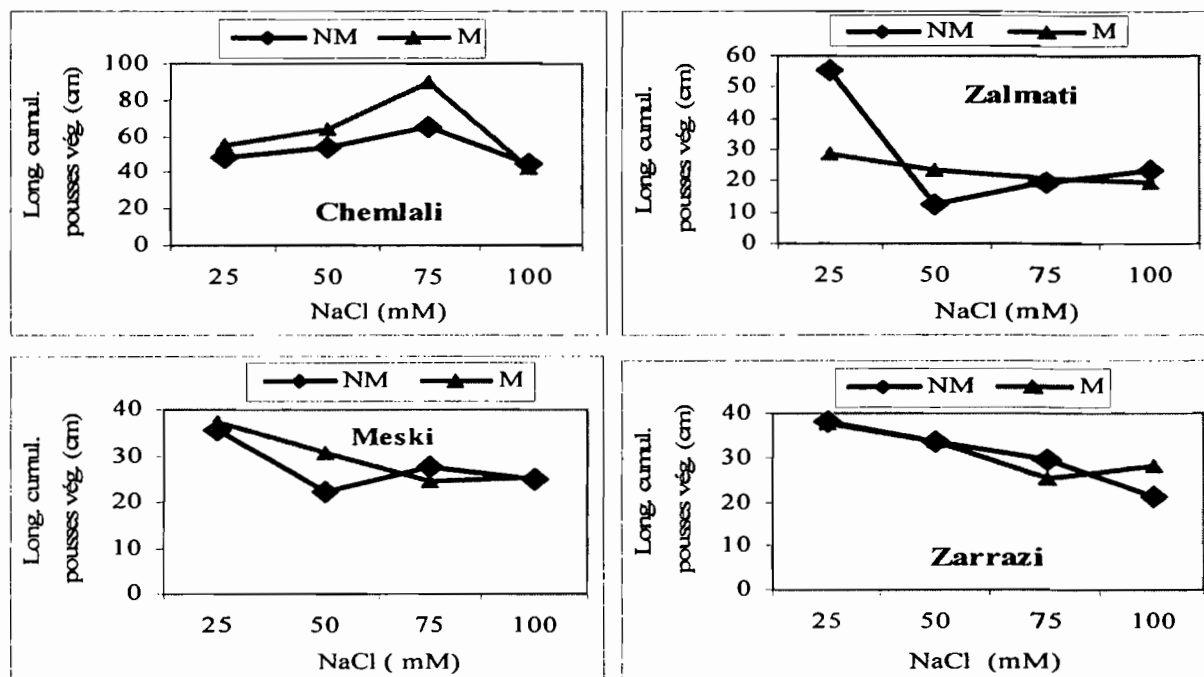


**Figure 2:** Effet de la mycorrhization sur la croissance végétative des plantules irriguées avec différentes concentrations en Na Cl (mM).

En terme de cet essai de mycorrhization des plantes d'olivier soumises à un stress salin, les résultats obtenus ont montré que la symbiose endomycorhizienne exercée par *Glomus intraradices* (Gi) a permis de réduire l'effet de NaCl chez la majorité des cultivars avec des doses inférieures à 75 mM. Dans le cas des doses faibles à modérées de NaCl (25 et 50 mM) Gi améliore la croissance des plantules sans toutefois être affecté par la salinité. Des résultats similaires ont été observés sur deux cultivars italiens Carolea et Nocellara del Bellice (Briccoli Bati et al., 1994), qui ont noté une diminution dans l'infection mycorhizienne avec l'augmentation de la salinité. Les mêmes auteurs ont rapporté que les fortes doses de sel ont réduit le statut minéral des plantes mycorhizées des deux cultivars. En effet, Requena et al., (1996; 2001) ont rapporté que la salinité affecte la viabilité des hyphes mycorhiziens qui constituent les principales sources de la propagation des champignons et des arbuscules qui représentent le site privilégié des échanges entre les deux partenaires de la symbiose Gianinazzi-Pearson et al. (1988). Cette amélioration de la croissance sous stress salin s'explique par le fait que Gi développe un système mycélien qui aboutit à l'amélioration du potentiel hydrique des plantes stressées. Par conséquent une meilleure absorption d'eau et de sels minéraux. Cette amélioration de la tolérance à la salinité des plantes mycorhizées a été déjà signalée sur d'autres espèces (Ruiz-Lozano and Azcon, 2000 ; Rabie and Almadini, 2005).

#### 4. Conclusion

Les tests d'inoculation de *Glomus intraradices* ont amélioré significativement aussi bien la croissance végétative que racinaire des cultivars Chemlali et Meski et un peu moindre pour le cultivar Zarrazi. Pour Zalmati il n'existe pas de différence significative entre les plantules inoculées et témoins. Les racines des plantes inoculées sont plus ramifiées. Généralement, chez les plantes mycorhizées et témoins, la croissance végétative, évaluée par la longueur cumulée des pousses végétatives, tend vers la baisse progressivement sous l'effet de la salinité. Cette diminution est plus prononcée chez les témoins. La salinité a diminué, en fonction du temps, l'infection mycorhizienne de Gi. Toutefois, l'effet de la salinité a été légèrement atténué avec *Glomus intraradices* chez tous les cultivars comparé avec le témoin, tout en signalant que l'effet de Gi diminue quand NaCl augmente dans le substrat d'élevage et que la dose 100 mM exerce un effet inhibiteur voire létale sur son installation.

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## Recrudescence du dépérissement de l'olivier causé par les champignons telluriques en Tunisie

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### Résumé

Cette présente étude a pour but d'élucider l'étiologie de la maladie du dépérissement de l'olivier sévissant dans plusieurs oliveraies tunisiennes et d'étudier sa répartition géographique. La maladie provoque le flétrissement, le dessèchement et la mort total des oliviers de différents âges et cause des dégâts considérables en pépinière et en plein champ. Les investigations au champ et au laboratoire ont montré que plusieurs champignons telluriques sont responsables des pourritures au niveau du collet et des racines des oliviers dépérissés. Il s'agit de *Fusarium solani*, *Fusarium oxysporum*, *Fusarium lateritium*, *Rhizoctonia bataticola*, *Cylindrocarpon destructives*, *Rhizoctonia solani*, *Verticillium dahliae*, *Corticium rolfsii*, *Armillaria mellea*, *Phytophthora* sp., et *Pythium* sp. Le pouvoir pathogène de ces champignons a été confirmé par inoculation des jeunes plants d'oliviers de la variété Chemlali. Récemment, une importante évolution spatio-temporelle de la verticilliose de l'olivier causée par *Verticillium dahliae* Kleb., a été enregistrée dans plusieurs régions du pays (Sfax, Sousse, Monastir, Sidi-Bouزيد, Kairouan, Mahdia, Tataouine, Zaghouane,...). Tous les nouveaux foyers sont découverts dans des périmètres irrigués où l'olivier est cultivé en intercalaire avec quelques espèces légumières sensibles à ce champignon.

**Most Clés :** Olivier; dépérissement; flétrissement, pourriture des racines, champignons telluriques.

## Increasing of olive trees dieback caused by Soilborne fungi in Tunisia

### Abstract

The objective of this study was to elucidate the etiology of the dieback and death of olive tree in several olive growing areas in Tunisia and to determine its geographical distribution. Olive trees are being severely affected by wilt, die-back and death. This disease cause serious problems of mortality in nurseries and olive fields. Our Surveys and laboratory isolation, revealed the presence of complex of soilborne fungi causing collar and root rot of olive trees. These fungal species are *Fusarium solani*, *Fusarium oxysporum*, *Fusarium lateritium*, *Rhizoctonia bataticola*, *Cylindrocarpon destructans*, *Rhizoctonia solani*, *Verticillium dahliae*, *Corticium rolfsii*, *Armillaria mellea* *Phytophthora* sp., and *Pythium* sp. The pathogenicity of these soil borne fungi proved that they are the causal agents of olive trees decline in Tunisia. Recently, an important spread of *Verticillium dahliae* kleb. was observed in several olive growing regions of Tunisia (Sfax, Sousse, Monastir, Sidi-Bouزيد, Kairouan, Mahdia, Tataouine, Zaghouane,...). The disease occurred mainly in some orchards where olive trees were intercalated with some sensitive vegetables to this pathogen.

**Keywords:** *Olea europaea*, die-back, wilt, decline, root rot, soil borne fungi.

### 1. Introduction

L'olivier (*Olea europaea*) occupe une importante place parmi les espèces fruitières cultivées en Tunisie. Elle occupe le quatrième rang mondial en nombre de pieds d'olivier avec environ 66 millions d'arbres, et le deuxième rang en termes de superficie, de l'ordre de 1,7 millions d'hectares, soit près du tiers des terres arables du pays (Jardak, 2008). Ces dernières années, plusieurs cas de dépérissement sont signalés sur des jeunes plants d'oliviers issus de boutures herbacées en pépinière et en plein champ et aussi sur des oliviers âgés. Les mauvaises reprises après plantations, les dépérissements et les mortalités les plus couramment observés sont en général causés par des agents pathogènes responsables de graves pourritures au niveau du collet et des racines. Plusieurs

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champignons telluriques tel que *Cylindrocarpon destructans*, *Phytophthora megasperma*, *P. palmivora*, *Pythium irregulare*, *Sclerotium rolfsii*, *Armillaria mellea*, *Rosellinia necatrix*, *Fusarium* spp., *Rhizoctonia* spp. et *Verticillium dahliae*, se sont montré à l'origine des symptômes de flétrissement et de dépérissement partiel ou total de l'olivier dans plusieurs pays du bassin méditerranéen. (Al Ahmed, 1984; Boulila, 1993, 1994 a et b, 2001; Sanchez Hernandez et al., 1995, 1997, 1998; Porras et al., 2003; Lucero, 2006; Jardak, 2008). La présente étude a pour objectif de déceler l'importance des dégâts causés par les principaux agents de dépérissement en Tunisie.

## **2. Matériel et Méthodes**

### **2.1. Importance de la maladie et description des symptômes**

Des prospections de terrains et des enquêtes ont été menées dans plusieurs régions oléicoles du pays dans le but d'étudier l'importance des dégâts sur les oliviers dépéris en plein champ et en pépinière, de préciser les caractères pathologiques et de repérer les principaux foyers en Tunisie. Cette étude consiste également à décrire les symptômes caractéristiques de la maladie et d'étudier l'évolution du dessèchement sur les arbres dépéris. Elle comprend d'abord une description de la morphologie externe (organes aériens,...) puis une étude de l'anatomie interne basée sur l'examen des tissus internes (système vasculaire) au niveau des organes attaqués.

### **2.2. Isolement et identification**

Des échantillons de bois ont été prélevés au niveau du collet, des racines de la partie vivante au-dessous de la zone nécrosée des rameaux attaqués, sur des oliviers malades au champ et en pépinière afin d'isoler d'éventuels agents pathogènes. Ils sont soigneusement désinfectés à l'alcool 95°, lavés à l'eau distillée stérile, puis découpés en petits fragments ensuite déposés dans des boîtes de Pétri contenant du milieu PDA et incubés à 25°C. Après cinq jours, chaque colonie fongique isolée (excepté les saprophytes) est transférée sur plusieurs milieux spécifiques (SNA, Prune medium, Water Agar) supplémenté de Streptomycine et/ou de Cefotam à raison de 200 mg l<sup>-1</sup>. L'identification des champignons isolés est basée sur les critères morphologiques des organes fructifères produits dans les différents milieux, comme décrit dans plusieurs clés d'identifications (Booth, 1966; Dhingra and Sinclair, 1978; Maas, 1998; Agrios, 2004; Nasraoui, 2000 and 2006; John F. Leslie, 2006). Cependant pour les Pythiacées, les colonies fongiques isolées sont transférées sur les milieux spécifiques des oomycètes: Oat Meal Agar (Triki et al., 2001) contenant 300 mg l<sup>-1</sup> de Streptomycine ou bien sur milieu Bonnet (Bonnet et al., 1980) incubé à 25°C. Leur identification est effectuée en se basant sur les clés de Ribeiro (1978); Waterhouse (1968); Van der Plaats-Niterink (1981) et Jeffers et Martin (1986).

### **2.3. Test de Pathogénie**

La pathogénie de tous les champignons isolés est confirmée par inoculation des jeunes plants d'oliviers de la variété Chemlali, âgés de deux ans. L'inoculation est effectuée par trempage des racines bien lavées à l'eau courante et légèrement blessées (au niveau de la racine pivotante) pendant une heure dans la suspension de spores de chaque champignon ajustées à 10<sup>6</sup> spores ml<sup>-1</sup>. Cependant, pour le cas de *R. bataticola* et *R. solani*, l'inoculation des jeunes oliviers est également effectuée par immersion du système racinaire dans des solutions de sclérotés et de mycélium respectivement. Les jeunes plants du lot témoin sont blessés puis trempés pendant une heure dans l'eau distillée stérile. Enfin, tous les plants sont transplantés dans des pots en polyéthylène contenant un substrat stérile (tourbe : sable, 1 :1 v/v), puis incubés dans une chambre de culture dans des conditions de forte humidité. Les plants sont irrigués deux fois par semaine.

### **2.4. Etude de quelques facteurs abiotiques contribuant à la manifestation de la maladie**

Dans le but de vérifier l'impact de certains paramètres abiotiques tels que la salinité, la texture du sol, et le mode de conduite (entretien,...) sur la manifestation de la maladie, une enquête de terrain a été effectuée dans plusieurs parcelles infestées et dans quelques pépinières situées dans des aires géographiques différentes.

### 3. Résultats et Discussions

#### 3.1. Importance de la maladie et description des symptômes

Le dépérissement de l'olivier est une grave maladie qui attaque souvent les jeunes plants en pépinière en plein champ dans plusieurs régions oléicoles de la Tunisie notamment dans le centre et le nord du pays (Fig. 1). Les investigations au champ et en pépinière montrent l'importance des dégâts sur les jeunes oliviers à cause de l'évolution rapide de la maladie. Les symptômes apparaissent généralement dès le début du printemps et sont presque identiques avec la majorité des champignons telluriques. Ils se manifestent au début par un changement de couleur au niveau de la frondaison (du vert sombre au vert clair puis au jaune). A un stade plus avancé, l'olivier souffre d'un jaunissement localisé à évolution ascendante à partir de la base de quelques rameaux suivi par un dessèchement localisé qui débute à partir des jeunes pousses (Figure 2). Enfin la maladie fini par l'envahissement total de l'arbre (évolution descendante) lorsque la dégradation atteint la totalité du collet et/ou des racines.

#### 3.2. Isolement et identification

Les isollements fongiques réalisés, à partir des échantillons de bois, de collet et de racines prélevés des oliviers malades, ont permis d'avoir plusieurs isolats fongiques qui se sont révélées appartenir à différents genres. L'identification morphologique basée sur l'utilisation des milieux sélectifs et/ou semi sélectifs suivant plusieurs clés d'identifications a montré que les champignons fréquemment isolés en pleins champs et en pépinières sont *Fusarium solani*, *Fusarium oxysporum*, *Fusarium lateritium*, *Rhizoctonia bataticola*, *Cylindrocarpon destructrices*, *Rhizoctonia solani*, *Corticium rolsii*, *Phytophthora* sp., et *Pythium* sp. (Booth, 1966; Waterhouse, 1968; Dhingra and Sinclair, 1978; Ribeiro, 1978; Bonnet *et al.*, 1980; Van der Plaats-Niterink, 1981; Jeffers et Martin, 1986; Maas, 1998; Agrios, 2004; Nasraoui, 2000 et 2006; John F. Leslie, 2006). L'espèce *Armillaria mellea* est souvent décelée sur des oliviers adultes souffrant d'asphyxie suite à une importante stagnation d'eau dans des sols lourds.

Des dégâts plus accentués se traduisant par des enroulements multiples au niveau du système racinaire sont également observés en plein champ sur des jeunes oliviers âgés de deux ou trois ans. Ces déformations racinaires sont notamment causées dès le stade pépinière, où le jeune olivier issu de bouture herbacée élevé émet au début une puissante racine pivotante, et se trouve par la suite rapidement limitée par les parois de conteneurs trop petits. Les étranglements et les lésions observées au niveau du système racinaire favorisent souvent l'invasion par les champignons telluriques. Ainsi, plusieurs cas de dépérissement sont observés sur des jeunes oliviers suite à un synergisme marqué entre *Fusarium solani* et *Rhizoctonia bataticola*.

#### 3.3. Émergence de la verticilliose de l'olivier en Tunisie

La Verticilliose de l'olivier causée par *Verticillium dahliae* Kleb. a été décrite pour la première fois en Tunisie dans une oliveraie de la délégation de Mahres à Sfax (Triki *et al.*, 2006). Les symptômes de cette maladie se manifestent de façon sectorielle, soit sur branche, soit sur charpentièr ou simplement quelques rameaux. La verticilliose induit sur les parties attaquées un flétrissement unilatéral puis les symptômes se généralisent. Les rameaux attaqués portent des feuilles qui s'enroulent en forme de gouttière vers leur face inférieure et perdent leur coloration verdâtre pour virer au brun clair, ce qui induit leur dessèchement complet. Sur les branches attaquées, les jeunes pousses peuvent apparaître partiellement ou totalement desséchées. Des coupes transversales ou longitudinales au niveau des tissus malades montrent souvent un brunissement du bois. Le système racinaire de quelques arbres malades présente parfois des altérations et un brunissement au niveau du cylindre central.

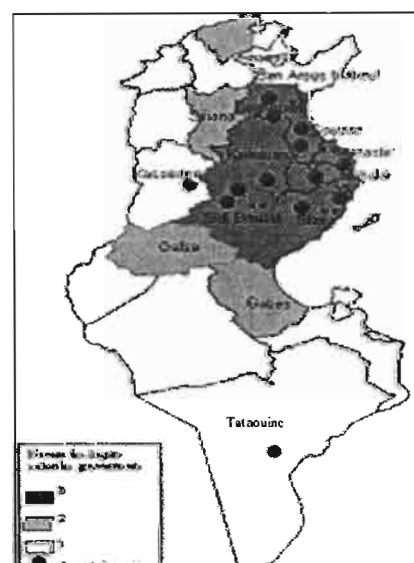


**Figure 1:** Cartographie des Foyers de dépérissement de l'olivier en Tunisie (● = foyers de Verticilliose de l'olivier).



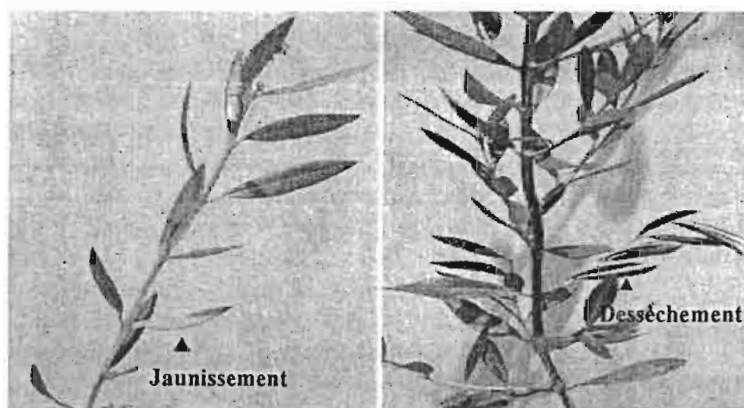


**Figure 2:** Evolution des symptômes de dépérissement sur un jeune olivier.

Les études épidémiologiques réalisées ces dernières années dans les principales oliveraies tunisiennes montrent une importante évolution spatio-temporelle de cette maladie dans plusieurs oliveraies situées dans différentes régions de divers étages bioclimatiques (Sfax, Sousse, Monastir, Sidi-Bouzyd, Kairouan et Zaghouane,...) (Fig. 1). Les investigations au champ et au laboratoire ont permis de mettre en évidence la présence de *Verticillium dahliae* Kleb. Tous ces nouveaux foyers sont découverts dans des périmètres irrigués où l'olivier est cultivé en intercalaire avec quelques espèces légumières (tomate, piment, pomme de terre, aubergine,...) sensibles à ce champignon.

Les travaux de caractérisation moléculaire des isolats de *Verticillium dahliae* sont basés sur l'amplification de séquences répétées (rep-PCR) et par l'amplification aléatoire de région de génome (RAPD). Parmi 20 amorces testées, uniquement dix ont été sélectionnées pour la qualité des profils et la productibilité des produits amplifiés (Fig. 3). En effet, les amorces sélectionnées ont révélé une variabilité de la structure génétique entre les isolats de *Verticillium dahliae* (Fig. 3). Ainsi, cette étude est fondamentale pour former des groupes d'isolats de structures génétiques homogènes liés aux sites géographiques de prélèvement des échantillons porteurs de la maladie.

L1 V1 V2 V3 V4 V5 V6 V7 L1 V8 V9 V10 V11 V12 V13 V14

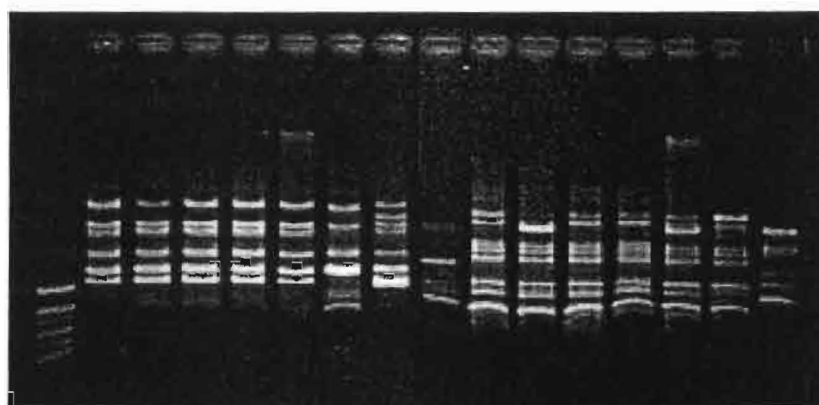


**Figure 3:** étude de la variabilité génétique de quelques isolats de *V. dahliae* par RAPD (Amorces OPD1 et OPK2 ; puits 2 à 8 et puits 10 à 16 = isolats de *V. dahliae*).

### 3.4. Tests de pathogénie

L'inoculation des jeunes plants d'oliviers de la variété Chemlali par les champignons telluriques isolés, confirme leur pathogénie par la production des symptômes caractéristiques de flétrissement, de jaunissement et de dessèchement à partir de la quatrième semaine de croissance. Toutefois, les cinq espèces fongiques *F. solani*, *R. solani*, *C. destructans*, *V. dahliae*, *Sclerotium rolfsii* et *R. bataticola* se sont avérées les plus agressives. D'ailleurs, les plantes inoculées par ces champignons sont totalement dépéries et présentent des fortes nécroses au niveau des collets et des racines. Il est également à signaler que les isolements fongiques effectués à partir des organes attaqués confirment la pathogénie de toutes les espèces fongiques inoculées.



### 3.5. Effet de quelques facteurs abiotique sur la manifestation de la maladie

Le phénomène de dépérissement des jeunes plants d'oliviers résulte d'une détérioration générale et graduelle des arbres pouvant se produire à cause de plusieurs facteurs biotiques et abiotiques ou de leur interaction. Les investigations au champ ont montré que les maximums d'attaques (80% environ) sont obtenus dans des sols lourds à forte rétention d'eau. Ces sols non filtrant et trop argileux favorisent la stagnation d'eau qui provoque la pourriture du collet et/ou des racines des jeunes oliviers et l'invasion par les champignons telluriques qui provoquent le dépérissement partiel ou total de l'arbre. D'autres facteurs comme la salinité de l'eau d'irrigation, la carence ou la toxicité d'un élément minéral et l'entretien de la parcelle (désherbage, façon culturales,...) peuvent contribuer fortement à l'installation de la maladie. En effet, le dépérissement peut être complexe et liée à l'invasion par un parasite de faiblesse qui précède un état de stress provoqué par un autre facteur non parasitaire (salinité, blessures,...). Il est à noter que le dépérissement des jeunes oliviers est souvent rencontré dans des parcelles ayant des espèces maraîchères sensibles à ces champignons telluriques en intercalaire avec l'olivier ou qui étaient cultivées précédemment.

### 4. Conclusion

Cette étude a montré que les nouvelles plantations d'oliviers sont menacées par des attaques de dépérissement en pépinière et en plein champ, dans plusieurs régions de la Tunisie. La plupart des symptômes observés sur olivier correspondaient à ceux décrits ailleurs dans plusieurs pays du bassin méditerranéen. Les investigations au champ et au laboratoire ont montré que la maladie est causée par un complexe de champignons telluriques qui sont capables d'induire des pourritures au niveau du collet et des racines. L'évolution rapide de la maladie dans l'espace et dans le temps justifie l'importance des dégâts en pépinière et en plein champ. En Tunisie, les hautes températures du sol (25-30°C) en printemps et en été et le recours à l'irrigation sont des conditions favorables à l'infection durant cette période. La présente étude a montré également que le dépérissement de l'olivier est sous l'influence de plusieurs facteurs abiotiques tel que la température, l'humidité, le type de sol, la salinité et la fertilisation. Ainsi, il est évident que les oliviers placés en situation difficile à cause d'un stress abiotique seront plus sensibles à une attaque par les champignons pathogènes que d'autres placés dans des conditions optimales. D'autre part, l'importante évolution spatio-temporelle de la verticilliose de l'olivier dans plusieurs dans différentes régions de divers étages bioclimatiques de la Tunisie nécessite l'étude de la diversité génétique des isolats de *Verticillium dahliae* et de leur pathogénie sur plusieurs variétés d'oliviers et hybrides issus de croisements dirigés (sensibilité variétale) dans le but de définir une stratégie de lutte efficace contre cette grave maladie.

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## Approche de la virulence de l'hylésine *Hylesinus oleiperda* (Coleoptera Scolytidae) vis-à-vis de quelques variétés d'olivier dans la région de Sfax

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### Résumé

Dans la perspective de rationaliser la lutte contre l'hylésine *Hylesinus oleiperda* (Coleoptera, Scolytidae), il convient de disposer, pour les nouvelles plantations oléicoles, de variétés d'olivier résistantes à l'attaque du scolyte. Disposant de deux collections variétales de l'OTD Bouzouita et Boughrara, cette étude a pour objectif de déterminer la virulence de l'hylésine vis-à-vis de quelques variétés autochtones d'olivier à huile et de table : Chétoui Tunis, Chemcheli Gafsa, Chemleli Sfax, Chemleli Ontha, Zarrazi Zarzis, Zalmati Zarzis, Ouesleti, Barouni du nord, Meski, Marsaline, Besbesi, Sayali et une variété étrangère la Picholine. Cette étude a reposé sur le dénombrement minutieux des plaques de l'hylésine sur 4 arbres par variété étudiée. Les résultats préliminaires obtenus dans les deux collections montrent que les variétés Chétoui Tunis et Chemcheli Gafsa sont les variétés tunisiennes les plus infestées par le scolyte. L'analyse statistique a permis de distinguer deux groupes bien distincts :

- Le premier groupe est composé de : Chemleli Sfax, Besbesi, Zarrazi Zarzis, Chemleli Ontha, Zalmati Zarzis, Marsaline, Sayali, Ouesleti et Picholine.
- Le deuxième groupe, le plus attaqué, est composé de Barouni, Meski, Chétoui Tunis et Chemcheli Gafsa.

Au champ, les résultats se confirment par les craquelures présentes sur le tronc des variétés les plus sensibles (Meski, Chétoui Tunis et Chemcheli Gafsa) alors qu'elles sont absentes sur les variétés de moindre sensibilité (Chemleli Sfax, Besbesi, Chemleli Ontha, Zarrazi Zarzis, Zalmati Zarzis, Marsaline et Sayali).

**Mots clés :** Hylésine, olivier, sensibilité variétale, lutte raisonnée.

### Abstract

In the perspective to rationalize the fight against *Hylesinus oleiperda* (Coleoptera, Scolytidae), it invites to arrange, for the new olive orchards, olive tree varieties resistant to the scolyte attacks.

Arranging two variety's collections of OTD Bouzouita and Boughrara, the aim of this study to determine the virulence of *H. oleiperda* face to some autochthonous varieties of olive tree: Chetoui Tunis, Chemcheli Gafsa, Chemleli Sfax, Chemleli Ontha, Zarrazi Zarzis, Zalmati Zarzis, Ouesleti, Barouni, Meski, Marsaline, Besbesi, Sayali and a foreign variety the Picholine.

This study consists in the precious numbering of plates of *H. oleiperda* on 3 to 4 trees by studied variety.

The exploratory results gotten in the two collections show that Chetoui Tunis and Chemcheli Gafsa are the most infested are the Tunisian varieties. The statistical analysis permitted to distinguish two distinct groups:

- The first group is composed of: Chemleli Sfax, Besbesi, Zarrazi Zarzis, Chemleli Ontha, Zalmati Zarzis, Marsaline, Sayali, Ouesleti and Picholine.
- The second group, the less attacked, is composed of Barouni, Meski, Chetoui Tunis and Chemcheli Gafsa.

In the field this results are confirmed by the existence of flakings on trunks of the most sensitive varieties (Meski, Chetoui Tunis and Chemcheli Gafsa). Whereas these flakings are absent on less sensitives ones (Chemleli Sfax, Besbesi, Chemleli Ontha, Zarrazi Zarzis, Zalmati Zarzis, Marsaline and Sayali).

**Key words:** *Hylesinus oleiperda*, olive tree, variety's sensitivity, reasoned struggle.

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## 1. Introduction

L'hylésine *Hylesinus oleiperda* est un coléoptère de la famille des *Scolytidae* qui s'attaque préférentiellement aux jeunes oliviers âgés de 4 à 25 ans et peut entraîner le dépérissement voir la mort totale de l'arbre.

Les attaques de l'hylésine sont caractérisées par l'apparition, sur l'écorce du tronc et des branches charpentières, des oliviers attaqués, de plaques de couleur marron portant des trous de pénétration et de sortie des adultes, qui en évoluant ce craquelle et cause l'affaiblissement puis le dépérissement de l'arbre.

Partout dans le monde, où il est signalé, les recherches sur ce scolyte sont presque inexistantes. Les seuls travaux sont conduits en Tunisie vers la fin des années cinquante par Touzeau (1957) puis furent repris par Jardak et al, (1997, 2002, 2004) à l'Institut de l'Olivier vers la fin des années quatre vingt dix.

Les moyens de lutte préconisés se limitent à quelques traitements chimiques à base de Deltaméthrine (le Décis) ou d'un mélange Décis- Diméthoate.

L'objectif de ce travail est de disposer, à partir des variétés locales, de plants d'oliviers plus ou moins résistants pouvant être préconisés pour l'installation des nouvelles plantations.

## 2. Matériel et méthodes

### 2.1. Biotope d'étude

Tous les travaux ont été conduits dans les parcelles de l'agrocombinat de Bouzouita sises à Boughrara. On a disposé, de deux collections variétales :

La première, de l'ordre de 6,5 ha, est composée de 600 plants issus de boutures herbacées âgés de 10 à 14 ans selon les années de plantation, à la densité de 8 sur 12. Elle compte une centaine de variétés locales et étrangères.

La seconde, de 12 ha est composée de 284 plants greffés, en 1990, sur des portes greffes Chemleli plantés selon l'écartement normal de la région de Sfax, soit 24 sur 24. On y compte une soixantaine de variétés locales et étrangères.

### 2.2. Les variétés étudiées

Les variétés retenues pour mener cette étude sont :

- Parcelle I, 11 variétés: Chétoui Tunis, Chemcheli Gafsa, Chemleli Sfax, Chemleli Ontha, Zarrazi Zarzis, Zalmati Zarzis, Ouesleti, Barouni du nord, Meski, Marsaline, Besbesi, Sayali et une variété étrangère la Picholine.

- Parcelle II, 8 variétés : Chemcheli Gafsa, Oueslati, Chétoui Tunis, Meski, Barouni du nord, Zalmati Zarzis, Zarrazi Zarzis et la Picholine.

Le nombre d'arbres analysés par variété est variable selon leur disponibilité dans chaque parcelle, tel que présenté dans le Tableau 1 :

**Tableau 1 :** Nombre d'arbres analysés par variété et par parcelle.

| Variétés                   | Parcelle I | Parcelle II |
|----------------------------|------------|-------------|
| Chemleli Sfax (Chl sf)     | 12         | *           |
| Meski (Mk)                 | 6          | 4           |
| Chemcheli Gafsa (Chm)      | 5          | 3           |
| Zarrazi Zarzis (Zr)        | 5          | 4           |
| Barouni du nord (Br)       | 5          | 3           |
| Chétoui Tunis (Cht)        | 4          | 4           |
| Zalmati Zarzis (zl)        | 4          | 4           |
| Chemleli Ontha (Chl Ontha) | 4          | *           |
| Marsaline (Mr)             | 3          | *           |
| Besbesi (Bs)               | 2          | *           |
| Sayali (Syl)               | 2          | *           |
| Ouesleti                   | *          | 4           |
| Picholine                  | *          | 4           |

### 2.3. Technique employée

L'étude de la virulence de l'hylésine vis-à-vis de différentes variétés d'oliviers, disponibles en même temps dans le même verger et dans les mêmes conditions de culture, repose sur le dénombrement des anciennes et nouvelles plaques sur le tronc et toutes les branches de chaque arbre analysé. Pour éviter tout risque d'erreur et de confusion, les plaquettes anciennes sont marquées par une croix alors que les nouvelles sont encerclées.

Parallèlement au dénombrement des plaques, on a pris soin de gratter quelques unes pour relever la structure des populations du phytophage au moment de l'étude (été 2006).

## 3. Résultats et discussion

### 3.1. Structure de la population de l'hylésine

Les travaux conduits par Jardak et al, (1997) signalent que l'hylésine effectue 2 générations par an. La première est au printemps et la seconde en automne.

En Juillet (période de déroulement de cette étude) l'hylésine se trouve sous sa phase de nuisible endophyte à l'intérieur de l'écorce.

L'observation à la loupe binoculaire des plaques, ramenées au labo après grattage de l'écorce, a montré la présence de tous les stades préimaginaux : des œufs, des larves, des nymphes en plus des femelles présentes encore dans les plaquettes.

Bien que tous les stades coexistent, la population de l'hylésine est constituée à 62.5% par des œufs vivants, 12.5% des larves vivantes, 8% des nymphes et 16.7% des adultes (Fig.1).

Une telle structure témoigne de la pleine activité de l'insecte en phase de reproduction.

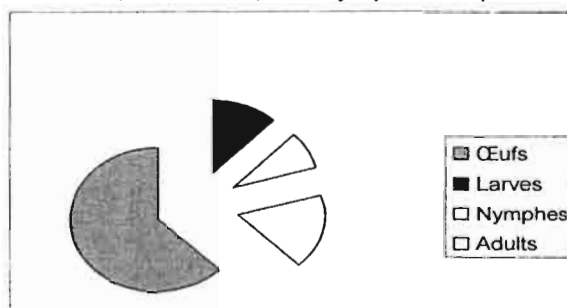


**Figure 1:** Structure de la population d'*Hylesinus oleiperda* durant l'été 2006 à Boughrara.

### 3.2. Sensibilité variétale

Le suivi des attaques de l'hylésine sur olivier dans les deux parcelles permet de distinguer deux types d'infestation :

Les anciennes attaques caractérisées généralement par la craquelure de l'écorce au niveau des galeries maternelles et larvaires.

Les nouvelles plaques qui ont lieu au printemps 2006 suite au développement d'une nouvelle génération.

Les niveaux d'attaques sont tout à fait différents entre les deux parcelles. La parcelle I est la moins infestée, avec des maxima ne dépassant pas 70 et 5 plaques anciennes et nouvelles/ arbre. Toutefois, les différentes variétés cultivées ne présentent pas la même intensité d'attaque.

### 3.2.1. Parcelle I

Les résultats du comptage des anciennes plaques sont illustrés par la Figure 2.

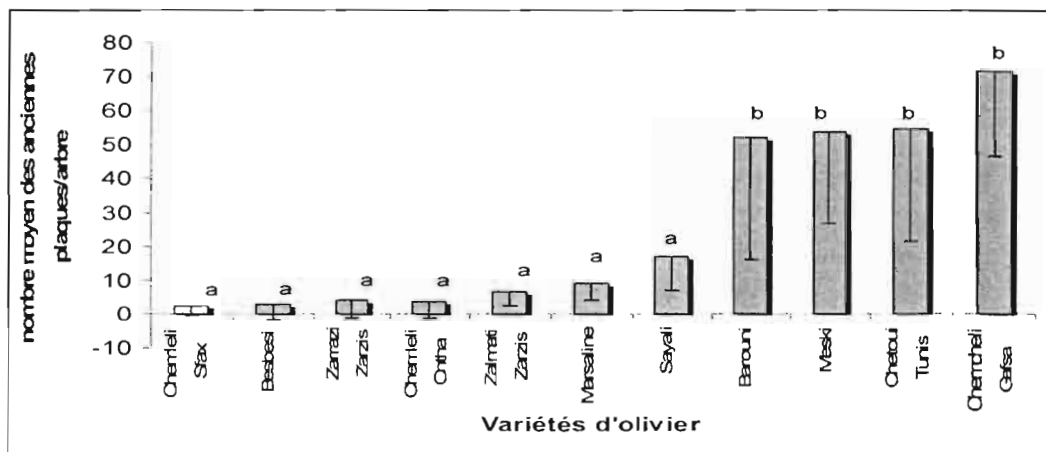


**Figure 2:** Variation du degré d'infestation par l'hylésine des différentes variétés d'olivier cultivées dans la parcelle I. Les variétés suivies de la même lettre ne sont pas significativement différentes au seuil de 1%.

Il ressort de la Figure 2 que nous sommes devant trois degrés d'infestations:

- Les variétés à faible degré d'infestation : Chemleli Sfax, Besbesi, Zarrazi Zarzis, Chemleli Ontha et Zalmati Zarzis.
- Les variétés ayant un degré d'infestation moyen : Marsaline et Sayali.
- Les variétés ayant un degré d'infestation élevé : Meski, Chétoui Tunis et Chemcheli Gafsa.

Toutefois, l'analyse statistique moyennant le test d'homogénéité (test Duncan, logiciel SPSS 11.0) entre les variétés, a révélé la présence de deux groupes bien distincts:

- Le premier groupe, le moins attaqué, est composé de Chemleli Ontha, Zarrazi Zarzis, Chemleli Sfax et Zalmati Zarzis.
- Le deuxième groupe est composé de Barouni, Chétoui Tunis, Meski et Chemcheli Gafsa.

Les variétés Marsaline, Sayali et Besbesi n'ont pas fait l'objet d'analyse statistique vu la rareté des représentants de chaque variété.

Au champ, les attaques se manifestent par les craquelures présentes sur le tronc des variétés censées être les plus sensibles (Meski, Chétoui Tunis et Chemcheli Gafsa) alors qu'elles sont absentes sur les variétés de moindre sensibilité (Chemleli Sfax, Besbesi, Chemleli Ontha, Zarrazi Zarzis, Zalmati Zarzis, Marsaline et Sayali).

Il en ressort aussi que la variété Chemcheli Gafsa est la plus recherchée par l'hylésine. Ce résultat élargi la liste des variétés sensibles étudiées par Ksantini et Jardak (2004), Jardak et al, (1997), Gouider (1983) et par Touzeau (1957).

La moyenne des plaques anciennes par variété représente le cumul dans le temps de l'ensemble des attaques du scolyte. Toutefois, un organisme vivant (plante, insecte, champignon...) se caractérise par l'évolution est non pas par la stabilité, alors, est ce que l'hylésine a gardé le comportement envers les variétés de l'olivier dans la parcelle I ?

Pour répondre à cette question on s'est référé au nombre des nouvelles plaques. Celles-ci ont eu lieu après l'envol printanier de 2006. Les résultats de ces comptages sont illustrés dans la Figure 3.

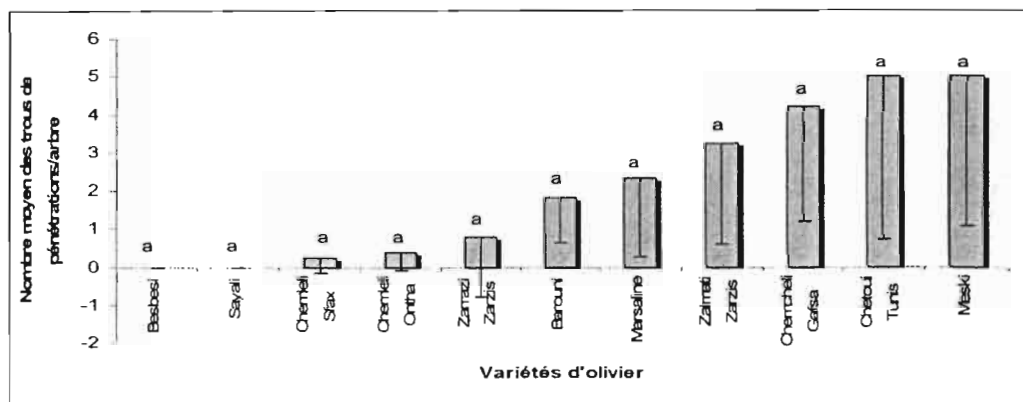


**Figure 3:** Variation des nouvelles plaques par variété dans la parcelle I. Les variétés suivies de la même lettre ne sont pas significativement différentes au seuil de 1%.

D'après la Figure 3, il apparaît que l'hylésine n'a pas changé de comportement avec le temps. Elle a toujours une préférence plus importante aux variétés Chemcheli Gafsa, Chétoui Tunis et Meski qu'aux variétés Barouni, Sayali, Marsaline, Zalmati Zarzis, Zarrazi Zarzis, Chemleli Ontha, Chemleli Sfax et Besbesi.

Cependant on note une légère variation de la réceptivité entre Chemcheli Gafsa et Chétoui Tunis : il y a plus de nouvelles plaques sur la Chétoui que sur la Chemcheli.

Toutefois, le test de Duncan appliqué aux 8 variétés déjà analysées révèle qu'elles appartiennent à un seul groupe d'infestation (de 0.4 à 5.8 plaques /arbre).

### 3.2.2. Parcelle II

Les résultats illustrés par la figure 4 montrent une homogénéité de l'infestation entre 7 variétés: Barouni, Oueslati, Picholine, Meski, Chemcheli Gafsa, Zalmati Zarzis, Zarrazi Zarzis, alors que la variété Chétoui Tunis est, cependant, la plus infestée. Ceci est confirmé par le test de Duncan qui révèle deux groupes bien distincts:

- Le premier groupe est composé de : Barouni, Oueslati, Picholine, Meski, Chemcheli Gafsa, Zalmati Zarzis, Zarrazi Zarzis.
- Le deuxième groupe est composé de Chétoui Tunis seul.

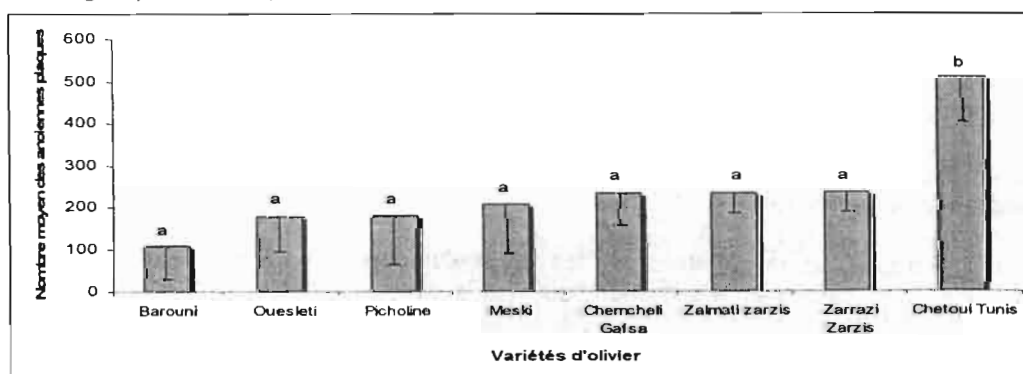


**Figure 4:** Histogramme des plaques anciennes /arbre/variété dans la parcelle II. Les variétés suivies de la même lettre ne sont pas significativement différentes au seuil de 1%.



En comparant le niveau d'attaque des deux parcelles expérimentales nous remarquons que toutes les variétés de la parcelle II sont beaucoup plus attaquées que celles de la parcelle I.

En effet la moyenne maximale des plaques par arbre dans cette dernière est de 71.8 alors que dans la première, la moyenne minimale des plaques par arbre est de 102. En outre on note des craquelures de l'écorce de toutes les variétés. Ces craquelures sont bien développées au point que le comptage des anciennes plaques devient très difficile.

La différence du degré d'infestation entre les deux parcelles peut être expliquée par les la vigueur des oliviers qui sont greffés sur Chemleli dans la parcelle II et issus de boutures herbacées dans la parcelle I.

Comme pour la parcelle I nous avons tenu à vérifier si l'hylésine a gardé le même comportement préférentiel des variétés de l'olivier dans le temps (Figure 5).

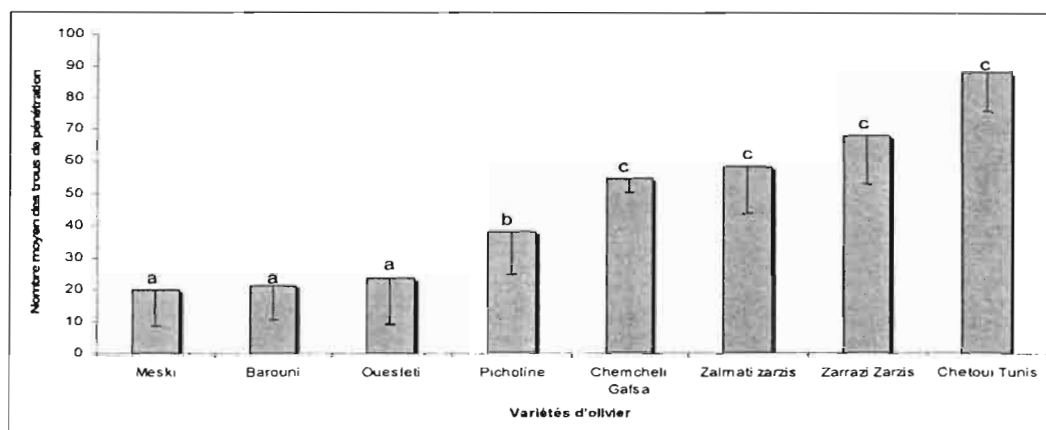


**Figure 5:** Variation des nouvelles infestations par variété dans la parcelle II. Les variétés suivies de la même lettre ne sont pas significativement différentes au seuil de 1%.

D'après la Figure 5, on constate que la sensibilité des variétés se confirme au niveau des nouvelles attaques. Ce qui conclut que l'hylésine n'a vrai semblablement pas changé de comportement envers les différentes variétés.

En effet, nous constatons, à l'opposé de la parcelle I, que les différentes variétés de la parcelle II comportent des degrés d'infestation variant de 18 à plus de 80 plaques/arbre.

L'analyse statistique, par le test Duncan fait ressortir 3 groupes d'infestation qui se résument comme suit :

- Les variétés fortement sensibles : Chétoui Tunis, Zarrazi Zarzis, Zalmati Zarzis et Chemcheli Gafsa.
- Une variété moyennement sensible : Picholine,
- Les variétés moyennement résistantes : Meski, Barouni et Ouesleti.

Paradoxalement avec la première parcelle, la variété Meski, se trouve la moins infestée. Elle semble avoir développé un mécanisme de défense contre pour les adultes de l'hylésine.

Dans ce contexte, par ailleurs, on a pu isoler quelques réactions développées par certaines variétés qui se traduisent par des sécrétions de résine chez la Marsaline, la Picholine, la Ouesleti. Ce constat fut même observé chez la Chétoui Tunis qui a excrété des taches huileuses et des taches blanches tout autour des plaques de ponte de l'hylésine.

Ces différents aspects de réaction aux attaques de l'hylésine méritent d'être étudiés pour comprendre les mécanismes de résistance développés par ces variétés dans leur aire de culture traditionnelle.

#### 4. Conclusion

En conclusion, à tout ce qui a été présenté précédemment, l'hylésine est un scolyte qui s'attaque à l'olivier avec des préférences variétales différentes.

Nous avons pu classer environ 12 variétés autochtones et une variété française la Picholine selon le degré d'attaque par l'hylésine. Toutefois, les résultats obtenus restent préliminaires et méritent d'être approfondis dans une étude plus détaillée et portant sur un nombre de répétition plus important et sur une période d'étude plus longue.

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## Rôle des biosenseurs pour une meilleure maîtrise endogène et exogène des systèmes de culture : application à l'olivier

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### Résumé

La biodiversité représente aujourd'hui un enjeu scientifique, social, économique et politique considérable. La biodiversité domestique recouvre la biodiversité des espèces cultivées. Pour une espèce donnée plus elle a de diversité, plus elle a de chance de trouver une solution aux différents problèmes qui se poseront (sécheresse, salinité du sol, maladie fongique...). C'est dans ce contexte général que nous allons présenter le rôle des biosenseurs dans la compréhension des stratégies adaptatives et à l'évaluation des réponses aux interventions de l'homme assurant respectivement une stabilité endogène et exogène du système de culture. Nous nous intéresseront particulièrement à l'olivier variété Chemlali une espèce ligneuse clé des agro systèmes semi arides et arides tunisiens. Malgré les nombreux travaux sur son adaptation à l'aridité les études sur son fonctionnement hydrique sont rares. Avec le développement techniques des méthodes d'évaluation du fonctionnement hydrique nous avons adopté une approche d'évaluation quantitative en continue du fonctionnement hydrique global de l'arbre grâce aux méthodes thermo électrique de mesure du flux de sève brute, ce ci nous a permis de quantifier la consommation en eau des arbres et d'identifier les relations entre la dynamique saisonnière de ces flux et les déterminismes majeurs de la production entre autres les échanges gazeux et leur régulation.

**Mots clés:** *Olea europaea* L., trunk shaker, mechanical beater, pneumatic combs, oil quality.

## Biosensors role for monitoring endogenous and exogenous stability of the olive culturing system

### Abstract

Today, biodiversity represents a considerable scientific, social, economic and political issue. The domestic biodiversity covers the biodiversity of the cultivated species. The more diversity a given species has, the more chance it has to overcome different problems (drought, soil salinity, fungal illness...). In this general context, we will present the biosensors role in the understanding of the adaptive strategies and the evaluation of the responses to man's interventions ensuring respectively an endogenous and exogenous stability of the culturing system. We are interested particularly in Chemlali variety, a woody species the key of arid and semi arid Tunisian agro systems.

In spite of the numerous works on its adaptation to aridity, the studies about its water functioning are scarce. With the technical development of the evaluation methods of water functioning, we have adopted a quantitative evaluation approach continuous to the global water functioning of the tree thanks to the thermo electrical methods such as brute sap flux, this enabled us to quantify water consumption for trees and to identify the relationships between seasoned dynamics of these fluxes and major determinisms and production between others gas exchanges and their regulation.

**Key words:** *Olea europaea* L., trunk shaker, mechanical beater, pneumatic combs, oil quality.

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## 1. Introduction

Aujourd'hui, la Méditerranée représente 98% de la production mondiale d'huile d'olive. La production d'huile d'olive joue un rôle déterminant pour les économies et l'emploi, ainsi que pour la biodiversité des régions méditerranéennes. Marqueur du territoire, l'olivier est un capital précieux et un patrimoine à sauvegarder et à enrichir.

Dans les pays du sud de la méditerranée l'évolution du climat montre que le réchauffement climatique est plus important que la moyenne. Si au niveau planétaire la hausse des températures durant le 20ème siècle a été de 0,75 °C, celle sur le Maghreb s'est située entre 1,5 et 2 °C selon les régions, soit plus du double de la hausse moyenne mondiale. Quant à la baisse des précipitations, elle varie entre 10 et 20 %.

Malgré cet environnement aride de plus en plus hostile découlant d'un changement climatique notoire, l'olivier est bien ancré dans le paysage tunisien et constitue le seul arbre cultivé pouvant valoriser cette région de plus en plus fragilisée par des pratiques agricoles erronées. La sauvegarde et la gestion rationnelle de cet arbre sont une des priorités nationales. L'eau étant la contrainte principale à la production, l'étude du fonctionnement hydrique de l'arbre pourrait orienter la conduite culturale rationnelle. Le développement technologique des équipements de contrôle de l'état hydrique de l'arbre a provoqué une révolution dans le domaine de la recherche ecophysiologique.

Un certain nombre de caractéristiques permettent de décrire l'état hydrique du végétal. Plusieurs travaux ont montrés que l'état énergétique de l'eau ( $\psi F$ ) et la transpiration sont très corrélés avec la croissance d'une part et le développement d'autre part. Ces deux paramètres peuvent intervenir dans la détermination du rendement en biomasse et en fruits chez une variété donnée. De plus, le maintien d'un bon état hydrique et une faible transpiration peuvent être des indicateurs d'une bonne adaptation des plantes à la sécheresse (Henchi et al. 1986). Ainsi, le maintien de la production dépend des mécanismes de tolérance physiologiques qui assurent l'hydratation cellulaire et diminuent la perte en eau en maintenant un état hydrique favorable à l'élaboration du rendement. Ce dernier est étroitement lié au rendement photosynthétique qui est un des premiers processus affecté par la sécheresse du notamment à la fermeture des stomates. En effet, cette fermeture qui a lieu pour réduire les pertes hydrique agit aussi sur le flux de carbone en limitant aussi son accès aux sites de carboxylation (Flexas et al., 1998 and Escalona et al., 2002). Ainsi, le suivi du statut hydrique des plantes en plein champ est d'un grand intérêt. Elle permettra de diagnostiquer l'installation d'un éventuel stress et de quantifier sa sévérité et d'orienter les interventions culturales qui ont un rapport avec l'état hydrique de la plante (contrôle de la surface transpirante par la taille, amélioration de l'état hydrique du sol...etc.). Dans ce contexte général s'inscrit notre travail qui a pour objectif de voir l'efficacité des senseurs biologiques pour le suivi du comportement de l'olivier dans des conditions culturales variées et diagnostiquer des situations de stress hydrique à la quelle il faut intervenir.

## 2. Matériels et méthode

Le travail est effectué dans une plantation d'olivier conduite en pluvial. Différentes densités de plantation supérieures à celles utilisées dans les plantations traditionnelles sont testées sur les plants agronomiques et biologiques et ce depuis la mise en place de la parcelle. Ces relevés ont montré un recul de la production et de la vigueur par arbre avec l'augmentation de la densité de plantation et ceci malgré une production par ha encore croissante avec la densité de plantation (Boujnah et al. 2006). L'objectif de ce travail est de voir le comportement hydrique et physiologique de l'arbre pour les différentes densités de plantation en vu de comprendre le comportement de l'arbre vis-à-vis de l'eau disponible (à travers les différents saisons et selon les densité de plantation) et de diagnostiquer un éventuel effet de compétition.

### 2.1. Conditions expérimentales

Le parcelle expérimentale est situé à Jemmel au centre Est de la Tunisie. Elle appartient au domaine du Centre de Formation Professionnelle Agricole de Jemmel (CFPA de Jemmel). La zone fait partie du climat méditerranéen semi-aride à étage supérieur. La pluviométrie moyenne annuelle est de 350 mm. La saison sèche s'étale sur huit mois et l'amplitude thermique est faible. Le sol est profond (50 à 60 cm), argilo- limoneux à accumulation calcaire, la texture est moyenne, le taux de matière organique est faible et le pH est autour de 8 (alcalin).

La parcelle expérimentale s'étend sur 3,5 ha. Elle est divisée en quatre lots. Chaque lot se distingue par une densité de plantation différente. Les densités testées sont toutes inférieures à celle utilisée dans l'entourage qui est de 40 arbres par ha (plantés en carré selon les écartements 16x16). Ces densités se présentent comme suit: 51, 70, 100 et 156 arbre /ha ce qui correspond respectivement aux écartements 14x14 m, 12x12 m, 10x10m et 8x8 m.

La variété cultivée est la Chemlali. Les arbres sont âgés de 20 ans. Cette variété est vigoureuse, productive, et résistante à la sécheresse.

## **2.2. Mesures réalisées**

Le potentiel hydrique foliaire:

Le potentiel hydrique foliaire est évalué par une chambre à pression (Modèle1000, Scholander et al., 1965). Il a été déterminé sur des feuilles adultes prélevées à la périphérie de la frondaison à hauteur d'homme et ce tous les 15 jours durant toute la période de l'essai.

### **2.2.1. La résistance stomatique:**

Ces mesures ont été au poromètre de type « AP4 Delta T Device ». Cet appareil nous permet d'évaluer le degré d'ouverture moyenne des stomates en déterminant la résistance à la diffusion de la vapeur d'eau émise par une portion de feuille insérée dans une chambre de mesure. Ces mesures ont été réalisées tous les 15 jours durant toute la période de l'essai.

### **2.2.2. Flux de sève**

La mesure du flux de sève xylémique est effectuée selon la méthode thermique de Granier (1985) à chauffage alternatif (Do et Rauchteau, 2002). Le fluxmètre est constitué par deux sondes cylindriques identiques, de longueur 20mm et de diamètre 1,8mm. L'une est chauffée d'une manière discontinue (20mn sans chauffage et 10mn avec chauffage). Chaque sonde contient un thermocouple Cuivre-Constantan permettant de mesurer l'écart de température entre les deux thermocouples. La tension électrique de chauffage est assurée par une batterie de 12 V. La saisie automatique des données est programmée suivant un pas périodique de 60s avec une moyenne enregistrée en mémoire toutes les 5mn à raison d'un cycle de chauffage/non-chauffage de 10mn/20mn. Les données enregistrées sont récupérées tous les 15 jours à l'aide d'une centrale d'acquisition de données (Data Logger). Seule la deuxième valeur de la phase chauffage est considérée pour la quantification des flux de sève, suivant la formule suivante :  $U = J.S$

-  $J$  ( $m^3.s^{-1}$ ) est la densité de flux estimée par le suivi d'un coefficient  $K$  qui dépend des différences de température entre les deux sondes du capteur, avec  $K = [T(0) - T(u)] / T(u)$  où  $T(0)$  est la différence de température à flux de sève nul,  $T(u)$  : la différence de température à flux de sève de densité  $u$  ;

-  $S$  ( $cm^2$ ) est la surface conductrice du bois, elle est déterminée par coloration des coupes de génératrice après l'expérimentation, elle est estimée à 70% de la section du bois. Les relevées se sont étalées sur toute la période expérimentale.

### **2.2.3. La photosynthèse:**

La photosynthèse nette est mesurée à l'aide d'un appareil de type LI-COR LI-6400. Il s'agit d'un système ouvert qui mesure à la fois la concentration en  $CO_2$  et  $H_2O$  de l'air de référence (atmosphère) et de l'air issu de l'échantillon situé dans la chambre. Le taux de photosynthèse nette est exprimé par la même unité de  $CO_2$  ( $\mu mol CO_2.m^{-2}.s^{-1}$ ). Ce paramètre a été évalué au cours du printemps qui est la période d'activité biologique la plus intense de l'olivier

## **3. Résultats et discussion**

### **3.1. Potentiel hydrique foliaire**

Les courbes de la Figure 1 illustrent l'évolution du potentiel hydrique foliaire de base chez l'olivier Chemlali planté à différentes densités. Ce paramètre mesuré en fin de nuit, alors que la transpiration est négligeable et que la plante a reconstitué ses réserves en eau, renseigne sur la disponibilité en

eau du sol et fournit une information sur l'état hydrique dans lequel se trouve le végétal, en raison d'une plus faible variabilité des conditions de milieu. En effet, on considère que la tension de sève dans le végétal est en équilibre avec le potentiel hydrique du sol dans la zone d'implantation des racines.

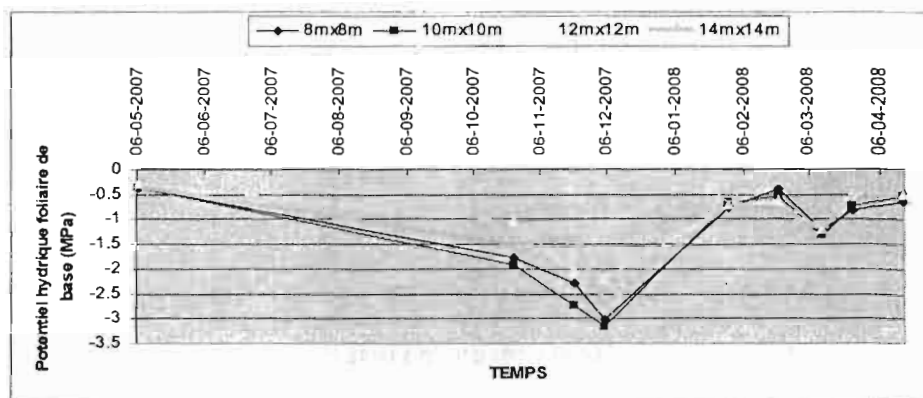


**Figure 1:** Evolution du potentiel hydrique foliaire de base en fonction du temps pour l'essai de révision des densités de plantation en culture pluviale de la parcelle expérimentale de Jemmel.

En fonction de la densité de plantation nous remarquons des potentiels hydriques plus bas chez les arbres plantés aux densités les plus élevées pour la période s'étalant de juin 2007 à janvier 2008 avec les valeurs les plus élevées pour les arbres plantés aux écartements 70 arbres/ha.

Par ailleurs, le potentiel hydrique foliaire minimal illustré par les histogrammes de la Figure 2 confirme ces résultats puisque cette densité s'est distinguée par le potentiel hydrique le plus élevé pendant la période estivale où les conditions hydriques sont à leur minimum. Durant la période printanière, qui correspond à la période d'activité la plus intense chez l'olivier puisqu'au cours de la quelle se déroule simultanément la croissance végétative et le développement fructifère et pour les deux potentiels hydriques (de base et minimum) les courbes se confondent. Ceci témoigne des grandes aptitudes de cette espèce à moduler son potentiel hydrique en fonction de la disponibilité de l'eau dans le sol. Le maintien de la production dépend des mécanismes de tolérance physiologiques qui assurent l'hydratation cellulaire et diminuent la perte en eau en maintenant un état hydrique favorable à l'élaboration du rendement dans ses différentes phases (organogenèse florale, floraison, fécondation... etc.). En conditions de déficit hydrique, la baisse du potentiel hydrique des plantes induit une importante perte de turgescence au niveau des feuilles (Boyer 1982 et Sonells et al. 2000). Le maintien d'un potentiel hydrique élevé est lié à l'aptitude à extraire l'eau du sol et à la capacité à limiter les pertes d'eau par transpiration (Turner 1986) Il caractérise une stratégie d'esquive à la déshydratation (Levitt, 1980).

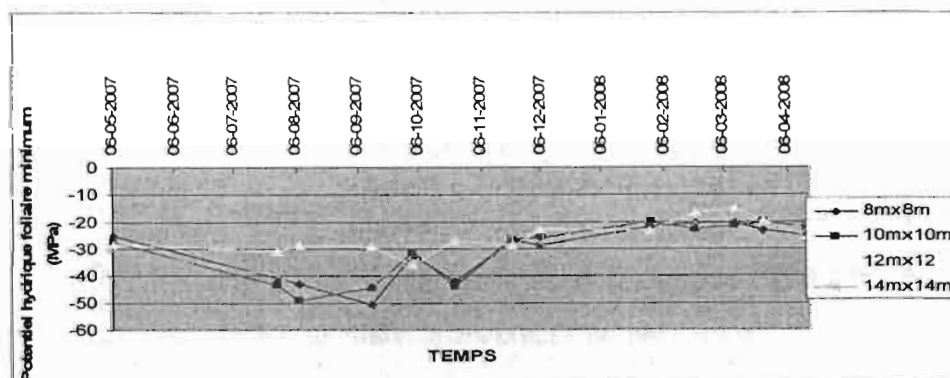


**Figure 2:** Evolution du potentiel hydrique foliaire minimum en fonction du temps pour l'essai de révision des densités de plantation en culture pluviale de la parcelle expérimentale de Jemmel.

L'absence de différence entre les autres écartements testés peu être attribué au comportement iso hydrique qui mène à des valeurs similaires du potentiel hydrique chez l'olivier (Escalona et al., 2002). Ceci sera dû à la régulation stomatique de la transpiration qui pourrait entraîner une baisse importante du potentiel hydrique menant à un endommagement des feuilles (Naor and Wample, 1994 and Schultz and Matthews, 1988). Les mesures de la conductance stomatique (Fig. 3)

confirment ces constatations puisque l'intensité des pertes hydriques par les stomates a varié en fonction de la situation culturale (densité de plantation). D'après Tyree and Sperry, 1988 la fermeture des stomates est une stratégie efficace qui permet d'éviter les pertes excessives d'eau pouvant mener à une basse importante du potentiel hydrique à des niveaux dangereux pour la plante. Chez l'olivier Natali et al. (1985) montrent que cette espèce semble réduire ses pertes hydriques par la fermeture des stomates jusqu'à un certain niveau par la suite il peut changer de stratégie en accentuant la transpiration ce qui augmente l'absorption par les racines.

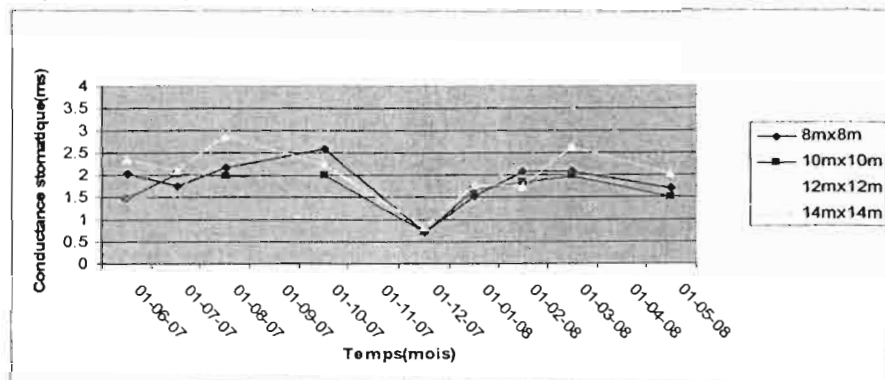


**Figure 3:** Evolution de la conductance stomatique en fonction du temps pour l'essai de révision des densités de plantation en culture pluviale de la parcelle expérimentale de Jemmel.

Malgré l'efficacité et la précision de ces paramètres hydrique pour l'étude du comportement hydrique de l'olivier ces deux méthodes présentent le désavantage d'être évaluées d'une façon discontinue, ceci peut être résolu par l'établissement d'une relation quantitative entre le statut hydrique de la plante et d'autres paramètres physiologiques qui peuvent être plus facilement relevés. C'est ainsi que nous avons mesuré le flux de sève en vu d'avoir une estimation instantanée et précise de la transpiration foliaire (Las Cano et al., 1992, East End and Gray, 1998, Braun and Schmid, 1999 and Escalona et al., 2002).

Les courbes de la Figure 4 illustrent l'évolution durant deux journées consécutives du flux de sève en fonction de la densité de plantation pour les quatre saisons. Ce paramètre permet de quantifier l'utilisation de l'eau par la plante. Nous remarquons des différences de consommation hydrique en fonction des saisons pour les différentes densités de plantation. Pour la saison automnales les différences entre les différentes densités ne sont pas significatives par contre une nette différence est observée durant la saison estivale où les conditions hydriques sont les plus sévère.

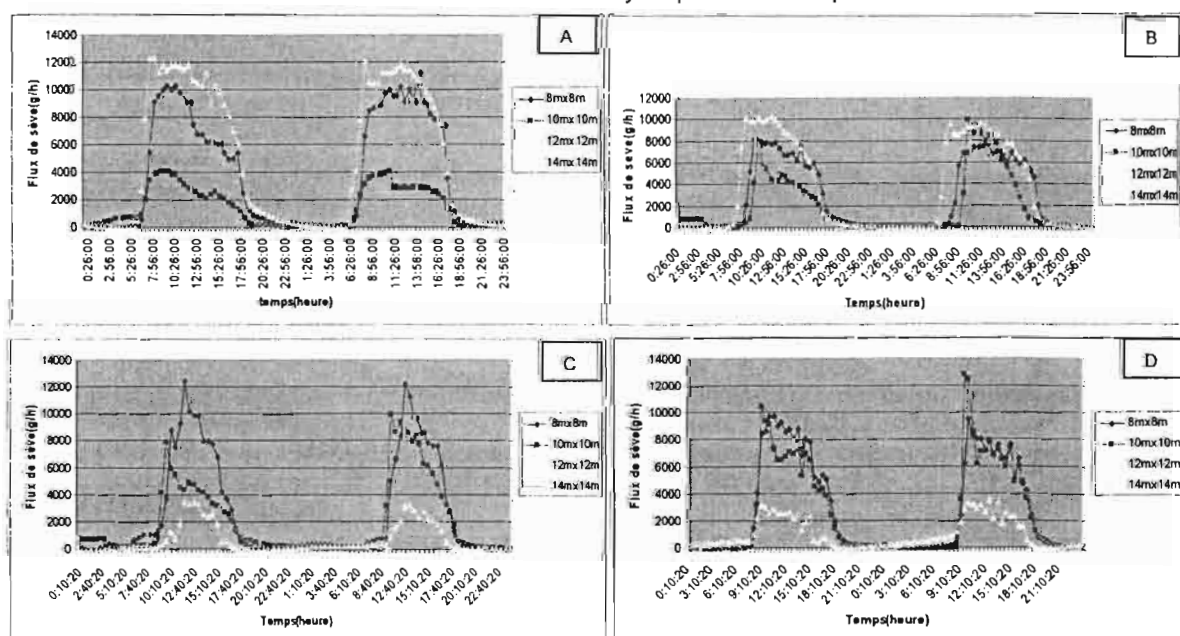


**Figure 4:** Evolution journalière de flux de sève au cours de deux jours consécutifs pendant la saison estivale (A) Automnale (B), Hivernale (C) et Printanière (D) pour l'essai de révision des densités de plantation en culture pluviale de la parcelle expérimentale de Jemmel.



L'estimation des pertes hydrique transpiratoires donne une idée sur l'intensité du stress et permet de palier à ces pertes par des interventions culturales au moment opportun. En effet la détection d'une baisse continue de la consommation hydrique journalière peut être considéré comme un signal de début de stress. Fernandez et al. (2001) ont mis en évidence le double avantage de cette technique pour l'olivier puisqu'elle peut être utilisée comme un outil de recherche pour fournir une mesure robuste de l'utilisation de l'eau et le fonctionnement hydraulique de l'arbre d'un côté et le potentiel d'être utilisé comme un dispositif pour fournir un signal à partir de laquelle les décisions peuvent être prises concernant la nécessité et le calendrier de l'irrigation.

Les courbes de la Figure 5 illustrent les variations de la photosynthèse en fonction de la densité de plantation et ce au cours de la saison printanière, période d'activité biologique la plus intense ce paramètre donne une idée sur le potentiel de croissance des plantes. En fonction de la densité de plantation nous n'avons remarqué au démarrage de l'activité biologique au printemps aucune différence entre les quatre densités de plantation, par la suite le taux de photosynthèse augmente pour atteindre des valeurs maximales au mois d'avril.

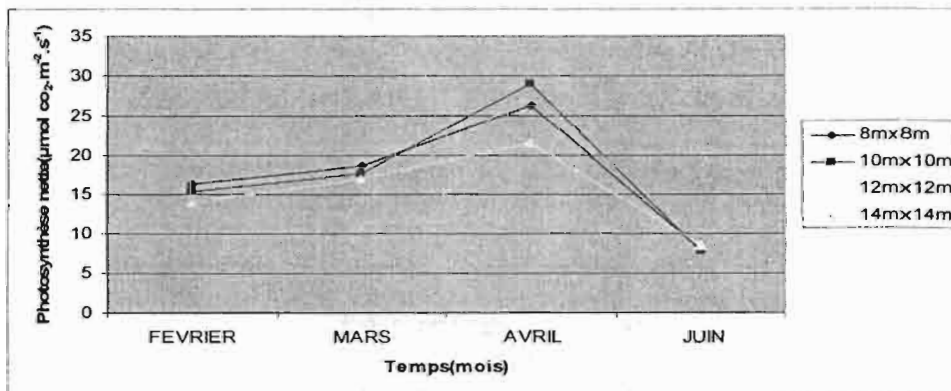


**Figure 5:** Evolution de la photosynthèse nette en fonction du temps pour l'essai de révision des densités de plantation en culture pluviale de la parcelle expérimentale de Jemmel.

Avec l'augmentation de la température au mois de juin nous assistons à une baisse de la photosynthèse avec des valeurs similaires pour tous les traitements. Pour ce paramètre les seules différences significatives ont été notées chez les arbres plantés aux plus faibles densités. En effet, au mois de mars les oliviers plantés à la densité 51 arbre /ha n'a subi aucune augmentation et au mois d'avril les arbres plantés à la densité 70 arbre par ha ont présenté les valeurs la plus faible de la photosynthèse. Etudiant la réponse de quelques espèces végétales, plusieurs auteurs notent que le stress hydrique affecte la physiologie de la plante en diminuant la photosynthèse nette et que ses effets sont variables avec le niveau de stress (Bensari et al. 1990 et Marsal et al. 2000). Chez l'olivier variété Chemlali, Laouar (1978) a montré que l'activité photosynthétique est maintenue maximale pour de légers déficits hydriques.

Plusieurs auteurs signalent que l'un des principaux processus touchés par la sécheresse est la photosynthèse, un fait dû principalement à la fermeture stomatique qui diminue la perte d'eau mais aussi le flux de carbone vers les sites de carboxylation (Flexas et al., 1998 et Escalona et al., 2002).

#### 4. Conclusion

L'utilisation des senseurs biologiques en se référant à la plante pour étudier le comportement adaptatif de l'olivier dans différentes situations culturales où l'eau peut être limitant (utilisations de densités de plantation plus ou moins élevées) est très important notamment en plein champs où le contrôle des facteurs du milieu est impossible. Elle nous a permis de nous rendre compte de l'importante capacité du cultivars d'oliviers testé (Chemlali) à mobiliser l'eau en fonction de ces besoins selon son état biologique et du rôle fondamental de la régulation stomatique dans le contrôle des pertes transpiratoire.

Par ailleurs, la le suivi journalier des pertes transpiratoire grâce aux mesures du flux de sève donne une idée précise sur la consommation hydrique de l'arbre et permet le diagnostic au moment opportun du stress. Il pourrait guider les interventions culturales. Les mesures de la photosynthèse

donne une idée sur le potentiel de croissance des plantes et pourrait servir à l'évaluation de l'efficacité des interventions appliquées.

Il ressort de ce qui précède que les senseurs biologiques - déjà important en recherche ecophysiologiques- peuvent devenir très utiles pour la gestion des systèmes de culture de plus en plus fragilisés par le changement climatique car elle permette le dépistage rapide et précis de la contrainte environnementale guidant ainsi le choix variétal adapté et la conduite adéquate au moment opportun.

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## Mecanisation de la récolte adaptée pour les oliviers âgés et de très grand taille

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### Résumé

En Italie du sud, en particulier dans la région de la Calabre et de Pouilles, une partie significative des oliveraies se caractérisent par la présence de vieux arbres de très grande taille (taille > 8 m). Habituellement, dans ces vergers, la récolte d'olives se fait par le ramassage périodique des fruits qui tombent naturellement par terre, avec des effets négatifs sur la qualité d'huile. Dans certains cas, c'est difficile de rajeunir ces oliveraies par des nouvelles plantations en raison de leur importance historique et/ou du paysage. Afin de trouver une alternative à cette façon de récolter les olives, en 2006, dans la région de Pouilles, un essai de récolte mécanisée des olives sur des vieux arbres de grande taille a été effectué. Les arbres des cultivars "Cellina di Nardò" et "Ogliarola Salentina" étaient âgés 60-100 ans avec une taille de 8 à 10 m et d'un volume de frondaison d'environ 450-500 m<sup>3</sup>/arbre. Dans la première moitié de novembre, la récolte mécanique a été effectuée: 1) en utilisant un batteur mécanique appliqué à un tracteur pour récolter la partie plus haute de la frondaison + les peignes pneumatiques manuels pour récolter les parties inférieures de la frondaison; ou 2) en utilisant un dispositif secoueur automate appliqué aux branches principales. Avec le batteur mécanique + peigne pneumatiques tenus à la main, le rendement de récolte (le pourcentage des olives récoltées sur toute la chevelure) était environ 90% et l'efficacité de récolte était autour 60 kg d'olives/heure/ouvrier. Avec le dispositif secoueur le rendement était d'environ 75% sur les variétés "Cellina di Nardò" et d'environ 40% sur les variétés "Ogliarola Salentina" tandis que la productivité de récolte était autour 190 kg d'olives/heure/ouvrier avec la variété "Cellina di Nardò" et autour 150 kg d'olives/heure/ouvrier avec la variété "Ogliarola Salentina". L'huile obtenue a été de qualité élevée. Les résultats indiquent des possibilités importantes pour la mécanisation de la récolte et la production d'huile de qualité dans les oliveraies caractérisées par des arbres de très grande taille.

**Mots clés:** *Olea europaea* L., dispositif secoueur de tronc, batteur mécanique, peignes pneumatiques, qualité de l'huile.

## Mechanization of harvesting in olive trees of very large size

### Abstract

In southern Italy, particularly in Calabria and Apulia regions, a significant portion of the olive orchards are characterised by old trees of very large size (height > 8 m). Usually, in these orchards olive harvesting is done by periodically collecting the naturally fallen fruits from the soil, with strong negative effects on oil quality. In some cases, these orchards cannot be replaced by young orchards because have a historical and/or landscape importance.

In order to find an alternative to the harvesting of olives from the soil, in 2006, in Apulia region, a trial for the mechanization of olive harvesting in orchards constituted by old, large trees was carried out. The trees, of the cultivars "Cellina di Nardò" and "Ogliarola Salentina", were 60-100-year-old and had a height ranging from about 8 m to about 10 m and a canopy volume of 450-500 m<sup>3</sup>/tree. In the first half of November, mechanical harvesting was performed 1) by using a mechanical beater applied to a tractor to harvest the high parts of the canopy + hand-held pneumatic combs to harvest the low parts of the canopy or 2) by using a self-propelled shaker applied to the main branches. With mechanical beater + hand-held pneumatic combs the harvesting yield (percentage of harvested olives with respect to the total present on the canopy, was about 90% and the harvesting working productivity was around 60 kg of harvested olives/h/worker. With the shaker the harvesting yield was about 75% on the cultivar "Cellina di Nardò" and about 40% on the cultivar "Ogliarola Salentina", whereas the harvesting working productivity was around 190 kg of harvested olives/h/worker with the cultivar "Cellina di Nardò" and about 150 kg of harvested olives/h/worker with the cultivar "Ogliarola Salentina". The oils obtained from the mechanically harvested olives always showed a high quality.

The results indicate possibilities for the mechanization of harvesting and the obtainment of oils of high quality in olive orchards characterised by trees of very large size.

**Key words:** *Olea europaea* L., trunk shaker, mechanical beater, pneumatic combs, oil quality.

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### **Thème 3**

**Impacts, enjeux, possibilités et limites de l'olivier en hyper-intensif**

## Manual and mechanical pruning tests in the super intensive olive growing

Giuseppe Zimbalatti, B. Bernardi

### Abstract

In the "super intensive" olive growing, the limited availability of low vigour varieties and the lack of dwarfing graft holders able to control the plants growth efficiently, make the tree-crowns need important pruning interventions in order to guarantee a sufficient illumination level, avoid the arising of competition phenomena and keep plants dimensions which can allow the passing of grape harvesters. This paper concerns the results relative to the first year of test, on manual and mechanical pruning, carried out in super intensive olive cultivation systems, with the purpose of evaluating the performance achieved through two different pruning systems, and making the comparison in terms of productivity and quality of the previously done work.

**Key words:** Pruning, Mechanization, Super intensive olive growing, Productivity.

### Les tests d'élagage manuels et mécaniques dans les cultures en hyper-intensif de l'olivier

#### Résumé

Dans le cadre de la production oléicole en super intensif, la disponibilité limitée des variétés à basse vigueur et l'absence de porte-greffes capables de contrôler efficacement la croissance des plants, implique la nécessité d'effectuer d'importantes opérations de taille sur la frondaison, dans le but de garantir un niveau suffisant de luminosité, de réduire le phénomène de compétition, et de préserver une dimension des plants permettant le passage des machines à vendanger. Le présent article expose les résultats relatifs à la première année d'expérimentation, sur la taille manuelle et mécanique, effectuées dans le cadre de la conduite en super intensif de la culture d'olivier, en vue d'évaluer les performances effectuées à travers deux différents systèmes de taille, et d'établir la comparaison en terme de productivité et de qualité avec les travaux antérieurs.

**Mots clés:** Taille, mécanisation, production oléicole en super intensif, productivité.

#### 1. Introduction

During the last ten years a new high density olive-growing (2000 and over plants/ha), called "super intensive" has become popular (Rallo *et al.*, 2006). The success of this model, centred on the high efficiency of the mechanical harvest carried out through the use of grape harvesters, is linked to the mechanization of pruning, since also this operation, just like harvest, has a great influence upon oil yield and production quality.

Tree-crowns, from the 5<sup>th</sup>-7<sup>th</sup> year, exceed the total volume of 10.000 m<sup>3</sup>/ha (Pastor *et al.*, 2006), so they need important pruning interventions in order to guarantee a sufficient luminosity level, avoid the arising of competition phenomena and keep small plants dimensions which can allow the passage of grape harvesters.

In the olive-grove, the employment of machines facilitates pruning operations, as it consists in a regular external cut of the tree-crown which can be done without particular problems (Giametta *et al.*, 1997). The use of machines for topping and hedging (Lodolini, 2006): operations which adjust respectively the crown height and thickness could be a decisive factor for the success of this model.

The present work has evaluated results relative to the first year of trials on manual and mechanical pruning, carried out in spanish super intensive olive cultivation systems. The aim of this research was to elevate the achieved performance and compare their productivity and quality with previous work.

## 2. Material and Methods

The aim of this study was to compare different work productivities achievable by means of manual and mechanical interventions on pruning operations. During the tests, the evaluation of the operational time was carried out according to the formalities provided by C.I.O.S.T.A. classification.

Tests on **manual pruning** were carried out in Andalucía (Córdoba). The plantation, made up of a eight-year-old "Arbequina" variety (selection Agromillora), had a planting distances of 3.75 x 1.35 (about 1,975 plant/ha). It presented a flat position, North-South direction and was provided with drop fertirrigation. The irrigation dose was comprised between 2,000 and 2,300 m<sup>3</sup>/ha-year. The formation was single axis with 4 metres high plants. The crown was 0.70 m from the ground, with an average volume of 11.85 m<sup>3</sup>. 15 rows, each one with an average length of 189 m, and 140 plants, were tested. The work group was made up of three operators, supported by power saws of a light type (model STIHL MS192T, with a volume of 30 cm<sup>3</sup> and power a of 1.3 kW), who had to carry out a hard intervention by means of shears since during the previous years this kind of operation had been done at irregular intervals (Figure 1).



Figure 1: Manual intervention

Tests on **mechanical pruning**, carried out in Cataluña (Reus) concern two different areas of the same olive-grove which has a planting distances of 3.00 x 1.35 meters (2,469 plants/ha), a flat position, East-West direction, and is provided with drop fertirrigation. The "Arbequina" plants (selection Agromillora) are 3.50 metres high and their crowns are 0.50 m from the ground. Two different kinds of interventions have been tested:

- **Thesis A:** cutting of both sides along the rows (hedging on two planes) with a 25° inclination from the vertical. During tests carried out, it was necessary to put a manual final touch to the low part of the tree-crown since the pruning bar could not reach it. The plants presented an average crown volume of 8.19 m<sup>3</sup>. 10 rows, each one about 169 m long with 125 plants, were tested (Figure 2).

- **Thesis B:** cutting of a single side, carried out in parallel with the vertical, as close as possible to the trunk and topping 2.20 m from the ground. This scheme is provided by the same operation every year, but on alternate sides. 10 rows, each one about 337.5 m long with 250 plants, were tested. The average crowns volume was 6.34 m<sup>3</sup> (Figure 3).

The work was made up of a single worker operating the pruning machine. The beginning of each survey was the moment when the machine was in front of the row to start pruning, and the final point was the moment when the work in the tested area ended. In both theses, every cut operation, was carried out by a single passage of the machine.

The pruning bar used in the tests, mounted towards the front on a 60 kW New Holland tractor, was comprised of a series of five sharp toothed wheels with the same diameter (0.55 m), placed on the same supporting shaft. These hydraulically operated wheels were made of tempered steel and had teeth with widia plates in order to facilitate the work; they had a rotation speed of 2.000-2.500 cycles/min and were able to cut branches with a diameter of over 0.8 m without problems. The single arm bar, thanks to an oleodynamic control system, can be lifted up vertically over 4 meters of height and



Figure 2: Hedging carried out with a 25° inclination off the vertical



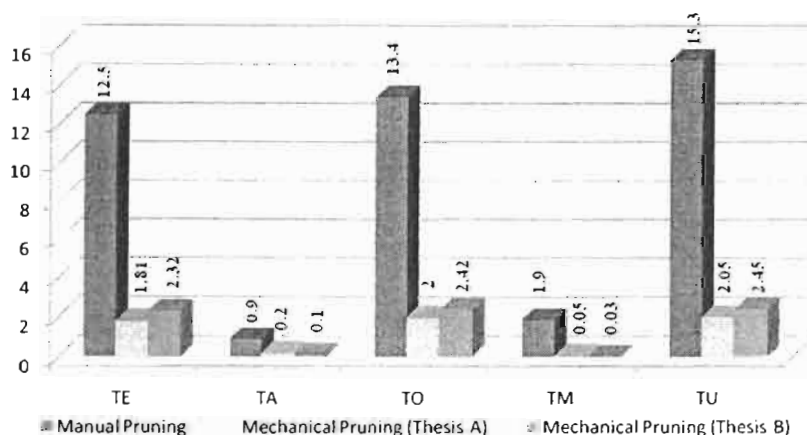
Figure 3: Hedging of a single wall and topping 2.20 m from the ground.



allows all the intermediate cut positions between the vertical one, for the wall pruning (hedging) and the horizontal one for the top pruning (topping). For the manual pruning, power saws of a light type model STIHL MS192T, with a volume of 30 cm<sup>3</sup> and a power of 1.3 kW were used.

### 3. Results

Results of pruning tests (graphic 1), show for manual interventions, a working effective time (TE) equal to 12.5 h/ha, and a total additional time (TA) of 0.9 h/ha, while the dead times (TM) have an influence of 1.9 h/ha. The total usage time (TU) is 15.3 h/ha. The equipment operating capacity is equal to 0.07 ha/h with a productivity of 0.023 ha/h per operator; the quantity of taking away wood is on the average equal to 17.5 kg/plant, equivalent to 34.5 t/ha. The diameter of 70% of pruned branches is shorter than 0.4 m since plants, in the super intensive model, keep small sizes. The average volume of the crowns is reduced to 4.04 m<sup>3</sup>. The width of the path existing between the rows changes from 1.65 m to 2.35 m.



**Graphic 1.** Comparison between working times collected for interventions of manual and mechanical pruning.

**Note:** TE = effective time, TA = additional time, TO = operational time, TM = dead time, TU = usage time. Working time expressed in h/ha.

With reference to mechanical pruning interventions, the elaborated data show that the lowest working operating times (TO), equal to 2.00 h/ha, were reported in thesis A, even if a possible manual final touch would have a considerable influence on this value. The additional times, requested only for the turns, have an average influence of 0.15 h/ha while the dead times are out of account. The working operating productivity is 0.50 ha/h in thesis A and 0.42 ha/h in thesis B. The quantity of taking away wood is equal to 3.0 kg/plant in thesis A and 4.5 kg/plant in thesis B, with an average value of 18.6 t/ha. The diameter of 80% of pruned branches is shorter than 4 cm. The average advance speed of the pruning machines along the rows is equal to 1.6 km/h. The average volume of the crowns is reduced to 4.68 m<sup>3</sup> in thesis A and 2.74 m<sup>3</sup> in thesis B.

Mechanical interventions have guaranteed a quite good structure continuity along the row, keeping the balance between geometric form, necessary to allow the passage of grape harvesters over the plants, and functional one, connected with productive aspects.

### 4. Discussion

The obtained results show a noteworthy economic profit achievable thanks to the employment of mechanical pruning: the working productivity is, on the average, about 22 times higher than manual pruning productivity. The advantage would remain, but considerably reduced, even if, (for thesis A) after the mechanical cut, there was a selective manual intervention of thinning in the low part of the tree crown.

The machine went on quickly with an average speed, as above-mentioned, of 1.6 km/h. However, the presence of a second cut bar (which, if necessary, can carry out the topping) would have allowed a better performance of the work, contributing, in thesis A, to the removal of that part of crown not subject to pruning.

In order to increase work speed and efficiency, it is important to stress that on the market we can find new machines able to remove only the hanging branches of the base crown which cannot be reached by the grape harvesters (Olint, 2006).

However this kind of pruning is not selective since both useful and useless wood is removed; so a reduction of buds, with a consequent fall of the production, can occur. However, since this, is only the first year of experiments, final remarks about productive aspects cannot be expressed yet.

The critical stage of the system is the time that plants take to recover a full production after pruning. This period of lower productivity must be reduced by stimulating the plant to balance the air-root relation as soon as possible. However a proper fertilization and irrigation, typical of the super intensive system, could be a good solution for this problem.

In conclusion, the use of mechanical pruning interventions in the super intensive models, can be an effective solution, taking in consideration the small size that plants must keep (Tous *et al.*, 2006); but further studies are necessary to define the best intervention scheme, the optimal turn and the proper cutting intensity (more or less severe, or rather close or less close to the trunk, taking in consideration the possible vegetative/productive answer of the super intensive olive-grove).

The authors have contributed equally to the present work.

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## La fertilisation raisonnée de l'olivier conduit en hyper-intensif

Ben Khelil Malek, Sanaa Mustapha

### Résumé

Jeunes Arbéquina oliviers, grandissant dans la région de Mornag, au Nord de la Tunisie, plantés en système hyper-intensif, ont été choisis pour une fertigation annuelle depuis 2006 de N, P<sub>2</sub>O<sub>5</sub>, K<sub>2</sub>O fractionnés selon les stades critiques de développement de l'arbre. Le fruit est choisi à maturité pour déterminer l'effet de la fertilisation combinée sur le rendement, la qualité des fruits et de l'huile d'olive. Les résultats ont montré une amélioration du poids moyen du fruit et du rendement cumulé des trois années successives de 20%, des arbres qui ont reçu les traitements par rapport au témoin. La teneur en huile a augmenté. Une amélioration d'acide oléique et une diminution de l'acide palmitique ont été constatées. Cependant les teneurs en carotène et en chlorophylle semblent ne pas être affectées par les traitements.

**Mots clés :** Olivier Arbéquina, hyper-intensif, fertigation, rendement, qualité des fruits

### Abstract

Young Arbequina olive tree, growing in North of Tunisia, planted under high density system, were selected to annual fertigation since 2006 of N, P<sub>2</sub>O<sub>5</sub>, K<sub>2</sub>O distributed according to the critical stage of development of the tree. The fruit was chosen at maturity to determine the effect of the combined fertilization in yield, quality of fruit and olive oil.

Results showed an improvement of the fruit mean weight and cumulative yield for three successive years of 20 % of the trees which received treatments with regard to witness. Content in oil was increased. An improvement of Oleic acid and decrease of the Palmitic acid were noticed. However contents in carotene and in chlorophyll seem not to be affected by treatments.

**Key words:** Arbequina olive tree, high density, fertigation, yield, quality of fruit.

### 1. Introduction

La Tunisie est classée au premier rang quant aux surfaces consacrées à l'olivier (30% des terres arables). La production nationale de l'huile d'olive représente 9 % de la production mondiale.

En Tunisie, l'intensification de la culture a commencé à naître ces dernières années par la variété espagnole Arbéquina adaptée à ce type de culture qui a été introduite en culture intensive et hyper intensive dans plusieurs régions du pays. Il en existe actuellement 3 000 ha plantés en hyper intensif, avec une production moyenne de 7 à 8 tonnes par ha (Msalle et al., 2004). Cependant cette évolution n'a pas été accompagnée par une amélioration de la fertilisation et les agriculteurs continuent à pratiquer une fertilisation inadéquate par rapport aux besoins nutritionnels de l'arbre.

La fertilisation raisonnée consiste donc à ajuster les apports en éléments fertilisants et les adapter aux besoins de la plante et au bon moment pour d'une part assurer une récolte suffisante et d'autre part limiter les intrants pour minimiser leurs effets secondaires sur le complexe sol-plante –climat.

La pratique de la fertilisation raisonnée se base sur l'analyse des éléments exportés par l'arbre (feuilles, fruits et bois de taille), ceci permet d'apprécier ce qu'il convenait de restituer au sol. Cette méthode permet d'évaluer les besoins globaux de l'arbre et permet d'estimer, au moins, ce qui est perdu annuellement par la culture et d'ajuster les quantités d'éléments fertilisants à apporter par la fumure en fonction de son stade de développement.

En effet, une amélioration du statut nutritionnel et surtout par une fertilisation raisonnée qui tient compte des caractéristiques du milieu dans lequel on travaille (sol, climat, proximité d'eau de surface ou de zones de captage) permet une amélioration de la productivité de l'olivier. En effet, le statut nutritionnel est le facteur le plus important qui régit le rendement de l'olivier d'après El Fouly (2005)

Cependant ce créneau de la fertilisation raisonnée reste inexploré et peu de travaux se sont intéressés pour améliorer le rendement de l'olivier en culture hyper intensive et surtout la composition chimique des olives (Fernandez- Escobar et al., 2006).

Ainsi, et compte tenu de l'importance de ce type de fertilisation dans le secteur oléicole, nous avons mené ce travail dans le but de déterminer l'effet de la fertigation en N,  $P_2O_5$ ,  $K_2O$  fractionnés selon les stades critiques de développement de l'arbre sur le rendement, la qualité des fruits et de l'huile d'olive Arbéquina conduit en mode hyper intensif.

## 2. Matériels et méthodes

Des jeunes oliviers de la variété 'Arbéquinal18' (*Olea europaea* L.) utilisés pour la production d'huile, plantés en système hyper-intensif (Figure1), à une densité de 2x4m dans une sous-parcelle expérimentale de Sadira sise à Mornag au Nord de la Tunisie de 1.5ha sur un sol sablo- argileux ont été choisis pour une fertigation annuelle depuis 2006 des trois engrais essentiels pour l'olivier. L'azote est apportée sous forme d'ammonitre (33.5%) , le phosphore sous forme d'acide phosphorique (50% de  $P_2O_5$ ) et le potassium sous forme de sulfate de potasse (50% de  $K_2O$ ). Les doses de fertilisants sont fractionnées selon les stades critiques de développement de l'arbre. Les arbres qui reçoivent le programme adopté par la société Sadira et qui est effectué sur le reste de la parcelle de 21ha sont considérés comme arbres témoins.



**Figure 1:** Arbéquina cultivée en hyper-intensif (4mx2m).

Pour chaque traitement, 4 blocs sont choisis de manière aléatoire. Chaque bloc comporte 12 arbres d'oliviers de même âge et représentatifs de la parcelle.

### 2.1. Evaluation des besoins globaux de l'arbre

Cette méthode permet d'estimer, au moins, ce qui est perdu annuellement par la culture et d'ajuster les quantités d'éléments fertilisants à apporter par la fumure en fonction de son stade de développement.

Méthodologie : Elle se base sur l'analyse des éléments exportés N, P et K par l'olivier (feuilles, fruits et bois de taille), ceci permet d'apprécier ce qu'il convenait de restituer au sol.

- Prélèvement des fruits : Un échantillon de 100g / bloc de fruits (le 16/11/2005), de la récolte de l'année 2005 est pris pour la parcelle d'étude, les fruits sont desséchés à l'étuve puis broyés par le mortier en porcelaine, ensuite on détermine les exportations des éléments minéraux dans le fruit.

- Prélèvement de gros bois de taille : On a déterminé les exportations de 5 arbres de chaque parcelle et puis on a extrapolé pour toute la parcelle de densité 1250 pieds /ha. Un échantillon de 60gr de chaque arbre est pris pour analyse des différents éléments minéraux : N ; P, K puis on a calculé le pourcentage en azote en  $P_2O_5$  et en  $K_2O$ .

- Prélèvement des feuilles, ceci pour déterminer les exportations des éléments minéraux dans les feuilles. Les feuilles sont collectées à la hauteur d'un homme sur la périphérie de l'arbre et sur les quatre points cardinaux (Est, Ouest, Nord et Sud). Les feuilles matures et saines sont prélevées de la partie médiane des pousses non fructifères d'après Fernandez- Escobar (1997). Un échantillon est pris de chaque bloc, composé de 100 feuilles pour la détermination des éléments minéraux.

Le dosage des éléments minéraux P et K est effectué selon la méthode de Pauwels et al. (1992) et l'azote total N est déterminé par la méthode Kjeldhal.

## 2.2. Estimation des besoins en fonction du rendement

Nous avons estimé les exportations pour un rendement moyen de 10T /ha pour la variété 'Arbéquina' en ne considérant que les exportations par les fruits, le gros bois de taille et les feuilles. En effet le petit bois de taille est resté sur place dans la parcelle. L'exportation par le gros bois de taille et les feuilles est évaluée en matière sèche de l'ordre de 2000 kg /ha (pour la parcelle de densité 1250 pieds/ha).

On donne les exportations en kg/ha de N, P2O5 et K2O trouvés par Xilloyannis et al. (2002) pour la variété Coratina conduite en mode intensif (Tableau1) et celles trouvées dans la parcelle de sadira à Mornag (Tableau 2).

**Tableau 1:** Exportations en kg/ha de N, P2O5 et K2O.

| Année après plantation    | Elément N | (Kg/ha) P2O5 | K2O   | zone                 | observation        |
|---------------------------|-----------|--------------|-------|----------------------|--------------------|
| ( 2 <sup>ème</sup> année) | 9,2       | 1,3          | 8,6   | sud Italie (lavello) | densité 6x3m       |
| ( 6 <sup>ème</sup> année) | 85,9      | 30,7         | 163,1 |                      | irrigué, 8000kg/ha |

**Tableau 2:** Exportations en N, P2O5 et K2O pour le fruit, les feuilles et le gros bois de taille des oliviers de Sadira pour une production de 10T/ha d'olives (46% d'humidité).

| Exportations en élément fertilisant Kg/ha | fruits | gros bois de taille+feuilles | Totaux des exportations |
|-------------------------------------------|--------|------------------------------|-------------------------|
| N                                         | 37     | 80                           | 120                     |
| P2O5                                      | 20     | 18                           | 40                      |
| K2O                                       | 123    | 36                           | 160                     |

Les analyses physico-chimiques du sol ont montré un niveau de fertilité satisfaisant, ne montrant pas de facteurs limitants et ne nécessitant pas une dose de fertilisation de correction pour les éléments précités, à ajouter aux exportations.

## 2.3. Fractionnement des doses et périodes d'apport

Le fractionnement de la dose d'ammonitre est donné en tenant compte des périodes critiques du besoin de l'olivier en cet élément qui sont : le débourrement, la floraison, le durcissement du noyau et la veraison.

La quantité de phosphore à apporter est appliquée avec le début de l'activité racinaire (fin hiver – début printemps), la floraison et le grossissement du fruit.

La quantité de sulfate de potasse est fractionnée pendant la période de grossissement du fruit. Ce fractionnement est déduit d'après la dynamique du potassium dans la feuille (Tableau 3).

**Tableau 3:** Pourcentage des engrais donnés suivant les stades de développement de l'olivier.

| Stade physiologique |                                                              | % de la dose de fertilisant |    |    |
|---------------------|--------------------------------------------------------------|-----------------------------|----|----|
|                     |                                                              | N                           | P  | K  |
| 1                   | Réveil végétatif et débourrement                             | 10                          | 30 | —  |
| 2                   | Début floraison                                              | 30                          | 30 | —  |
| 3                   | Nouaison                                                     | 20                          | 15 | 25 |
| 4                   | Début de grossissement des fruits et sclérification du noyau | 20                          | 15 | 50 |
| 5                   | Veraison                                                     | 20                          | 10 | 25 |

Le Tableau 4: suivant présente les quantités apportées par stade physiologique. La quantité de la dose à apporter par fertigation dépendra de la solubilité du fertilisant utilisée.

**Tableau 4:** Quantités des engrais donnés suivant les stades de développement de l'olivier.

| Stade physiologique |                                                              | Quantité d'engrais par stade physiologique (Kg /ha) |                               |                  |
|---------------------|--------------------------------------------------------------|-----------------------------------------------------|-------------------------------|------------------|
|                     |                                                              | N                                                   | P <sub>2</sub> O <sub>5</sub> | K <sub>2</sub> O |
| 1                   | Réveil végétatif et débourrement                             | 12                                                  | 12                            | —                |
| 2                   | Début floraison                                              | 36                                                  | 12                            | —                |
| 3                   | Nouaison                                                     | 24                                                  | 6                             | 40               |
| 4                   | Début de grossissement des fruits et sclérification du noyau | 24                                                  | 6                             | 80               |
| 5                   | Veraison                                                     | 24                                                  | 4                             | 40               |

La récolte a été effectuée pour chaque arbre séparément, pour déterminer le rendement moyen pour chaque bloc (moyenne de 12 arbres) considéré comme une répétition.

Le poids moyen a été déterminé par une pesée de trois sous échantillon de 100 fruits frais à l'aide d'une balance de précision. L'échantillon récolté pèse 2 Kg, puis 960 g d'olives sont triturés. Après centrifugation, le résidu est mis dans une éprouvette graduée de 1000ml pour décantation, puis on lit directement la teneur en huile (sachant que la densité de l'huile = 0.920) exprimée en pourcentage de la matière fraîche. L'acidité a été déterminée selon la méthode Wolf (1986). La composition en acides gras a été réalisée par les méthodes décrites in Regulations EEC/2568/91 et EEC /1429/92 dans le (EUC, 1991). La teneur en chlorophylle et la teneur en  $\beta$  carotène exprimées en ppm sont déterminées dans la solution de cyloxane à 470nm, mesurées par spectrophotomètre à différentes longueurs d'ondes 472, 630, 670, 710nm. Les coefficients d'extinction K 232 et K270 sont calculés à des longueurs d'ondes d'absorption respectivement 232 et 270 nm par spectrophotométrie en utilisant une solution de 1% d'huile en cyloxane.

Les analyses statistiques des données ont été effectuées grâce au logiciel STATITCF (ver.V). L'ensemble des mesures a fait l'objet d'une analyse de variance à un facteur au seuil de risque de 5%. Il est complété par une comparaison multiple de moyennes par le test de Newman et Keuls (au seuil de 5%).

### 3. Résultats et Discussions

#### 3.1. Effet de la fertilisation raisonnée sur le rendement en olives

D'après la Figure 2 qui représente le rendement final en olives, on note une amélioration du rendement pour les arbres qui ont reçu le programme tri-factoriel (N, P, K) par rapport au témoin (le programme de fertigation adopté par la société) pour les trois années d'étude. En effet le rendement des arbres du traitement NPK pour l'année 2006 a été de 10,54 Kg/arbre par rapport au témoin 9,2 Kg/arbre. Cependant, on note une diminution du rendement pour les deux traitements pour l'année suivante.

Les rendements obtenus sont de 7,07 Kg /arbre pour le Témoin et de 8,32 Kg /arbre pour le traitement NPK. On note que la diminution du rendement est plus faible pour le traitement NPK par rapport au témoin relatif à une amélioration de la nutrition minérale de l'arbre apportée au stade critique de développement. Pour l'année 2008, on note aussi une diminution du rendement des arbres témoins égal à 6,3 Kg /arbre, cependant le rendement est maintenu presque stationnaire pour le traitement NPK (8,13 Kg/ arbre) par rapport à l'année précédente. L'analyse de la variance ne montre pas de différence significative entre le traitement NPK par rapport au témoin pour les trois années d'étude.

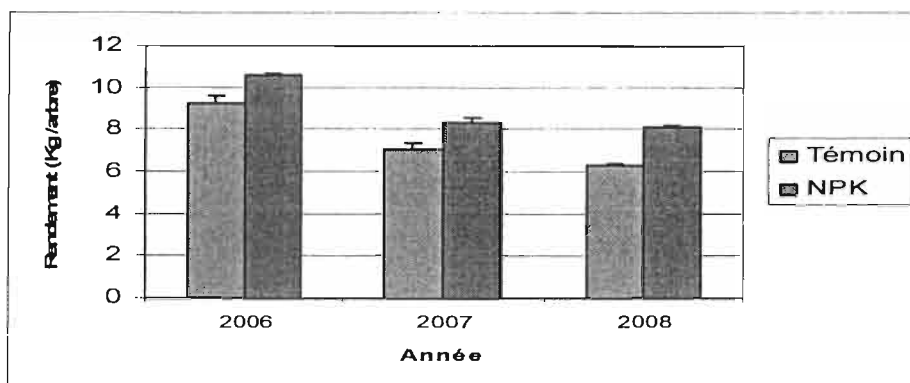


**Figure 2:** Le rendement des olives à la récolte pour les arbres ayant reçus les traitements NPK et le témoin effectués pour les trois années d'étude.

D'après la Figure 3 qui donne le rendement cumulé des trois années pour les deux traitements, on note une amélioration de 20% du rendement pour le traitement NPK par rapport au témoin.

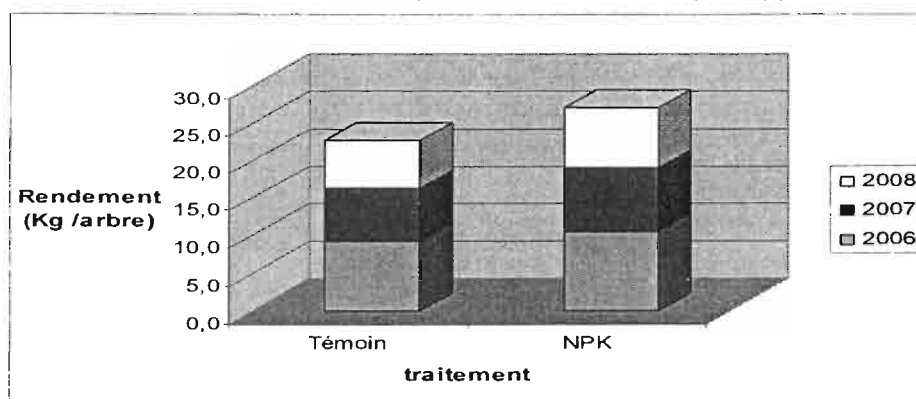


**Figure 3:** Le rendement cumulé des olives à la récolte des trois années pour les arbres ayant reçus les traitements NPK et le témoin

### 3.2. Effet de la fertilisation raisonnée sur la teneur en huile

La teneur en huile pour les oliviers du traitement NPK pour l'année 2006 a été de 17,2 % par rapport au témoin égal à 16,54%. Cependant, on note une diminution de la teneur en huile pour les deux traitements pour l'année suivante. Les teneurs obtenues sont de 15,7 % pour le traitement NPK par rapport au Témoin qui était de 15,2 % pour l'année 2007 (Fig. 4).

Cette diminution est due aux conditions de croissance et des conditions climatiques au moment du processus de la lipogenèse qui sont essentiellement les pluies torrentielles de septembre et d'octobre qui ont engendré une augmentation du poids des fruits au dépend de la lipogenèse. D'après (Lavee, 1997) la quantité potentielle de l'huile qui s'est accumulée dans l'olive à la récolte est déterminée par la variété, mais elle peut varier sensiblement selon les conditions de croissance, du climat et de la charge de l'arbre en fruits.

A la troisième année d'étude la teneur en huile a été aussi plus élevée pour le traitement NPK (16,51%) par rapport au Témoin (16,07%) (Fig. 4). Cette amélioration de la teneur en huile pour les trois années d'étude est due à l'amélioration du statut nutritionnel azoté et phospho-potassique de l'olivier constatée par les traitements apportés à la parcelle de Sadira surtout au moment de la croissance végétative améliorant la capacité photosynthétique de l'arbre et des fertilisants apportés au cours du développement du fruit et de la lipogénèse. En effet, l'accumulation de l'huile dans l'olive est un processus qui dépend de la quantité des carbohydrates qui alimentent les fruits au moment de la lipogenèse. Ces carbohydrates proviennent principalement des feuilles mûres, mais aussi du fruit lui-même (Conde et al., 2008). Cependant l'analyse de la variance ne montre pas de différence significative entre le traitement NPK par rapport au témoin pour les trois années d'étude.



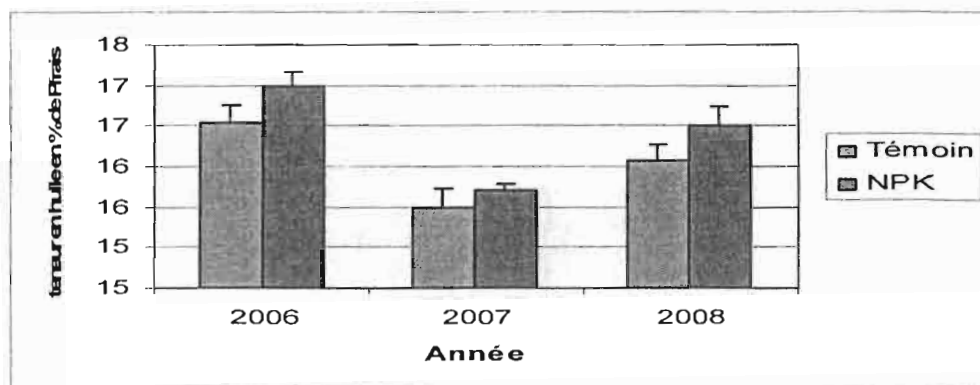


Figure 4: La teneur en huile (% du poids frais) pour les traitements NPK et le témoin pour les trois années d'étude.

### 3.3. Effet de la fertilisation raisonnée sur le poids moyen des olives

Le poids frais des olives à la récolte a été égal à 2,05 g pour le traitement NPK et était plus élevé par rapport au témoin égal à 1,9g au cours de la première année, cependant il a diminué à la deuxième année pour le traitement NPK égal à 2,86g par rapport au témoin égal à 2,97g. Quant à la troisième année, les teneurs ont été presque similaires et égales à 2,25g pour le témoin et 2,28 g pour le traitement NPK (Fig. 5). Toutes ces valeurs sont supérieures au poids frais moyen de la même variété cultivée en Espagne, qui est de 1,86g (Tous et Romero, 2002). Cette différence peut être expliquée par l'influence du milieu et par l'apport des intrants (eau et fertilisants) (Aouini, 2006). Cependant l'analyse de la variance ne montre pas de différence significative entre le traitement NPK par rapport au témoin pour les trois années d'étude.

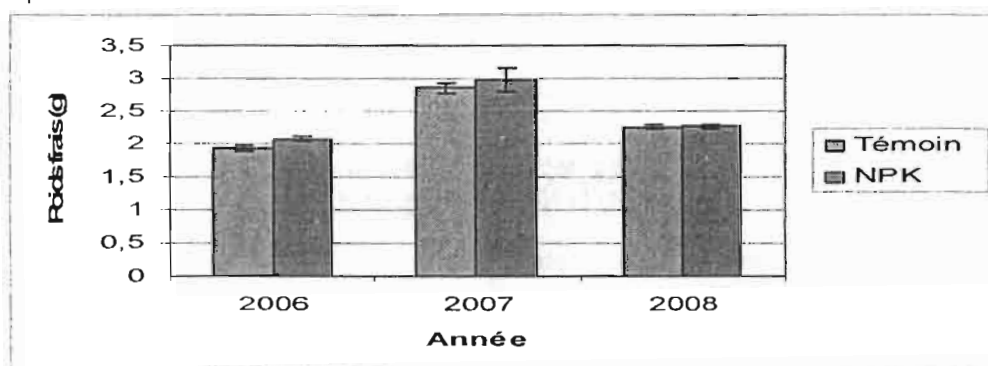


Figure 5: Le poids frais des olives à la récolte pour le traitement NPK et le témoin pour les trois années d'étude

### 3.4. Effet de la fertilisation raisonnée sur les critères de qualité de l'huile

#### 3.4.1. Composition acide

L'analyse chromatographique de l'huile d'olive a permis de donner la composition en acides gras totaux de l'huile allant de l'acide palmitique (C16:0) à l'acide eicosénoïque (C20:1). Tous les échantillons du traitement NPK et du témoin sont dans les normes proposées par le COI (1988) et sont riches en acide oléique (C18:1) et faibles en acide palmitique. Cependant, on note une légère augmentation de l'acide oléique et linoléique (C18:2) pour le traitement NPK par rapport au témoin pour les trois années d'étude et qui se rapproche du pourcentage de l'acide oléique de l'Arbéquina en Espagne qui est de 65,7% et linoléique 14,8% (Tous et al., 1999). Cette augmentation est significative au seuil de 5%.

Aussi, on note une diminution significative de l'acide palmitique pour le traitement NPK par rapport au témoin constatée pour les trois années d'étude (Tableau 5). La variété 'Arbéquina' semble avoir une meilleure adaptation dans le nord du pays.

**Tableau 5:** Composition en acide gras totaux de l'huile de l'olivier de la variété 'Arbéquina' selon les traitements effectués pour les trois années de l'étude.

| Année              | C16:0         | C16:1          | C17:0    | C17:1    | C18:0          | C18:1            | C18:2           | C18:3          | C20:0          | C20:1 |
|--------------------|---------------|----------------|----------|----------|----------------|------------------|-----------------|----------------|----------------|-------|
| Témoin 2006        | 18,8a         | 2,87a          | 0,10     | 0,21     | 1,67a          | 58a              | 15,7            | 0,62           | 0,29           | 0,22  |
| NPK                | 17,1b         | 2,48b          | 0,12     | 0,21     | 1,73b          | 61,7b            | 15,20           | 0,62           | 0,28           | 0,21  |
| Témoin 2007        | 16,77a        | 1,81a          | 0,08     | 0,21     | 1,68a          | 67,33a           | 10,56           | 0,43a          | 0,24           | 0,14a |
| NPK                | 15,70b        | 1,91b          | 0,08     | 0,22     | 1,88b          | 68,5b            | 10,53           | 0,40b          | 0,26           | 0,21b |
| Témoin 2008        | 18,53a        | 2,52a          | 0,08     | 0,18a    | 1,75a          | 62,28a           | 14,13           | 0,47           | 0,25           | 0,19a |
| NPK                | 18,16b        | 2,31b          | 0,07     | 0,16b    | 1,71b          | 63,8b            | 14,02           | 0,47           | 0,24           | 0,17b |
| <b>Norme (COI)</b> | <b>7.5-20</b> | <b>0.3-3.5</b> | <b>-</b> | <b>-</b> | <b>0.5-5.0</b> | <b>55.0-83.0</b> | <b>3.5-21.0</b> | <b>&lt;1.0</b> | <b>&lt;0.6</b> |       |

Les moyennes suivies de lettres différentes pour chaque année sont significativement différentes au seuil de 5% selon le test de Newman et Keuls

### 3.4.2. Caractéristiques physico-chimiques

L'acidité de l'huile a été variable, en diminuant pour le traitement NPK pour les années 2006 et 2008 par rapport au témoin alors qu'elle a augmenté pour l'année 2007. L'acidité la plus élevée a été observée pour l'année 2008, où elle était égale à 0,28% pour le traitement NPK. Elle ne semble pas être affectée par les traitements effectués malgré les différences significatives observées vu que parfois elle augmente et tantôt elle diminue entre les années d'étude (Tableau 6).

La teneur en chlorophylle a varié entre 0,73 ppm à 1,17 ppm pour les trois années de l'étude. Ces teneurs ont été légèrement plus élevées pour le traitement NPK par rapport au témoin pour les deux dernières années alors que l'inverse est observé pour l'année 2006 où elles ont augmenté significativement (Tableau 6).

Les teneurs en carotène semblent ne pas être affectées par le traitement NPK, où on note parfois une faible augmentation, et tantôt une diminution des teneurs en carotènes du NPK par rapport au témoin (Tableau 6).

L'extinction spécifique mesurée à 232nm et 270 nm, montrant l'état d'oxydation de l'huile, a augmenté significativement pour le traitement combiné NPK pour les deux premières années pour 232 nm par rapport au témoin. En effet ceci est dû à l'augmentation de la teneur en acide oléique constatée, sujet de cette oxydation qui favorise la formation d'hyperoxyde linoléique, cependant aucune variation est obtenue pour ceux de K270 nm.

**Tableau 6:** Caractéristiques de l'huile selon les traitements effectués pour les trois années de l'étude.

| Année       | Acidité (%) | Chlorophylle (ppm) | Caroténoïde (ppm) | Extinction Spécifique K 270 | Extinction Spécifique K 232 |
|-------------|-------------|--------------------|-------------------|-----------------------------|-----------------------------|
| 2006 Témoin | 0,28a       | 1,18a              | 14,33             | 0,12                        | 1,71a                       |
| NPK 2007    | 0,24b       | 1,01b              | 14,49             | 0,11                        | 1,78b                       |
| Témoin      | 0,17a       | 0,73               | 26,83a            | 0,11                        | 1,45a                       |
| NPK         | 0,19b       | 0,75               | 28,49b            | 0,11                        | 1,49b                       |
| 2008 Témoin | 0,3a        | 0,84               | 15,33a            | 0,12                        | 1,93                        |
| NPK         | 0,28b       | 0,87               | 13,66b            | 0,11                        | 1,94                        |

Les moyennes suivies de lettres différentes pour chaque année sont significativement différentes au seuil de 5% selon le test de Newman et Keuls.

#### 4. Conclusion

Nous pouvons conclure que la fertilisation raisonnée a permis l'amélioration de certaines caractéristiques des olives (poids moyen, rendement en olive) et de l'huile d'olive (la teneur en huile, augmentation de l'acide oléique, baisse de l'acide palmitique...). Cette amélioration de la qualité en huile et du rendement pour les trois années d'étude est due aux traitements NPK, donnés à juste dose et au bon moment.

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## Comparative study of Tunisian and foreign olive cultivars suitability for high density planting system

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### Abstract

In 2000, a new concept of olive orchard based on high densities was introduced in Tunisia. Limited cultivar choice is considered as the main inconvenient of high density planting system. In deed, only Arbequina and Arbosana are used in these types of orchards. Therefore, two comparative trials were set up in 2003 and 2004, respectively in Takelsa (north of Tunisia) and Sfax (south of Tunisia), to evaluate the suitability of 4 olive cultivars (Arbosana, Arbequina, Chemlali and Chetoui) to this planting system (1250 trees ha<sup>-1</sup>).

Results obtained here showed that local varieties (Chemlali and Chetoui) are more vigorous than Arbequina and Arbosana. However, the latest varieties presented the highest yield in comparison to the other varieties. Arbosana had the highest accumulated olive yield after 4 harvests in Takelsa while in Sfax; Arbequina presented the highest accumulated olive yield after 3 harvests. However, the highest yield efficiency was obtained by Arbosana variety in the two locations. The Chetoui variety, despite its lower production and accentuated alternate bearing, showed some interesting agronomic characteristics related with the high density planting system like the lower width canopy and the erected port.

**Keywords:** High density planting system, olive trees.

## Etude comparative de l'aptitude des cultivars d'oliviers Tunisiens et étrangers au système de plantation en hyper-intensif

### Résumé

Un nouveau concept de la culture de l'olivier basé sur l'emploi de hautes densités a été introduit en Tunisie l'an 2000. Le choix limité de variétés est considéré comme étant l'inconvénient principal de ce système de culture. Pour ceci, deux essais ont été installé en 2003 et en 2004 à Takelsa (le nord de la Tunisie) et Taoues (Sfax, sud de la Tunisie) pour évaluer le comportement de 4 variétés (Arbequina, Arbosana, Chemlali et Chetoui) à la culture super intensive (1250 arbres/ha).

Les résultats obtenus dans ce travail indiquent que les variétés locales (Chemlali et Chetoui) sont plus vigoureuses que les variétés Arbequina et Arbosana. Toutefois, les variétés Arbequina et Arbosana présentent une productivité plus élevée. En effet, la variété Arbosana a présenté la production cumulée la plus importante à Takelsa après 4 récoltes alors qu'à Taoues, c'est la variété Arbequina qui a présenté la production cumulée la plus élevée après 3 récoltes. Cependant, l'efficacité de production la plus élevée a été obtenue chez la variété Arbosana dans les deux sites sujets de notre étude. Malgré la faible production et l'alternance de la production de la variété Chetoui, elle a présenté des caractéristiques agronomiques intéressantes en relation avec le système de plantation hyper intensif tels que la faible largeur de la frondaison et son port érigé.

### 1. Introduction

The high density system developed in Spain 13 years ago was introduced in Tunisia in 1999 by some private investors. Since this date, this system of plantation was a matter of debate concerning the choice of cultivar and the choice of planting density. The high density planting system was developed to facilitate the use of the over-head mechanical harvester which may reduce costs relative to hand harvesting.

The life time of this new planting system as compared to other planting density remains the basic question by the detractors of this new planting system. Higher densities may allow higher productions during the first years. Later, the vigor will be difficult to be controlled which may reduce the light interception with the consequent significant decrease of production. So, the cultivar choice is considered as the main criterion when deciding to establish a high density orchard. Actually, Arbequina and Arbosana are considered as the most suitable cultivars for high density planting (Tous et al., 2007, De la Rosa et al., 2007). However, little is known about the behavior of these varieties under Tunisian conditions. Furthermore, no work has been done on the behavior of Tunisian varieties conducted under high density planting system, which limit the variety choice in high-density olive orchards.

The aim of this work was to study the behavior of Arbequina, Arbosana, Chemlali and Chetoui varieties grown under high density planting system in two different regions (Takelsa in the north and Sfax in the south).

## 2. Material and Methods

The olive tree orchards were located in Takelsa (North of Tunisia, 36° 47'; 10° 37') and Sfax (South east of Tunisia, 35° 02'; 10° 28'). Olive varieties planted in these orchards are Chemlali, and Chetoui from Tunisia, and Arbequina and Arbosana from Spain. Olive trees are 6 and 5 years old, respectively and planting density is 1250 trees/ha with a frame of 2 x 4 m. After planting, a trellis system was placed and each tree was tied to a stake, for central leader training. During the first's years, trees were lightly pruned to maintain the central leader. The statistical design was randomized complete blocks with two rows of ten trees per variety in Takelsa and 4 rows of five trees in Sfax. All measurements were carried out on the totality of trees composing each block.

Vigour (height and canopy volume), tree yield and estimated yield per ha, yield efficiency (yield/canopy volume), oil content, and oil characteristics (fatty acid composition) were determined. It must be pointed out that for studying oil characteristics, 3 fruit samples (3 kg) per block and per cultivar were taken each year from 2005 in Takelsa and 2006 in Taoues.

## 3. Results and Discussion

Results obtained in this work indicated that all studied cultivars bear in the second year after planting except for Chetoui in both localities (Table 1). In deed, Chetoui variety bears in the third and the fourth year after planting in Takelsa and Taoues (Sfax), respectively. Furthermore, Chetoui variety showed a tendency to an accentuated biennial bearing from the third year in Takelsa (Table 1). The most productive variety in the second year after planting was Arbosana in both localities. In the third year after planting Arbosana was also the most productive variety in Takelsa followed by Arbequina, Chetoui and Chemlali. However, in Taoues, Arbequina was the most productive followed by Arbosana, Chemlali and Chetoui. Without taking into account the production obtained the fifth year after planting in Takelsa, only "Arbequina" planted in Taoues showed a highest accumulated yield as compared to the same variety planted in Takelsa. With respect to the other varieties, similar accumulated yield were obtained in both localities. It must be pointed out that trees were severely damaged by *Spilotea oleaginae* during the fourth year after planting in Takelsa which reduced significantly the olive yield.

Estimated yield per ha for the first three harvests for Arbequina 118 and Arbosana in Taoues and Takelsa were inferior to those obtained with the same varieties in Cordoba (Spain) for the same period after planting (De la Rosa et al., 2007). This result may be due mainly to the differences in trees density which were about 1975 trees per ha in Cordoba. However, when compared to the production obtained by the same varieties in Tarragona (Spain), productivity in Tunisia was much higher; beside the tree density employed in Tarragona was 2 fold superior to that employed in Tunisia (Tous et al., 2007).

**Table 1:** Average yield (Kg of olives/tree) and accumulated yield of studied varieties from the second to the fifth and the fourth year after planting in Takelsa and Taoues.

|           | Takelsa              |      |      |     |             | Taoues               |      |      |             |
|-----------|----------------------|------|------|-----|-------------|----------------------|------|------|-------------|
|           | Years after planting |      |      |     | Accumulated | Years after planting |      |      | Accumulated |
|           | 2                    | 3    | 4    | 5   |             | 2                    | 3    | 4    |             |
| Arbequina | 2.06                 | 6.73 | 2.23 | 9.4 | 20.42       | 1.83                 | 3.21 | 11.6 | 16.6        |
| Arbosana  | 3.62                 | 7.13 | 2.7  | 13  | 26.48       | 3.06                 | 2.18 | 7.88 | 13.12       |
| Chemlali  | 2.42                 | 5.28 | 1.18 | 7.7 | 16.57       | 1.1                  | 0.16 | 9.82 | 11.07       |
| Chetoui   | 0                    | 7.07 | 0    | 6.4 | 13.5        | 0                    | 0    | 7.55 | 7.55        |

With respect to the vegetative characteristics, results obtained in both trials indicated that Arbequina I18 and Arbosana are low vigorous as compared to Tunisian varieties (Figure 1). In the fifth year after planting in Takelsa, Chetoui showed the highest vigour followed by Chemlali, Arbosana and Arbequina. However in Taoues, the highest vigour was obtained by Chemlali followed by Chetoui, Arbequina and Arbosana. Canopy volume of Arbequina reached in the third year after planting in Taoues ( $4.97 \text{ m}^3$  per tree) were superior to that obtained in Takelsa ( $3.59 \text{ m}^3$  per tree). Arbosana was the less vigorous variety in both localities. Tous et al. (2007) reported that Arbequina I18 and Arbosana were less vigorous than Koroneiki, Jonanenca and Fs17 planted in high density planting system.

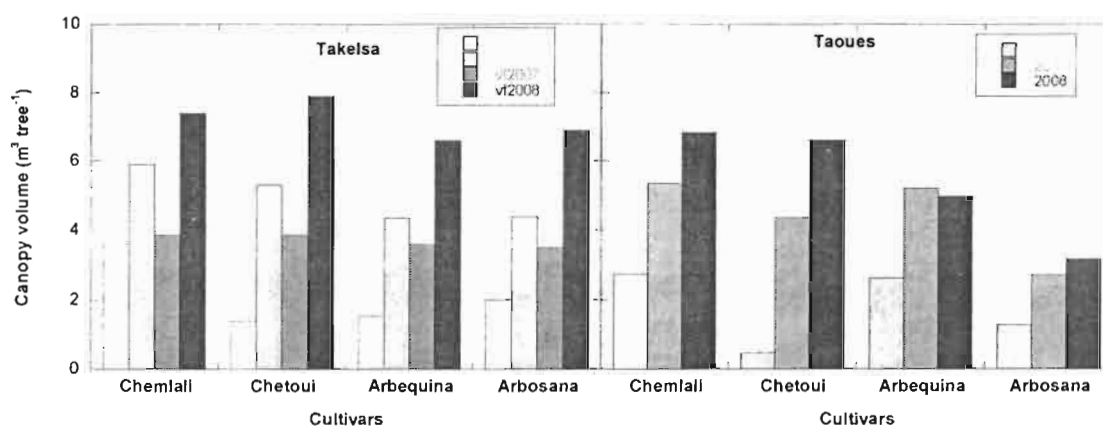


**Figure 1:** Canopy volume of studied varieties for the years under study.

The highest yield efficiency ( $\text{Kg per m}^3$  of canopy) was obtained with Arbosana followed by Arbequina, Chemlali and Chetoui in both localities (Fig. 2). Similar results were obtained by Tous et al. (2003) indicating the highest yield efficiency of Arbequina I18 and Arbosana. The highest yield efficiency is mainly due to the lower vigour of these varieties as compared to Tunisian studied cultivars (Fig. 1).

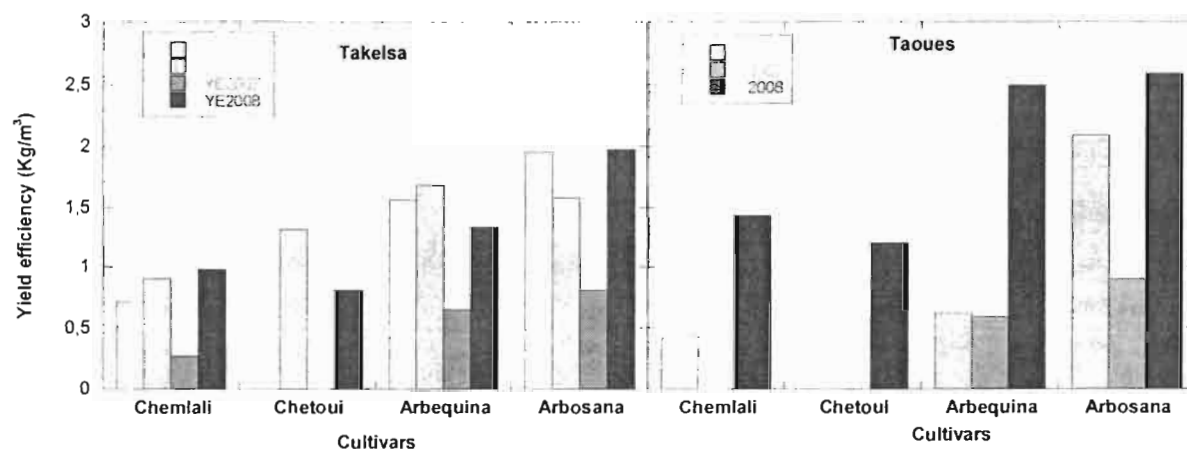


**Figure 2:** Yield efficiency for studied varieties for the years under study.

All studied varieties planted in Taoues showed higher oil content on dry weight basis as compared to those planted in Takelsa, except for Chetoui which showed similar oil content in both localities (Table 2). In contrast to the oil content, moisture content was higher in olives harvested from Takelsa for all studied varieties except Arbequina. Chetoui showed the highest fruit weight in both localities followed by Arbequina, Arbosana and Chemlali.

**Table 2:** Average fruit characteristics for the years under study.

| Varieties     | Takelsa           |                  |              | Taoues            |                  |              |
|---------------|-------------------|------------------|--------------|-------------------|------------------|--------------|
|               | Oil content (NMR) | Fruit weight (g) | Moisture (%) | Oil content (NMR) | Fruit weight (g) | Moisture (%) |
| Arbequina I18 | 47.6              | 1.94             | 47.64        | 51.79             | 2.07             | 56.42        |
| Arbosana      | 46.24             | 1.22             | 57.08        | 52.5              | 1.55             | 53.34        |
| Chemlali      | 44.98             | 0.89             | 54.97        | 48.6              | 0.85             | 50.73        |
| Chetoui       | 52.6              | 2.62             | 62.34        | 51.26             | 2.28             | 55.93        |

Arbequina I18 and Chemlali were characterized by low oleic acid content and a high palmitic and linoleic acid (De la Rosa et al., 2007; Grati-Kammoun and Khelif, 2001). Results obtained here indicated that oleic, palmitic and linoleic acid content of Arbequina planted in Takelsa were similar to those obtained in Cordoba (De la Rosa et al., 2007). While, in Taoues, the oleic acid content decreased and the palmitic and linoleic acid increased as compared to those obtained with the same variety in Takelsa. By contrast, Chemlali and Chetoui showed highest and lowest oleic acid content in Taoues, respectively in comparison with those obtained with the same varieties in Takelsa. These results may be explained by the origin site of each variety which was the north for Chetoui and the south for Chemlali. Contrary to these varieties, Arbosana showed similar oil characteristics in Taoues and Takelsa (Table 3). Several authors reported that olive oil composition in fatty acids varies according to the geographical origin (Ben Temime et al., 2004, Ryan et al., 1998).

**Table 3:** The fatty acid composition of the oil of the different studied varieties. Values are the average of three harvests in Takelsa and two harvests in Taoues except the chetoui.

| Varieties     | Takelsa |       |       | Taoues |       |       |
|---------------|---------|-------|-------|--------|-------|-------|
|               | C16:0   | C18:1 | C18:2 | C16:0  | C18:1 | C18:2 |
| Arbequina I18 | 17.63   | 63    | 14.21 | 18.53  | 58.8  | 16.5  |
| Arbosana      | 16.37   | 68.77 | 9.57  | 16.93  | 67.12 | 10.56 |
| Chemlali      | 18.19   | 60.15 | 15.9  | 16.07  | 63.8  | 14.57 |
| Chetoui       | 16.95   | 66.77 | 12.81 | 12.47  | 60.91 | 22.14 |

In summary, Arbosana showed a good suitability for high density orchards mainly due to its lowest vigour when compared to Arbequina and Tunisian varieties, high productivity and stable oil characteristics in different environments. Furthermore, Arbosana were characterised by its high oleic acid content. Arbequina seem to be better adapted in the north than the south of Tunisia due to the decrease of oleic acid content recorded in the south in comparison with the north (Takelsa). However, it must be pointed out the high productivity of this variety in both localities. The employment of Tunisian cultivars (Chetoui and Chemlali) in high density planting system seem to be difficult due to the high vigour of these varieties, the alternate bearing of Chetoui and the instability of fatty acid composition when these varieties are planted in non original sites.

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## Effect of topping pruning on pollen viability and germination of Arbequina and Arbosana varieties grown in high density planting system

Ameur Teycir, Larbi Ajmi, Msallem Monji, and BenDhiab Ali

### Abstract

The main objective of this work is to study the effect of topping pruning on pollen quality, into the canopy of Arbequina and Arbosana varieties conducted under HDP. For this purpose an experiment was conducted in Mornag (North of Tunisia, 450 nm; 24.2° C; 36.42 North), planted in 1999 with a density of 1250 trees/ha. Trees were divided into three layouts: the bottom layout (0-1 m), the medium (1-2 m) and the top one (up to 2 m). The pruning was carried out mechanically at a height of 220 cm above the soil at February 2007. The pollen sampling was carried out at white button stage (May 2008). In the same time, the light interception by the canopy (PAR) and branch growth were measured. Our results show that the highest rates of pollen viability were recorded at the more lighted parts of the canopy of the control trees of Arbequina and Arbosana varieties. The germination rate followed the same trend in both varieties, with a decrease of 30.27 and 12.87% respectively at the bottom layout (0-1m) and the medium one (1-2 m) as compared to the upper layout of Arbosana variety. In the second year after the "Topping", pruning improved significantly the viability and germination rate in the lower parts of the canopy of the two studied varieties. However, a significant decrease of pollen viability and germination was recorded in the pruned part of the canopy, as compared with controls.

**Keywords:** high density, « Arbequina », « Arbosana », quality, pollen, pruning, « Topping ».

### Résumé

La lumière constitue un élément essentiel pour l'olivier surtout pour les plantations hyper intensives. La qualité du pollen est fortement influencée par son microenvironnement. La taille « Topping » est appliquée comme moyen de correction en haute densité. A cet effet un essai été réalisé dans un site expérimental à Morneg (Nord de la Tunisie, 450 nm ; (13,9 et 24,2 °C); 36,42 Nord) à une densité de 1250 arbres/ha en Arbequina et Arbosana (plantation 1999). Les arbres ont été subdivisés en trois étages : le premier étage (0-1 m), le deuxième étage (1-2 m) et le troisième étage (supérieur à 2 m). La taille est réalisée mécaniquement à une hauteur de 220cm parallèlement au sol (au mois de Février 2007). Les prélèvements des échantillons du pollen, le suivi de l'interception lumineuse et de la croissance végétative ont été effectués au stade bouton blanc (mai 2008). Nos résultats montrent qu'au niveau des arbres non taillés, la qualité du pollen ainsi que la croissance végétative sont meilleure au niveau des zones les mieux éclairées de l'arbre, avec une diminution de 30,27 et de 12,87%, respectivement au niveau des étages 0-1m, 1-2 m par rapport à l'étage supérieur chez la variété Arbosana. A la deuxième année de son application, la taille a contribué à l'amélioration significative de la fertilité du pollen au niveau des étages inférieurs des arbres taillés pour les deux variétés, mais pas celui de l'élongation végétative. Au même temps, la qualité du pollen a été négativement affectée au niveau de la zone de coupe, en comparaison avec les témoins.

### 1. Introduction

The olive production sector in Tunisia has known a marked intensification and mechanization from 1999 (Msallem et al., 2004; Msallem et al., 2008; Caballero, 2009). This intensification was based mainly on the use of the high density planting (HDP) system developed in Spain since 1995. The HDP system orchards cover actually 4500 ha in Tunisia (Msallem et al., 2008) and is based on a high production per hectare, early fruit-setting cultivars, rapid recovery of investment and a low cost of production through mechanization of the harvest (Msallem et al., 2004; Tous, 2007; Léon et al., 2007). In these orchards, the production distribution is a function of the location, its cardinal directions and its height all over the olive tree (Acebedo et al., 2000). In fact, the light penetration in the canopy of the tree is under the influence of inter-tree and intra-tree shading in HDP system (Faust, 1989, Connor, 2006).

In the HDP system, it is necessary to define an optimal density and ensure a good control of the dimensions of trees to avoid the negative effects of the shading (León et al., 2007; Pastor et al., 2007). The "Topping" pruning is applied as a correction technique in HDP (Pastor, 1989; Dag, et al., 2006; Dias et al., 2009). Depending on this pruning types (frequency, period of application, position, and severity), the physiological reaction and the reproductive performance of the olive tree will change (Faust, 1989; Cronje et al., 2004).

Phenological behaviors of olive tree are also largely influenced by the micro-environmental factors (temperature and exposure) (Defni et al., 2000; Delphi et al., 1997). The influences of these factors are explained by the probable relation of heat accumulation with bud's location by the canopy (Cesaraccio, 2005). Viability has been defined as "having the capacity to live, grow, germinate or develop" (Lincoln *et al.* 1982 cited by Dafni et Firmage, 2000). So, the evaluation of pollen viability is the first step in understanding the chances of the pollen to germinate on the stigma as a crucial stage toward fertilization.

A pollen grain quality is determined by pollen viability, germinability and/or ability to fertilize flowers. The main objective of this study is to carry out first the effect of HDP system and second the reaction to the application of the "Topping" pruning on pollen potential's performance of "Arbosana" and "Arbequina" olive oil varieties, and the relationship with the vegetative growth and the light distribution by the canopy.

## 2. Material and Methods

This work was carried out in Mornag in a high density planting orchard (1250 trees/ha) planted at 1999 with Arbequina and Arbosana varieties. The dormant pruning trial was carried out in this site in February 2007. The experimental design consisted of two randomized complete blocks with 5 trees each one for every variety. Three trees / variety / treatment (Topping and control) were considered. Each tree was divided into three layouts: the bottom one (0-1 m), the medium one (1-2 m) and the top one (up to 2 m). On each layout, three branches for each quadrant (North, East, West and South) and three from the interior were selected and labeled in March 2008. In total, 15 twigs for each layout were observed during the reproductive season until fruit set stage. The pollen sampling was carried out at white button stage (May 2008) from this five locations/layout/variety/treatment. At the same time, the light interception by the canopy (PAR) and branch growth were measured. Statistical analyses were performed using the SPSS 13.0 for Windows.

## 3. Results and discussion

Results obtained here showed that photosynthetic active radiation (PAR) decreases significantly from the top to the medium and low parts of the tree (Figure 1). In fact, PAR measured above vegetation (up to 3m) was 1220.33 $\mu\text{E}/\text{m}^2/\text{s}$  and 1376.52 $\mu\text{E}/\text{m}^2/\text{s}$  for Arbosana and Arbequina varieties respectively. From the upper part to the lower one of the tree, PAR decreased continually by 10-19%, 39-31.5% and 70.5-59% respectively, for Arbosana and Arbequina. León and al. (2007) reported that trees planted in high density are likely to suffer from the mutual shading problems by the seventh years.

The topping pruning did not affect significantly the PAR interception by the canopy (Fig. 1). In deed the light interception only increased (10.7 and 23.7%) in the upper part of the canopy of pruned trees as compared to the control trees (Figure 1). However, a small decrease of light interception was recorded in the medium and the bottom layout in pruned trees in comparison with control trees. Porpiglia and Barden (1981) found that light intercepted by the canopy increased during the first years, but tend to decrease the following years, depending to the pruning severity level.

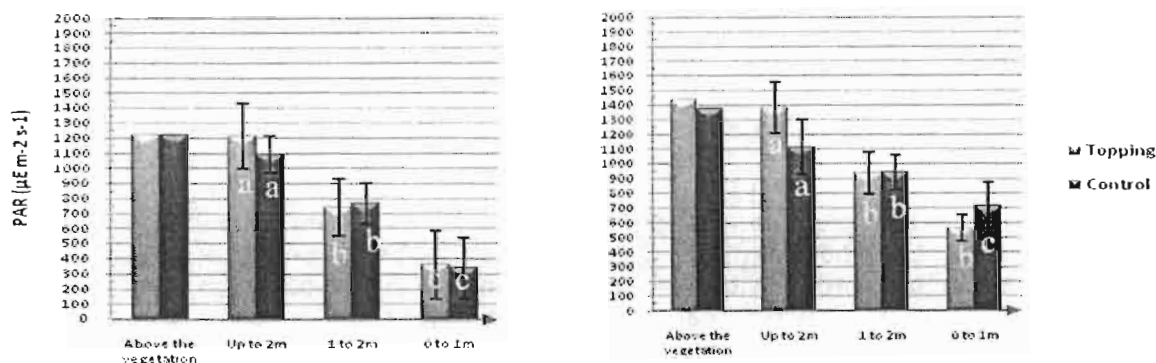


**Figure 1:** Par intercepted by the 'Arbosana' (A) and 'Arbequina' (B) olive oil canopy of 'pruned' and 'unpruned' trees conducted in high density planting system (Means with different superscript letters (a,b,c).

Results show that Pollen viability varied significantly with the location by the canopy and the cultivars. The topping pruning application did not influence significantly pollen viability rate in the medium and lower parts of the Arbosana, but a significant increase in the lower layout in case of Arbequina was noted. In contrast with the upper and lower layout of the canopy of Arbosana, a significant increase of pollen viability was found in the upper part of the canopy of pruned trees in comparison with the controls (Figure 2).

Viability rate was explained by pollen grain origin, its position in the inflorescence and its location in the branch, but also the microenvironment during pollen formation (Villemur, 2006; Dafni and Firmage, 2000).

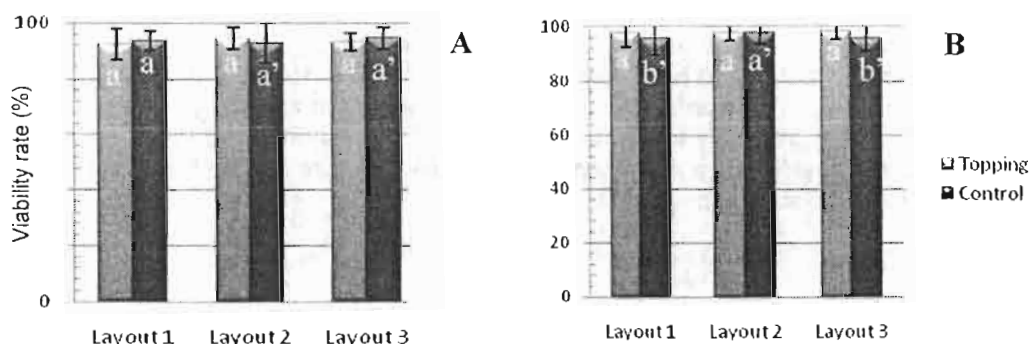


**Figure 2 (A and B):** Viability rate distribution by the canopy of 'pruned' and 'unpruned' trees 'Arbosana' (A) and 'Arbequina' (B) varieties conducted in high density planting system (Means with different superscript letters (a,b,c) are significantly different at  $P < 0.05$  (Duncan's test)).

Considering germination rate, our results showed that it varied significantly by the treatment and the employed variety and the combined effect. In fact, germination rate was closely associated with the PAR levels intercepted by the canopy. It was higher in the branches localized up to 2m with 43.9%-30.2% for Arbosana and Arbequina. In the medium and lower parts of Arbosana and Arbequina, the potential of pollen to germinate was 38.3%-31.2% and 30.6%-24.6%, respectively.

Similar results were found with Frangivento and Frantoio olive cultivars (Cimato 1980; 1982 cited by Acebedo et al. 2000).

The "Topping" pruning application, led to the improvement of pollen germination rate in the lower part of the canopy by 6.7% and 39% in comparison with control trees, for Arbosana and Arbequina cultivars, respectively. In contrast with the lower part of the canopy, a significant decrease of germination rate was observed in the upper layout for two varieties. This result could be explained by the stress effect of the pruning technique which was done in the upper part of the canopy.

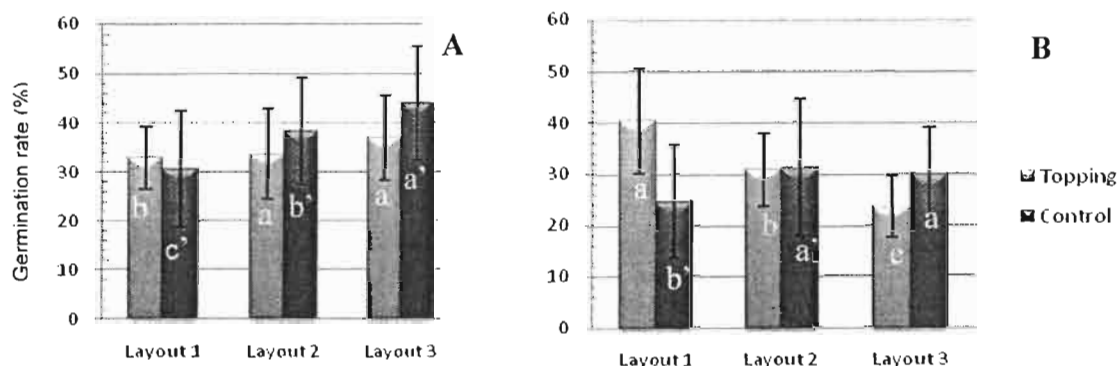


**Figure 3 (A and B):** Germination rate distribution by the canopy of 'pruned' and 'unpruned' trees 'Arbosana' (A) and 'Arbequina' (B) varieties conducted in high density planting system (Means with different superscript letters (a,b,c) are significantly different at  $P < 0.05$  (Duncan's test)).

With respect to vegetative growth, results obtained in this work indicated that the top of the tree grew significantly more than other parts of the tree for pruned and controls trees. However, it must be pointed out that the difference observed between the two higher layouts in control trees was not significant.

Results shown in Fig.4 indicated that the length of branch in the upper layout was higher than the other parts of the canopy. The length of new shoots varied significantly with the same trend under the combined effect of the layout and variety.

Faust (1989) suggested that the growth in the top of the tree would shade the rest of the tree at the same time; growth at the base of the canopy would diminish. The reductions in the resources available for pollen production could be explained by the limited growth vegetative (Delphi et al., 1997).

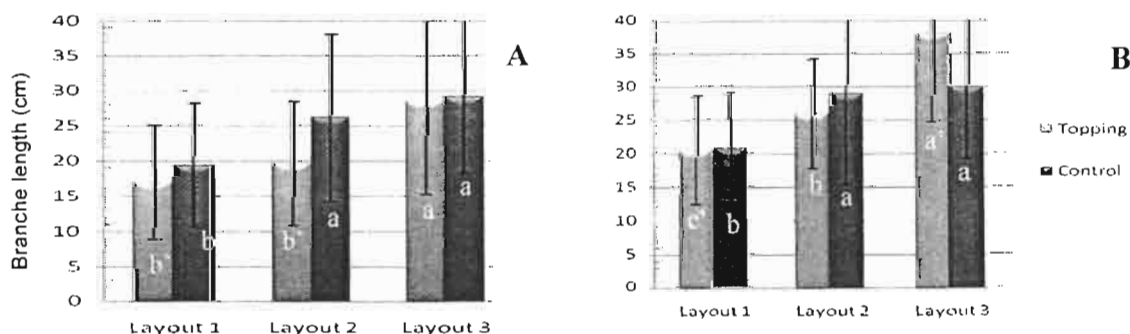


**Figure 4 (A and B) :** Shoot growth according to the three layout by the canopy of 'pruned' and 'unpruned' trees 'Arbosana' (A) and 'Arbequina' (B) varieties conducted in high density planting system (Means with different superscript letters (a,b,c) are significantly different at  $P < 0.05$  (Duncan's test)).

The topping pruning increased the number of flowers per inflorescence in the different layouts of the canopy of the two studied varieties except the upper layout of the pruned Arbequina. The decrease of the number of flowers per inflorescence in the upper layout of the pruned Arbosana may be explained by the highest flowering intensity (data not shown).

Similar results were found by Acebedo (2000) indicating that flowering intensity was highest in parts of unpruned trees receiving the highest levels of radiation. Furthermore, Cesaraccio et al. (2005) indicated that the branch exposures have certain effect on reproductive cycle and phenological behavior of olive tree in term of timing of reproductive stages.

The fruit set percentage of Arbequina and Arbosana varieties were higher in the top layout as compared to the medium and bottom ones (table 1). Pastor et al. (2007) found a linear response between the number of inflorescence per shoot and the relative photosynthetic active radiation. For Arbequina cultivar, Acebedo et al. (2000) concluded that the effect of shoot location on fruit setting was quit significant between the top and the lower part of the tree.

The topping pruning decreased the fruit set percentage of Arbequina and Arbosana. Decreases were more pronounced in Arbosana. Fruit set decrease in Arbequina variety may be due to the stimulation of vegetative growth after pruning topping application. With respect to Arbosana variety, the higher decrease of fruit set in pruned trees, may be due to either stimulation of vegetative growth and to the higher percentage of flowers buds in comparison with control trees.

Contrary to our results, several authors have indicated that pruning increased fruit set which might result from increased water to the tree and nitrogen supply to the leaves (Chandler, 1919 cited by Faust, 1989). Aldrich and Grim (1938) cited by Faust (1989) found an increased N supply in fruiting spurs of pruned trees.

**Table 1:** influence of topping treatment on flowers numbers per inflorescence and fruit set for Arbequina and Arbosana varieties.

| Treatment | Layout      | Number of flowers/inflorescence |                  | Fruit set (%)      |                    |
|-----------|-------------|---------------------------------|------------------|--------------------|--------------------|
|           |             | Arbosana                        | Arbequina        | Arbosana           | Arbequina          |
| Control   | (0-1 m)     | 11,42 a<br>±3,88                | 11,79 a<br>±3,35 | 20,75 b<br>±14,22  | 14,50 b<br>±8,96   |
|           | (1-2 m)     | 11,59 a<br>±3,30                | 11,94 a<br>±3,97 | 22,50 ab<br>±16,03 | 15,52 b<br>±9,57   |
|           | (up to 2 m) | 12,93 a<br>±5,65                | 11,26 a<br>±3,94 | 24,30 a<br>±14,00  | 23,83 a<br>±16,30  |
| Topping   | (0-1 m)     | 12,17 a'b'<br>±7,07             | 11,40 a' ±4,47   | 12,74 b'<br>±8,04  | 13,43 b'<br>±8,85  |
|           | (1-2 m)     | 14,12 a'<br>±6,23               | 13,00 a' ±4,67   | 12,92 b'<br>±7,77  | 15,22 b'<br>±9,98  |
|           | (up to 2 m) | 9,97 b'<br>±7,73                | 12,43 a' ±4,39   | 21,35 a'<br>±15,87 | 22,99 a'<br>±12,59 |

#### 4. Conclusion

In conclusion our preliminary results indicate that the high density planting system limit the potential of pollen grain to fertilize in the lower part of the canopy.

The application of the topping improves pollen potential to have great number of fertilized flowers.

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## Mechanical harvesting of table and oil olives

Louise Ferguson Vossen Pm\*, Rosa U\*\*, Guinard Jx\*\*\*, Burns J\*\*\*\*, Krueger Wh\*, Glozer K\*

### Abstract

Mechanical harvesting must be developed for successful table and olive oil production in California. Both canopy contact shaking head and trunk shaking harvesters can produce processed black ripe olives that trained sensory panels and consumer panels cannot distinguish from hand-harvested olives. However, both types of harvesters remove and capture less than the 80% efficiency required. No successful abscission compounds to decrease fruit removal force have been identified. Therefore, as with oil olives the tree shape must be modified for successful Mechanical harvesting must be developed for successful table and olive oil production in California. Both canopy contact shaking head and trunk shaking harvesters can produce processed black ripe olives that trained sensory panels and consumer panels cannot distinguish from hand-harvested olives. However, both types of harvesters remove and capture less than the 80% efficiency required. No successful abscission compounds to decrease fruit removal force have been identified. Therefore, as with oil olives the tree shape must be modified for successful table olive harvesting. Recent results demonstrate training to an espalier shape with and without a trellis does not decrease yield and the tree that can be harvested with both canopy contact and trunk shakers. However, the harvesters differ in their removal patterns, efficiency, and tree damage.

**Key words:** *Olea europaea*; flower development; SSH; gene expression, Gene Ontology

### Résumé

La récolte mécanique des olives doit être développée aussi bien pour les oliviers à tables ou à huiles. Des tests de dégustations effectués par des panels de dégustateurs ou par des consommateurs n'ont pas réussi à détecter les olives de tables noirs récoltés mécaniques par vibration au niveau de la frondaison ou du tronc des olives récoltées manuellement. Cependant les deux types de récolteurs mécaniques ont une efficacité de récolte inférieure au 80% demandé à ce genre d'opération. Aucun produit d'abscission efficace n'a pu être identifié pour augmenter cette efficacité. Par contre la forme de la frondaison des oliviers à tables doit être adaptée pour la récolte mécanique comme cela a été fait pour les oliviers à huile. Les résultats récents montrent qu'une forme en mur fruitier avec utilisation ou non de structure ne diminue pas le rendement. Les arbres peuvent être récoltés en utilisant une vibration au niveau du tronc ou au niveau de la frondaison. Néanmoins les deux types de récolteur diffèrent d'un point de vue de leur efficacité et des dégâts occasionnés sur les arbres.

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### **Thème 4**

**Changement climatique, gestion de l'eau et développement durable de l'olivier**

## **Tendances de floraison des oliviers et caractéristiques climatiques dans deux régions Méditerranéens (Italie et Tunisie)**

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### **Résumé**

L'influence de la latitude sur les dates de floraison chez olivier a été étudiée à travers quinze stations de culture de cette espèce en méditerranée: quatre en Tunisie et onze en Italie (de Zarzis, 33° au Sud, jusqu'à Perugia, 43° au Nord). La floraison été suivie grâce à la technique de captage pollinique sur une période de 10 ans (1999 à 2008). La tendance du déroulement de la floraison a mis en évidence une étroite relation entre la phénologie de l'espèce, les températures et les caractéristiques géographiques, même si les variations météorologiques annuelles portent à considérer que la précocité ou le retard phénologique sont des événements locaux.

**Mots clés :** Olivier, floraison, latitude, climat.

## **Flowering trends in olive and climate features in two mediterranean countries (Tunisia and Italy)**

### **Abstract**

Research on the influence of latitude on flowering phenology has been realized to investigate the different magnitude of biological response. This type of analysis was conducted considering one of the more important plant species of Mediterranean shrub, the olive, in fifteen monitoring stations, four of which located in Tunisia and eleven in Italy, from the southern area of Zarzis (33°) to the northern Perugia (43°). The flowering was studied utilizing an aerobiological monitoring method through pollen traps inside olive groves from 1999 to 2008. The flowering trends during the study period evidenced very close relationships between plant's phenology, temperature trends and geographical features even if the yearly mesoscale meteorological variations force to consider phenological advances or delays as local events.

**Key-words:** Phenology, Olive, Climate, Latitude, Mediterrany.

### **1. Introduction**

The investigation efforts on the theme of climate change have necessarily to consider the importance of plants, since they are very sensible to climate and manifest with their phenological developments its variations. In these terms we can consider plants as bio-indicators so as biological systems used for evaluation of deviations from the stable environmental condition, independently of its organization level and its function in the ecosystem (Menzel 2002; Osborne et al. 2000; Schwarz 2000; Parmesan et al. 1999). The climate variability and the climate change can influence the vegetative-reproductive cycle of a plant species (Menzel et al. 2006). Plants can highlight the alterations caused by different factors; a response to any kind of disturbance must thus be interpreted and evaluated because it summarizes the synergic action of all environmental components. The possible consequences are numerous, from risks on a small scale, like late spring frost, to more drastic consequences, likes a change of growth area. The actual climatic change could have the sufficient impact to determine a modification in area of distribution of the most sensible natural species and a temporal shifting of phenophase manifestations. Obviously, phenological responses to climatic variables are rather complex and varied in different species; nevertheless, some simple empirical temperature-based models have shown good forecasting results for different phenological phases. As a consequence of the present hypothesis, if phenological developments are highly influenced by climate, the same climate depends on geographical characteristics (latitude, altitude, closeness to water surfaces and so on) which may variously influence it.

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In particular one of these characteristics, the latitude, was considered through phenological response to climate changes (Parmesan 2007; Kleunen and Fischer 2008) in order to investigate the possible different magnitude of biological response in relation to lower or higher latitudes. The present study, although at a lower scale, permitted to collect phenological data on olive flowering through the aerobiological monitoring in study areas located on a close interval between the longitude of 9°50' and 16°00' but at various latitudes, approximately from 33° of the southern (Zarzis) to 43° of the northern (Perugia). The two stations represent the geographical limit of olive cultivation, considering that Zarzis is situated in a Tunisian very dry climatic area (the desert side), while Perugia with its continental climate constitute the north Italian limit, especially during winter, to the productive olive grove cultivation. In this manner the investigation permitted to compare the obtained flowering dates to evidence a possible relationship between latitude gradient and plant response. Moreover, another aim of the research was to investigate on the presence of flowering trends in the different geographical areas and on the hypothetical relationships with the same latitude.

## 2. Materials and methods

In the present investigation 15 olive monitoring stations were evaluated, considering that beside the two cited Zarzis and Perugia, other 13 olive cultivated areas were monitored: Taous, Mornag, Jammel in Tunisia, Agrigento, Trapani, Reggio C., Catanzaro, Cosenza, Lecce, Salerno, Brindisi, Benevento and Foggia in Italy (Fig. 1). All these areas were evaluated because their significance by a olive productive point of view. Moreover, in all these areas different olive cultivars were cultivated, in particular in the four Tunisian ones the principal cultivars are Chetoui and Chemlali, in Sicily the cultivars Biancolilla and Nocellara are the most famous while in the other Italian areas the more important cultivated varieties are: Frantoio, Carolea and Coratina.

The olive flowering phenomenon was monitored utilizing two aerobiological methods principally used in this scientific field of research, one developed by Hirst (a volumetric method) and the other by Cour (Cour 1974; Fornaciari et al. 2000a, b; Galan et al. 2004). Really in all the monitoring stations the quantitative evaluation of pollen emission was utilized to determine the start of flowering SF, defined as the day in which one pollen grain/m<sup>3</sup> was reached wherever five subsequent days contained one or more pollen grains/m<sup>3</sup>, the maximum of flowering (peak of flowering) MF, defined as the day of maximum pollen concentration and the end of flowering EF, defined as the last day with pollen concentration.

The phenological data (flowering dates) were collected from 1999 to 2008 in every monitoring station and intra-inter variability analyses were carried out.

The presence of monotonic positive or negative trends of the flowering dates during the 10-year study (1999-2008) in all the monitoring areas were at first tested with the nonparametric Mann-Kendall test and secondly the slope of a linear trend was estimated with the nonparametric Sen's method (Gilbert 1987).

The meteorological variables considered were obtained both in Tunisia and in Italy by the Stations of the National Meteorological centres located near the pollen monitoring areas. They include the minimum, maximum and average temperatures, the range of temperature between the maximum and the minimum levels, precipitations intended as quantity of rainfall, the number of days with precipitations and the evapotranspiration. The daily values of temperature accumulation in Growing Degree Days (GDD) were calculated with different threshold temperatures (7, 9, 11, 13° T), while the GDD amounts were considered, for each one of the monitoring station, from the first day of January to the mean flowering date during the 10-year study period.

Up to three different GDD calculation methods were utilized to determine the daily GDD in each area; Single Triangle and Sine Methods; "Cesaraccio" Method.

The determination of the most suitable Threshold Temperature between those utilized for heat accumulation and the evaluation of the different GDD calculation methods for the different study years and monitoring areas were carried out through the method of the RMSE considering its reliability in the threshold temperature estimation (Snyder et al. 1999).

To obtain specific results, about the relationships between flowering dates and the most suitable GDD amounts for each one of the monitoring areas, 15 singular regression analyses were carried out utilizing the temperature summations as independent variable.

### 3. Results

The relationship analyses between flowering dates and GDD amounts for each one of the monitoring areas are shown in the 15 singular regression analyses carried out and reported in Table 1. All the statistical analyses evidenced good percentages of explained variability ( $R^2$  higher than 0.35) and high significances of the independent variables (prob level < 0.05). The main results evidenced by this type of analysis were represented by the specific slopes of flowering related to the GDD amounts calculated for each one of the monitoring areas. The slope value permitted to consider mathematically the ratio between variation in days and GDD amount, all the slopes were negative indicating that the increase of one GDD unit provokes the flowering advance of a day portion. The average slope of -0.05 defines the advance of about 1.2 hour ( $24h * 0.05$ ) in relation to the increase of a GDD unit. In this sense the slope calculated in relation to the latitude of the respective monitoring stations evidenced as the biological response of olive plants are quite constant independently from the different study areas.

In Figure 2 the relationships between full flowering dates, temperature amounts (GDD amounts) and latitude are explicated by linear regressions considering contemporarily the biological, meteorological and geographical data of all the monitoring areas. The statistical results evidenced the high positive influence of latitude on flowering dates and on the other hand, its negative influence on temperature amounts.

Taking in consideration the first regression, the slope of full flowering dates per latitude degree is 4.38 with a standard error of 0.45. The value of R-Squared is 0.88 while the correlation between the two variables is 0.94. A significance test that the slope is zero resulted in a t-value of 9.75. The significance level of this t-test is 0.0000. Since  $0.0000 < 0.0500$ , the hypothesis that the slope is zero is rejected. The estimated intercept is -32.34. In the regression that considers as dependent variable the GDD amounts the slope of temperature summation per latitude degree is -26.23 with a standard error of 5.98. The value of R-Squared, is 0.60. The correlation between variables is -0.77. A significance test that the slope is zero resulted in a t-value of -4.39. The significance level of this t-test is 0.0007, the hypothesis that the slope is zero is rejected. The estimated intercept is 1815.16.

### 4. Discussion and conclusion

We could conclude that averagely the relationships between plant's phenology, temperature trends and geographical features are very close. However, the yearly mesoscale meteorological variations avoid the possibility to forecast flowering dates of northern areas on the base of southern ones, considering that the meteorological and consequently the phenological advances or delays are local events.

Finally, the regression analysis between full flowering dates and GDD amounts at the different latitudes permitted us to evidence the response of olive species in geographic regions subjected to different rates or patterns of climate change. The magnitude of biological response at different latitude was investigated and the slope results of respective monitoring stations evidenced as the flowering phenomenon of olive plants are quite constant, practically the same temperature increase provokes similar olive flowering advance in the different areas. As reported in a recent study (Parmesan 2007) while an effect of latitude is present (justified by complessive regression analysis) the same latitude is not so important as predictor of the magnitude of olive phenological response to climate change in the Mediterranean area investigated.

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## **Climatic change impacts on olive orchards in Tunisian arid area: the resort to the irrigation with brackish wastewater**

Bechir Ben Rouina, Chedlia Ben Ahmed & Saïd Jilani

### **Abstract**

Since the beginning of 1980, Tunisian arid climate has not stopped to show a worrying evolution. In comparison with the period extended between 1950 and 1980, the rise of the average annual temperatures exceeded  $1,5^{\circ}\text{C}$  ; whereas the averages of precipitations regressed of at least 30 mm year<sup>-1</sup> and the episodes of one year dryness and more became numerous. In corollary with these two phenomena, the already excessive evapotranspiration rate raised at least to 35 mm of water per degree Celsius, which makes the atmosphere hotter and less saturated with water vapour.

Under these hostile conditions the soil is the basic element in the life of plants by playing a great role as a source of water and nutrients. The rains sustain the water reserves which will be restored at the plant when they will be in need. To face this climate effects: increasingly more hostile and drier with the plants and in order to ensure a better productivity of the olive trees, the resort to the irrigation of the olive groves is developed. However, confronted with fresh water scarcity, the use of treated wastewater water is extended and risks of generating serious problems threatening at the same time the soil and the plant, and consequently, the fragile ecosystem sustainability following grounds salinization and plant toxicity by salts.

## **Impacts du changement climatique sur l'oléiculture en milieu aride tunisien : le recours à l'irrigation avec les eaux non conventionnelles**

### **Résumé**

Depuis le début des années 1980, le climat aride tunisien ne cesse de montrer une évolution inquiétante. En comparaison à la période étendue entre 1950 et 1980, la hausse des températures annuelles moyennes a excédé  $1,5^{\circ}\text{C}$  ; alors que les moyennes des précipitations ont régressé d'au moins 30 mm an<sup>-1</sup> et les épisodes de sécheresse d'une année et plus sont devenues nombreuses. En corollaire à ces deux phénomènes, l'évapotranspiration potentielle déjà excessive, se trouve majorée d'au moins 35 mm d'eau par degré Celsius, ce qui rend l'atmosphère plus chaude et moins saturée en vapeur d'eau.

Dans ces conditions hostiles à la vie des végétaux, le sol est l'élément de base dans la vie des plantes en jouant un rôle de source d'eau et de nutriments. Les pluies entretiennent les réserves hydriques de celui-ci qui vont être restituées à la plante au fur et à mesure qu'elle aura besoin lors de ces différentes phases de développement. En culture pluviale sous des conditions de précarité hydrique, ce rôle de puits devient très important puisque l'olivier est en deçà de ses besoins en eaux estimés à 450 mm de pluie par an et la qualité du sol devient un facteur capital de régulation de l'alimentation hydrique de celui-ci.

Pour faire face à ce climat de plus en plus hostile aux cultures et en vue d'assurer une meilleure productivité des arbres, le recours à l'irrigation des oliveraies est développé. Cependant, confrontés à la rareté de l'eau douce, l'utilisation tout azimut des eaux non conventionnelles est en extension et risque de poser de sérieux problèmes menaçant à la fois le sol et le végétal et par voie de conséquence, la durabilité de l'écosystème fragile suite à la salinisation des terrains et à la toxicité des sels.

**Mots clés :** Olivier, climat, sol, aridité, eaux salines, eaux usées traitées.



## 1. Introduction

The Middle East and North Africa are considered as the driest region in the world and freshwater availability in those areas is very low (World Bank, 1996). According to several authors (Ben Ahmed et al., 2006; 2008; 2009a; 2009b; 2010; Bedbabis et al., 2009; 2010a; 2010b; Ben Rouina et al., 2006; 2010) the use of brackish water or recycled wastewater in agriculture may help in reducing the problem of the limited availability of freshwater. In Tunisia, 106 wastewater treatment plants generated approximately 238 Mm<sup>3</sup> year<sup>-1</sup> of treated wastewater (TWW) in 2009 (ONAS, 2010). About 65 millions cubic meters (30%) of these TWW quantities were recycled on agriculture and supply to irrigate 9600 hectares. Many studies (Ben Ahmed et al., 2009a ; Bedbabis et al., 2009; 2010a ; 2010b ; Rejeb, 1992) showed that the application of treated wastewater at a reasonable rate improved growth and productivity of some crops and trees. However, the main problem that can arise from excessive and continuous application of TWW is phytotoxicity due to high content of salts and heavy metals, which may pose a health risk for human beings or livestock (Rejeb, 1992). In Sfax, TWW is from domestic provenance (88%) and industrial (12%) sources and is typically reclaimed at the secondary level by using biological processes. These processes consist of eliminating biodegradable material by transforming it into microbial residues. Secondary treatments remove dissolved and colloidal organic matter by using an aerobic-biological treatment. The aerobic-biological treatment is performed in the presence of oxygen by microorganisms which metabolize the organic matter, thereby producing more microorganisms and inorganic end-products (CO<sub>2</sub>, NH<sub>3</sub> and H<sub>2</sub>O). Studies on the effect of TWW on olive's growth and productivity are scarce. The topic should be better investigated in countries like Tunisia where olive trees play an important role for the economy but water availability for agricultural purposes is very limited. The objective of our study is to verify if irrigation with TWW can be considered a good source of water and fertiliser's source for "Chemlali" olive trees with no phyto toxicity effects.

## 2. Materials and Methods

### 2.1. Experimental plots

Olive trees (*Olea europaea* L.), planted in a sandy soil at 'El Hajeb' experimental station at Sfax, Tunisia (34°43N, 10°41E) were used from February 2004 to 2009. Eighteen-years 'Chemlali' olive trees spaced 24 m × 24 m were selected for this experiment (17 trees ha<sup>-1</sup>). A randomized block design with three blocks and two treatments (TWW and WW irrigation) were used. Both plots contained twenty four olive trees (8 × 3 replications) irrigated by submergence at an annual rate of 5000 m<sup>3</sup> ha<sup>-1</sup> with treated wastewater (TWW) or well water (WW). Irrigation with TWW started in February 2003 and extended until now (2010). The region is characterized by an arid climate of Mediterranean type. The annual rainfall and temperature averages over 52 years were 250 mm and 23 °C, respectively.

### 2.2. Water sampling and nutrient analyses

The characteristics of TWW and WW were reported in table (1). The pH of the TWW and WW were 7.6 and 7.9, respectively. These were within the pH 6 - 9 range appropriate for irrigation reuse (ONA, 2010). EC values were 6.3 and 4.6 mS cm<sup>-1</sup> for TWW and WW, respectively. These values are indicators of a high level of salinity.

### 2.3. Soil sampling and mineral analysis

Soil samples were collected in plastic bags and stored in a portable cooler. Composite soil samples were collected quarterly from each plot at both 0 - 40 cm and 40 - 80 cm depth twice by year (July and December). The samples were air-dried at room temperature, ground and crushed to pass a 2 mm sieve, and mixed thoroughly for analysis. Soil pH was measured in suspension (soil: water = 10: 25) by a pH meter. Electrical conductivity (E. C.) was measured in a saturated paste using a conductivity meter. Organic matter was determined using volumetric method. Further, 10 soil samples were selected and analysed for texture using the Robinson pipette method. For mineral nutrients and heavy metals analyses, the soil samples were extracted with 100 ml ammonium acetate and analysed following the same methodologies as for the same water. For P analysis a soil samples were dissolved in 100 ml of calcium carbonate, agitated for one hour, and filtered. The assimilated phosphorus was determined using a JENWAY colorimeter.

**Table 1: Characterization of Irrigation Waters (Mean Values).**

| Elements                      | Unit                | Well Water | Treated Wastewater | Tunisian standards |
|-------------------------------|---------------------|------------|--------------------|--------------------|
| pH (H <sub>2</sub> O)         |                     | 7.9        | 7.6                | 6.5 – 8.5          |
| E. C.                         | mS cm <sup>-1</sup> | 4.6        | 6.3                | 7.0                |
| Salinity                      | g L <sup>-1</sup>   | 3.50       | 4.66               | -                  |
| P total                       | mg L <sup>-1</sup>  | 0.8        | 10.3               | 0.05               |
| K <sup>+</sup>                | mg L <sup>-1</sup>  | 35         | 38                 | 50.0               |
| Na <sup>+</sup>               | mg L <sup>-1</sup>  | 355        | 470                | 300.0              |
| Cl <sup>-</sup>               | mg L <sup>-1</sup>  | 1580       | 1999               | 600.0              |
| Ca <sup>2+</sup>              | mg L <sup>-1</sup>  | 184.5      | 95.8               | -                  |
| Mg <sup>2+</sup>              | mg L <sup>-1</sup>  | 126.2      | 83.8               | -                  |
| SO <sub>4</sub> <sup>2-</sup> | mg L <sup>-1</sup>  | 162.3      | 787.0              | 1000               |
| Zn <sup>2+</sup>              | mg L <sup>-1</sup>  | 0          | 0.42               | 5                  |
| Mn <sup>2+</sup>              | mg L <sup>-1</sup>  | 0          | 0.5                | -                  |
| DCO                           | mg L <sup>-1</sup>  | 0          | 73                 | 90                 |
| DBO                           | mg L <sup>-1</sup>  | 0          | 22                 | 30                 |

## 2.4. Leaf mineral analyses and growth control

Thirty-two fully developed leaves were detached from the middle portion of the current year's shoot from the external and internal canopy in the four cardinal directions (8 shoots / tree). They were collected in paper bags and stored in a portable cooler at monthly intervals during the two years. Successively, the leaves were dried in an oven (60 °C) and analyzed according to the methods described by Pauwels et al. (1992). Mineral elements (P, K, Na, Cl, Mn, Zn, Cd and Pb) analysis was carried out after dry - ashing at 450 °C in a muffle oven (HEROTEC) and digestion of the ashes with 1 M HNO<sub>3</sub>. N was determined by using the Kjeldahl method; K and Na were determined by atomic emission spectrophotometry (JENWAY PFP7, Milan, Italy); heavy metals (Mn, Zn, Fe, Cu, Cd and Pb) by atomic absorption spectrophotometry (Perkin Elmer A Analyst 300, PerkinElmer Inc., Wilesley, MA, USA). P in leaves was determined by a vanado-molybdate colorimetric procedure with a JENWAY 6405 UV / Vis spectrophotometer (Milan, Italy). Finally, Cl was determined titrimetrically with AgNO<sub>3</sub>.

Eight shoots of the current season were tagged, two shoots for each cardinal direction (8 shoots/ tree). Data on shoot length were collected yearly from February 2003 and the number of flowers/ inflorescence was recorded on the representative chosen shoots. Olives were harvested manually to guarantee the accuracy and weighed to obtain the yield.

## 2.5. Statistical analysis

Statistical analysis (General Linear Model) was used to study the effect of treated wastewater on mineral nutrients and heavy metal content in soil. Data were analysed using the Super anova statistical software SPSS for Windows 14.0. The means were separated by Fisher's protected univariate least significant difference (LSD) test at  $p < 0.05$ .

## 3. Results

### 3.1. Environmental effects on olive tree growth

The olive tree development depends on the interaction of major ambient environmental conditions (rainfall pattern, air temperature, solar radiations intensity, atmospheric humidity, air CO<sub>2</sub> concentrations, etc...). Several researches have studied the relationship between olive tree activity and environmental conditions; they have showed that drought and heat are the most important environmental stress factors that disrupt the plant activity.

Water availability is the principal cause conditioning crops growth. It interacts directly in the plant activity via photosynthesis and transpiration process and affects its growth and fruit set up. In arid area, climate change affects average temperatures and temperature extremes (fig. 1a), timing and spatial patterns of precipitation (Fig. 1b), soil moisture, runoff, and the frequency of disturbances, such as drought. Between 1981 and 2009 global warming is twice more important in arid area of Tunisia than the world average (1.5 - 2 ° C against 0.75 ° C). This climate warming is accompanied by a regression between 10 and 20 % of average annual precipitation.

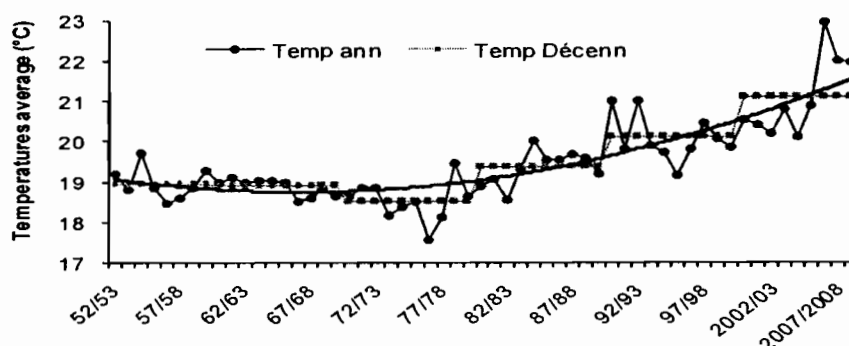


**Figure 1:** Yearly average of temperature (a) and rainfall (b) at the Chaâl farm between November 1952 and August 2009.

### 3.2. Soil texture

Soil samples from both experimental plots were classified as sandy-silty soils. Mechanical analysis of soil samples are described in Table 2, with means sand content varying from 68.3 to 67.6%, silt content varying from 22.8 to 25.0%, and clay content varying from 7.4 to 8.9% in both TWW irrigated and WW irrigated plots, respectively.

**Table 2:** Characterization of soil texture (depth of 0 to 80 cm).

| Treatment Element  | Treated wastewater irrigated soil |      |      |                   | Well water irrigated soil |      |      |                   |
|--------------------|-----------------------------------|------|------|-------------------|---------------------------|------|------|-------------------|
|                    | Clay                              | Silt | Sand | CaCO <sub>3</sub> | Clay                      | Silt | Sand | CaCO <sub>3</sub> |
| Mean (%)           | 8.9                               | 22.8 | 68.3 | 12.9              | 7.4                       | 25.0 | 67.6 | 10.3              |
| S. D. <sup>a</sup> | 4.8                               | 0.9  | 5.5  | 4.9               | 2.2                       | 0.8  | 14.5 | 4.5               |

<sup>a</sup> Standard deviation

### 3.3. Effect of treated wastewater reuse in soil

At the beginning of the experimental period, soil organic matter contents were similar for both treatments: 0.42% and 0.45%. Soil macronutrients concentrations were comparable and no statistical differences were shown. For both soil irrigated treatments, 2.6 g kg<sup>-1</sup> for N, 143 mg for K and 70 mg for P (Table 3). After 6 years of irrigation, soil O.M, N, P and K concentrations increased due to TWW effects (table 3). In particular, the increase of O.M, N and K was highly significant ( $p < 0.001$ ). These findings are in the agreement with previous study [7, 8, 10]. However, the increase of Na (315 mg kg<sup>-1</sup> vs. 180 mg kg<sup>-1</sup>) and Cl (650 mg kg<sup>-1</sup> vs. 212 mg kg<sup>-1</sup>) concentrations was detected after 6 years of irrigation with TWW with respect of WW treatment, as reported previously (Ben Ahmed et al., 2010; Bedbabis et al, 2009; 2010a). At the beginning of experiment, heavy metals concentrations ranged from 25 to 28 mg kg<sup>-1</sup> for Zn and from 120 to 123 mg kg<sup>-1</sup> for Mn. Significant increase of Mn (168 mg kg<sup>-1</sup> vs. 105 mg kg<sup>-1</sup>) and Zn (44 mg kg<sup>-1</sup> vs. 26 mg kg<sup>-1</sup>) were found, after 6 years of irrigation with TWW. This might be attributed to cumulative addition of these metals to the soil through irrigation, as reported in previous investigations (Rejeb, 1992; Bedbabis et al, 2009; 2010a; 2010c). However, significant increase of all organic and mineral elements was detected after six years of irrigation with TWW and denotes the fertilizer values of this kind of water.

### 3.4. Effect of treated wastewater reuse in leaf nutrient concentrations

Irrigation with TWW caused a significant increase ( $p \leq 0.001$ ) of leaf N, P, K, Na, Mn and Fe concentrations compared to WW treatment (Table 3). Furthermore, the comparison between these mineral concentrations between February 20003 and February 2009, showed a significant high differences. The increase was a consequence of the larger amount of these mineral nutrients supplied by TWW compared to WW. Olive trees generally exhibit significant variations in the seasonal and the annual levels of leaf nutrients which depend on the biological cycle and the annual yield status. In fact, at spring, an important decrease of N, K and P concentrations was found in high (On)

or low (Off) yield periods. This decrease indicated the mobility of these nutrients in order to allow the development of vegetative and reproductive structures.

After six years of TWW irrigation, high significant differences have been observed ( $p \leq 0.001$  for Na and  $p \leq 0.01$  for Cl) for leaf Na and Cl concentrations between the two treatments after six years (Table 3). In particular, Na varied between 0.03% at the beginning of experimentation and 0.12% in 2009. The same tendency was observed with Cl which concentrations increase from 0.30% to 0.47% in the same periods. TWW irrigated trees had a higher leaf Na and Cl concentrations than WW irrigated trees, as a consequence of higher NaCl concentration in the TWW which is basically from domestic provenance (88%) sources. Leaf Fe, Mn, Cu and Zn concentrations were statistically different between the two treatments (Tab. 3) and amounted to the values of 10 – 50 ppm for Mn, Zn and Cu and between 50 – 150 ppm for Fe, usually reported for olive tree [16].

**Table 3:** Effects of irrigation treatments (well water, WW and treated wastewater, TWW) on some soil and leaves chemical properties.

| Element                             |       | Unit                  | Chemical composition of the soil (depth 0 – 80 cm) |        |              | Chemical composition of olive tree leaves |        |        |              |
|-------------------------------------|-------|-----------------------|----------------------------------------------------|--------|--------------|-------------------------------------------|--------|--------|--------------|
|                                     |       |                       | WW                                                 | TWW    | Significance | Unit                                      | WW     | TWW    | Significance |
| 2003<br>February<br>(Initial)       | O. M. | (%)                   | 0.42                                               | 0.45   | ns           | -                                         | -      | -      | -            |
|                                     | N     | (g Kg <sup>-1</sup> ) | 2.6                                                | 2.6    | ns           | (%)                                       | 1.25   | 1.99   | ***          |
|                                     | P     | (ppm)                 | 70.3                                               | 69.9   | ns           | (%)                                       | 0.08   | 0.01   | *            |
|                                     | K     | (ppm)                 | 143.3                                              | 142.1  | ns           | (%)                                       | 0.72   | 1.03   | ***          |
|                                     | Na    | (ppm)                 | 106.0                                              | 105.3  | ns           | (%)                                       | 0.04   | 0.03   | ns           |
|                                     | Cl    | (ppm)                 | 62.2                                               | 62.5   | ns           | (%)                                       | 0.27   | 0.30   | ns           |
|                                     | Zn    | (ppm)                 | 25.0                                               | 28.0   | ns           | (ppm)                                     | 15.9   | 18.6   | *            |
|                                     | Mn    | (ppm)                 | 120.2                                              | 123.0  | ns           | (ppm)                                     | 26.4   | 27.2   | ns           |
|                                     | Cu    | (ppm)                 | -                                                  | -      | -            | (ppm)                                     | 7.0    | 8.5    | ns           |
|                                     | Fe    | (ppm)                 | -                                                  | -      | -            | (ppm)                                     | 50     | 65     | *            |
|                                     | Cd    | (ppm)                 | <0.004                                             | <0.004 | ns           | (ppm)                                     | <0.004 | <0.004 | ns           |
|                                     | Pb    | (ppm)                 | <0.004                                             | <0.004 | ns           | (ppm)                                     | <0.004 | <0.004 | ns           |
| 2009<br>February<br>(after 6 years) | O. M. | (%)                   | 0.74                                               | 1.30   | ***          | -                                         | -      | -      | -            |
|                                     | N     | (ppm)                 | 3.1                                                | 12.1   | ***          | (%)                                       | 1.53   | 2.15   | ***          |
|                                     | P     | (ppm)                 | 61.1                                               | 82.3   | **           | (%)                                       | 0.09   | 0.14   | ***          |
|                                     | K     | (ppm)                 | 215                                                | 493    | ***          | (%)                                       | 0.89   | 1.42   | ***          |
|                                     | Na    | (ppm)                 | 180                                                | 315    | ***          | (%)                                       | 0.06   | 0.12   | ***          |
|                                     | Cl    | (ppm)                 | 212                                                | 650    | ***          | (%)                                       | 0.32   | 0.47   | **           |
|                                     | Zn    | (ppm)                 | 26.3                                               | 43.9   | *            | (ppm)                                     | 14.3   | 21.5   | **           |
|                                     | Mn    | (ppm)                 | 105.2                                              | 168.7  | *            | (ppm)                                     | 22.5   | 52.1   | ***          |
|                                     | Cu    | (ppm)                 | -                                                  | -      | -            | (ppm)                                     | 12.1   | 20.0   | **           |
|                                     | Fe    | (ppm)                 | -                                                  | -      | -            | (ppm)                                     | 65.5   | 112.0  | ***          |
|                                     | Cd    | (ppm)                 | <0.004                                             | <0.004 | ns           | (ppm)                                     | <0.004 | <0.004 | ns           |
|                                     | Pb    | (ppm)                 | <0.004                                             | <0.004 | ns           | (ppm)                                     | <0.004 | <0.004 | ns           |

Data represent mean values of all observations. Significance referred to the comparison between treatments (WW and TWW) within the same year. Differences between means: non-significant (ns), significant at  $p \leq 0.05$ , (\*) at  $p \leq 0.01$ (\*\*) and at  $p \leq 0.001$  (\*\*\*) according to LSD test.

In the low yield period (2003), olives production was 1469 kg ha<sup>-1</sup> in TWW treatment compared with 2469.2 kg ha<sup>-1</sup> in WW treatment ( $p \leq 0.01$ ). In the high yield period (2006), olives production was again significantly higher for TWW with 2918 kg ha<sup>-1</sup> compared to 1535.1 kg ha<sup>-1</sup> of WW plot. The high yield obtained in TWW irrigated plot was probably a consequence of the presence of nutrient elements such as N, P and K, and the irrigation treatment worked as fertirrigation. After six years of study, the mean values of yields were respectively 1843. Kg ha<sup>-1</sup> and 1002.2 Kg ha<sup>-1</sup>, respectively on TWW and WW plots (Table 4). Based on these results, an efficient fertilization schedule, in particular with N, P and K, may provide a good tool to decrease biennial bearing. Although it is reported in other studies that saline water reduces yield (Ben Ahmed et al., 2009a; Bedbabis et al, 2009; 2010a; Ioannis, 2009), in the present study the fertilization effects seem to balance the saline effects resulting in a higher yield.

**Table 4:** Shoot growth (cm) and olive yields (kg ha<sup>-1</sup>) in well water (WW) and treated wastewater (TWW) irrigated plots.

|                        | Yearly shoot growth (cm) |            | Yearly yield importance (kg ha <sup>-1</sup> ) |        |
|------------------------|--------------------------|------------|------------------------------------------------|--------|
|                        | WW                       | TWW        | WW                                             | TWW    |
| High value (Max)       | 38.1 ± 5.0               | 42.5 ± 7.9 | 1535.1                                         | 2918.5 |
| Low value (Min)        | 10.7 ± 2.2               | 12.5 ± 2.6 | 269.2                                          | 368.8  |
| Mean value (Six years) | 25.4 ± 3.3               | 27.6 ± 4.1 | 1002.2                                         | 1843.4 |

#### 4. Conclusion

Continuous application for six years of TWW irrigation increased soil fertility; with high significant improves in N, P, K and Fe soil concentrations. Results showed that TWW can be considered as a complementary source of fertilization. However, significant increases in E.C, Na and Cl levels might lead to soil salinization over the long term, and menace to causes soil degradation and plant phytotoxicity.

In conclusion, our data showed that irrigation with TWW caused a high significant improve of shoot growth and olive yield increasing. TWW worked as fertirrigation supplying N, P, K, and Fe in large amounts. Finally, the application of TWW caused an increase of MN and Zn in soil and leaves but within the usual range detected in plants.

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## Automatic irrigation scheduling of olive orchard from sap flow records with the heat dissipation method. 1- Physiological validation

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### Abstract

We evaluated the potential of sap flow values estimated from records with the heat dissipation method for automating irrigation scheduling in an olive orchard (*Olea europaea* L, cv. Meski) near Enfidha, Tunisia. Two drip irrigation treatments were imposed with an automatic irrigation controller, T1 (100% sap flow) and T2 (60%ET<sub>o</sub>). Data on sap flow, predawn, midday and stem water potential, net photosynthesis, stomatal conductance, transpiration rate and chlorophyll fluorescence were recorded in representative trees of both treatments, during the 2008 irrigation season (April to August). Results showed that through the irrigation dose calculated from sap flow records we saved 30% of water delivered for irrigation. But, water potentials were decreased and noted that olive tree were not stressed especially stem water potential, leaf gas exchange parameters were affected significantly by about 15%. An automatic irrigation controller can be easily used in olive orchards to save water, extend irrigation areas with little decreased in plant physiology behaviour.

**Keywords:** Sap flow, Gas exchange, Automatic irrigation, Olive.

## Planification d'irrigation automatique des vergers d'oliviers basée sur le flux de sève en utilisant la méthode de dissipation thermique dans un espace de climat méditerranéen

### Résumé

Nous avons évalué le potentiel du flux de sève estimées à partir de la méthode de dissipation thermique pour programmer et automatiser l'irrigation dans un verger d'olive de table (*Olea europaea* L, cv. Meski) à l'Enfidha, Tunisie. Deux traitements d'irrigation par le système goutte à goutte ont été imposés avec un contrôleur automatique d'irrigation, T1 (100% de sève) et T2 (60%ET<sub>o</sub>).

Les données sur le flux de sève, le potentiel hydrique de base, midi et celui xylémique, la photosynthèse nette, la conductance stomatique, la transpiration et la fluorescence chlorophyllienne ont été enregistrés dans les arbres représentatifs des deux traitements, pendant la saison d'irrigation (avril à août). Les résultats ont prouvé que l'irrigation par la dose calculée à partir du flux de sève, nous avons économisé plus de 30% de l'eau délivrée pour l'irrigation. Mais, les potentiels hydriques foliaires particulièrement xylémiques ont été diminués notant que l'olivier n'était pas soumis à une contrainte hydrique. Les paramètres d'échange gazeux ont été affectés d'environ de 15%.

Un contrôleur automatique d'irrigation peut être facilement employé dans les vergers d'olivier en intensif pour gérer l'irrigation avec peu d'effet sur le comportement physiologique des plantes.

### 1. Introduction

Approaches for irrigation scheduling take into account physiological variables related to the water use by the tree (Nicolas et al., 2005 ; Tognetti et al., 2009). Among them, sap flow and trunk diameter variations are considered by many as two of the most promising plant-based indicators for the automatic control of irrigation in fruit tree orchards (Ortuno et al., 2005). Sap flow readings in branches, trunks and roots are being widely used to determine both water consumption and the dynamics of transpiration, as well as hydraulic conductance and water uptake by main roots (Fernández et al., 2001; Nicolás et al., 2005). Sap flow records have been reported as useful to determine actual olive water needs (Fernandez and Moreno, 1999 ; Fernandez et al., 2001) to the point that they have been used to develop an automatic irrigation controller, AIC (Fernández et al.,

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2008b). For the olive tree, Karray et al., (2006) studied the transpiration in agro-system of olive tree with the heat dissipation method, and horticultural species under rainfall condition. Recently, Rousseaux et al. (2009) have estimated olive water needs by the heat balance method developed by Sakuratuni et al. (1981). The heat dissipation method (Granier, 1987) has been used successfully to study transpiration and some physiological aspects of both forest and fruit trees (Sellami and Sifaoui, 2003).

The main objectives of this study were to evaluate the heat dissipation method for scheduling irrigation automatically in an intensive olive orchard under arid climate in Tunisia. Then to test and validate this method using some physiological behaviours of olive tree especially leaf water potential, photosynthesis, transpiration and stomatal conductance

## **2. Material and method**

### **2.1. Site description and environmental measurements**

This experiment was conducted at the irrigated olive orchards Enfidha, Tunisia (36°08'N, 10°22'E, 23 m). Our study site within the olive trees was a 27-year-old olive (*Olea europaea* L. cv. Meski) plantation, with approximately 7m x 7m trees spacing with a density of 204 trees per hectare. The soil was classified as sandy-loam. Soil water content at wilting point and field capacity were 11% and 26% respectively.

### **2.2. Irrigation management and experimental design**

Water for irrigation was delivered daily using a localized irrigation system with two lines of nozzles at 1.0 m from the trunk each. Each tree was equipped by eight nozzles, with four nozzles per side of 8 L h<sup>-1</sup> each (at 0.5 m and at 1.0 m from the trunk per side).

T1: Nineteen olive trees that received a daily irrigation amount of 100% of transpiration losses measured by sap flow meters ;

T2: Nineteen olive trees full irrigated that received a daily irrigation amount of 60% ET<sub>0</sub>.

### **2.3. Sap flow measurements**

The measurement of sap flow by the method of Granier (1985, 1987) is based on two cylindrical probes, 2 mm in diameter and 20 mm in length inserted radially in the xylem and spaced vertically by 8 cm. The upper probe is continuously heated, whereas the lower probe is unheated and the resulting temperature difference is measured with copper constantan thermocouples placed in each probe. Under zero flow conditions, the temperature around the heated probe increases to the point where heat dissipation by xylem conduction is in equilibrium with the heat energy supplied, which gives a maximum temperature difference. When sap is flowing, heat dissipation increases by convection and the temperature difference decreases.

### **2.4. Leaf water potential**

During the irrigation season, leaf water potential was measured using a portable pressure chamber according to Scholander et al. (1965) following the recommendations of Turner (1981). The data were collected during sunny days at predawn and midday. Leaf and xylem water potentials were established according to Nicolas et al. (2005) by sampling a total of 9 fully sunlit and covered leaves of each treatment.

### **2.5. Photosynthesis, stomatal conductance and transpiration**

Leaf CO<sub>2</sub> assimilation rates, stomatal conductance and transpiration was measured using a portable system IRGA (LI-COR, LI-6400). Data were collected other month from April to August. From each tree, three two-year old leaves from different vegetative shoots were measured. All measurements were carried out in the morning between 11:00 and 13:00 h on cloudless days. Photosynthetic photon flux density was fixed at 1500 μmol m<sup>-2</sup> s<sup>-1</sup>. External CO<sub>2</sub> concentration was fixed at 400 μmol m<sup>-2</sup> s<sup>-1</sup> and flow rate of air passing through the chamber was 200 μmol s<sup>-1</sup>. Temperature and humidity rate through the chamber were 25 °C and 60 %. After the data were collected, the leaves were cut and leaf area was estimated with a digital camera system (LI-COR, LI-3200).



### 3. Statically analysis

Data were statistically analysed using the SPSS statistical software Version 16.0 (SPSS Inc., Chicago). Standard errors (S.E.) of the means were calculated. Duncan's multiple range test was used to compare the means.

## 4. Results

### 4.1. Climatic parameters

Maximum daily air humidity and minimum temperatures were 85% and 11°C during the spring measurement period (i.e., April), while maximum daily temperature and minimum air humidity values were 33°C and 30% respectively during the summer measurements (i.e. August, Fig. 1a). Minimum and maximum solar radiation were 6 and 16  $\text{w m}^{-2}$  at first April and mid-May, they were stabilized with high values during summer (i.g., July and August, Fig. 1b). Evapotranspiration showed two periods; during first April to first June was approx between 1.6 and 2.9  $\text{mm day}^{-1}$ . During the second period (mid June to end August),  $\text{ET}_0$  ranged between 2.3 and 3.7  $\text{mm day}^{-1}$  (Fig. 1c). During the full irrigated season from April to August total evapotranspiration was 420 mm, with a daily mean value of  $\text{ET}_0$  approx 2.8  $\text{mm day}^{-1}$ .

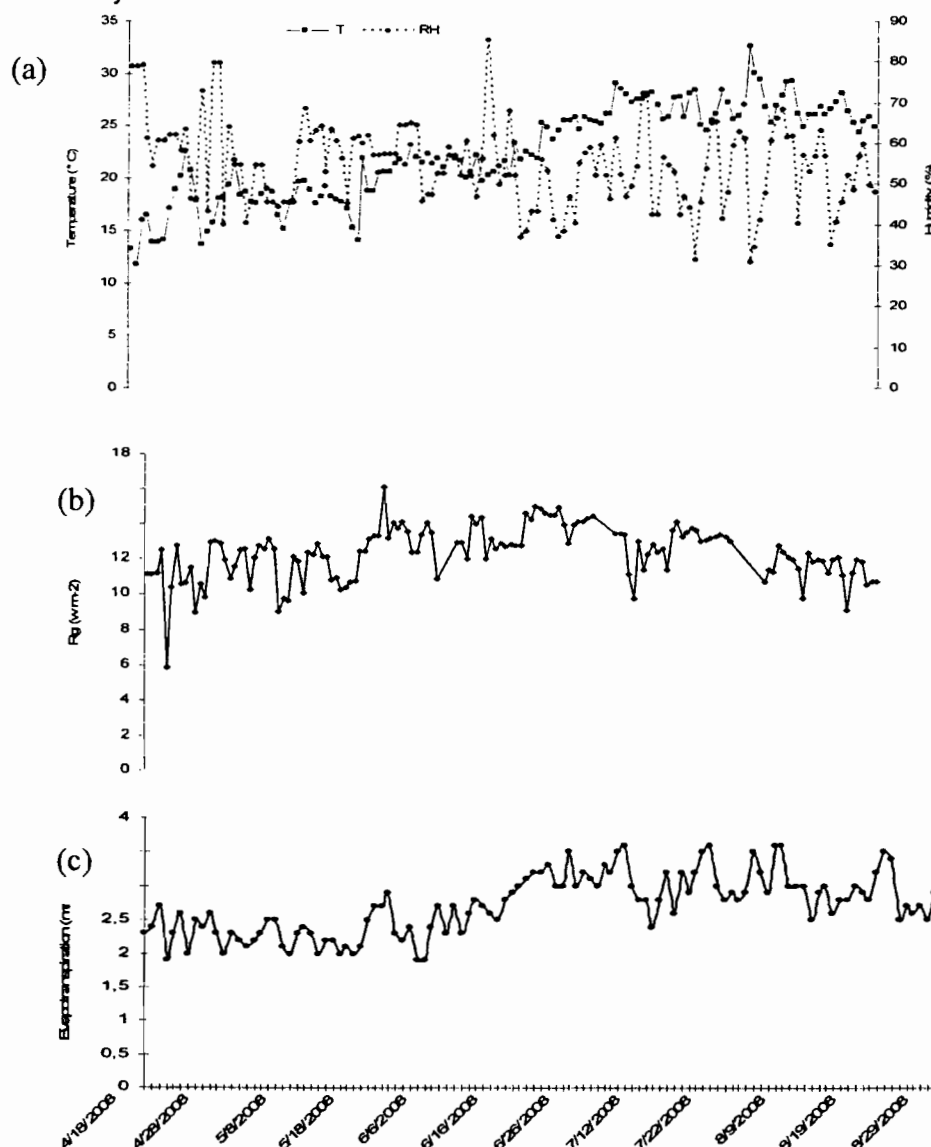


**Figure 1:** Daily climatic parameters' (a) temperature and air humidity, (b) global radiation, (c) evapotranspiration.

## 4.2. Irrigation doses and sap flow measurements

Irrigation dose in T2 presented similar values during first April until mid-June approx 490 L Tree Week<sup>-1</sup>. They increased to reach a maximum value of 700 L Tree Week<sup>-1</sup> at first August (Fig. 2a). Similarly with significant differences, irrigation dose from sap flow measurements increased from 180 L Tree Week<sup>-1</sup> in April to reach a maximum level approx 400 L Tree Week<sup>-1</sup> at August (Fig. 2a). During the full irrigated season from April to August total irrigation dose were 6300 and 11300 L Tree<sup>-1</sup> in T1 and T2 respectively. The mean daily irrigation doses were 42 and 75 L Tree day<sup>-1</sup> in T1 and T2 respectively. For the sap flow treatment, maximum soil evaporation measured by lysimetry method was approx 10 % of ET<sub>0</sub>. Then total irrigation dose in T1 was about 7800 L Tree and the mean daily dose was 52 L Tree day<sup>-1</sup>. Average weekly values of canopy transpiration estimated by sap flow were at their minimum during spring (April) and at their maximum during the warmest summer period

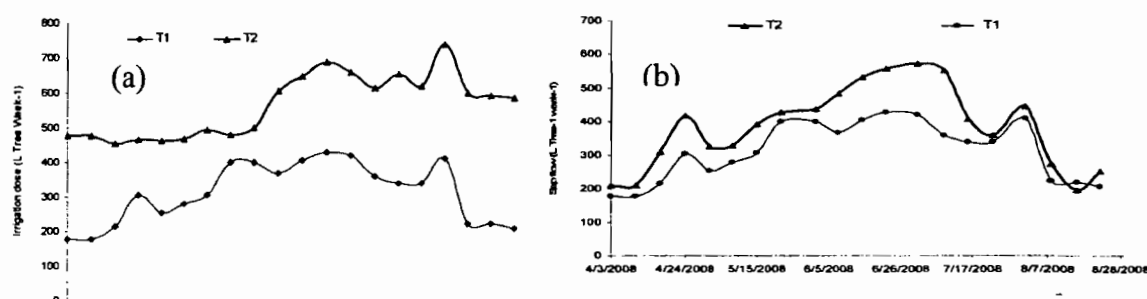


**Figure 2:** Weekly evolution of (a) irrigation dose and (b) sap flow of irrigation treatments T1 and T2.

## 4.3. Leaf water potential

Pre-dawn leaf water potential ( $\Psi_{pd}$ ) increased progressively and continuously during the full irrigation season, reaching the highest values at first May about -0.45 and -0.35 MPa in T1 and T2 respectively.  $\Psi_{pd}$  increased slightly to -0.6 and -0.4 MPa in T1 and T2 respectively with significant differences between the two treatments (Fig. 3a). In all treatments, xylem and leaf water potentials have similar seasonal pattern, high water potentials values were observed in full irrigated treatment T2 with significant differences (Fig. 3b and Fig. 3c).

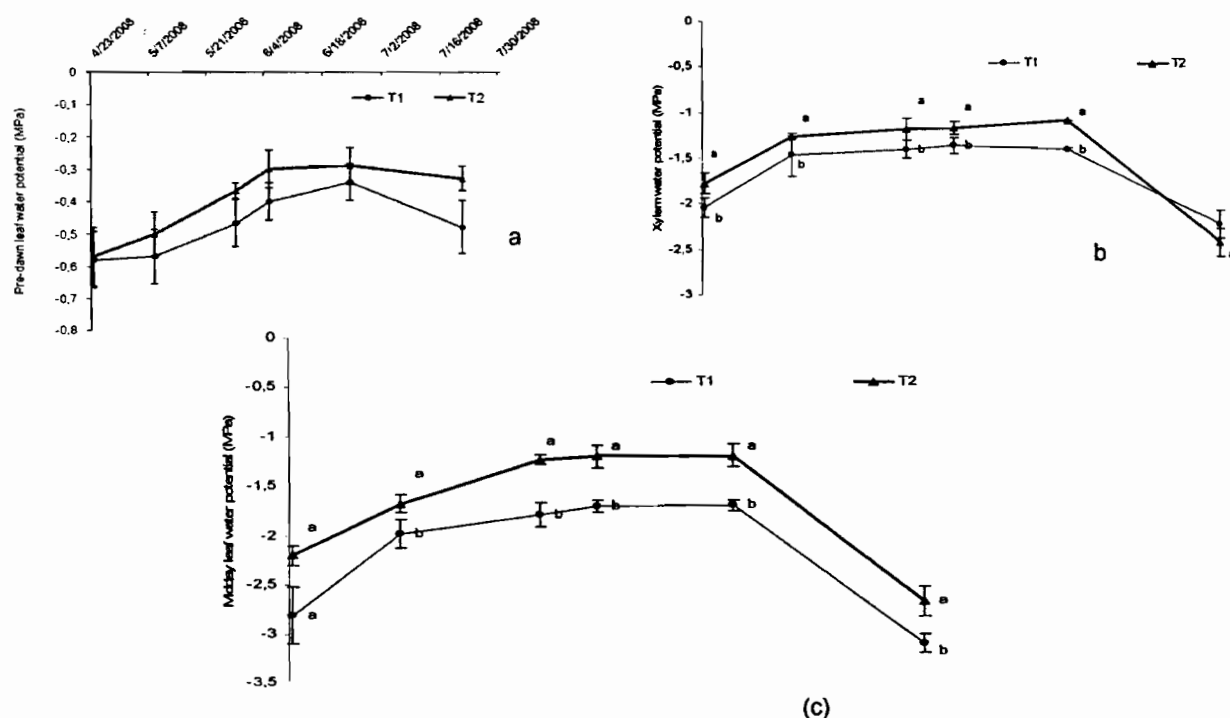


**Figure 3:** Seasonal pattern of leaf water potential (a) predawn leaf water potential, (b) xylem water potential, (c) midday leaf water potential of irrigation treatments T1 and T2.

#### 4.4. Photosynthesis, stomatal conductance and transpiration

Seasonal patterns of photosynthesis rate measured during the full irrigated period were similar with high values observed in T2 ( $10\text{--}16.8\ \mu\text{mol m}^{-2}\text{ s}^{-1}$ ) then in T1 ( $10\text{--}14\ \mu\text{mol m}^{-2}\text{ s}^{-1}$ ).  $\text{CO}_2$  assimilation rate were lower in April, increased by about 60% in May and reached a maximum in June and then increased in August (Fig. 4a). In all photosynthesis measurements full irrigated treatment T2 had significantly higher  $\text{CO}_2$  assimilation rates throughout the season by about 15%. Stomatal conductance followed similar trends with  $\text{CO}_2$  assimilation rates (Fig. 4b). The  $g_s$  increased from April to the end of June, then decreased during August. The maximum  $g_s$  values were  $0.24$  and  $0.17\ \mu\text{mol m}^{-2}\text{ s}^{-1}$  in T2 and T1 respectively. Similarly to photosynthesis and stomatal conductance patterns, transpiration rate ( $E$ ) increased from April to June, and then decreased in August. The maximum  $E$  values were  $7.3$  and  $5.3\ \text{mmol m}^{-2}\text{ s}^{-1}$  in T2 and T1 respectively (Fig. 4c).

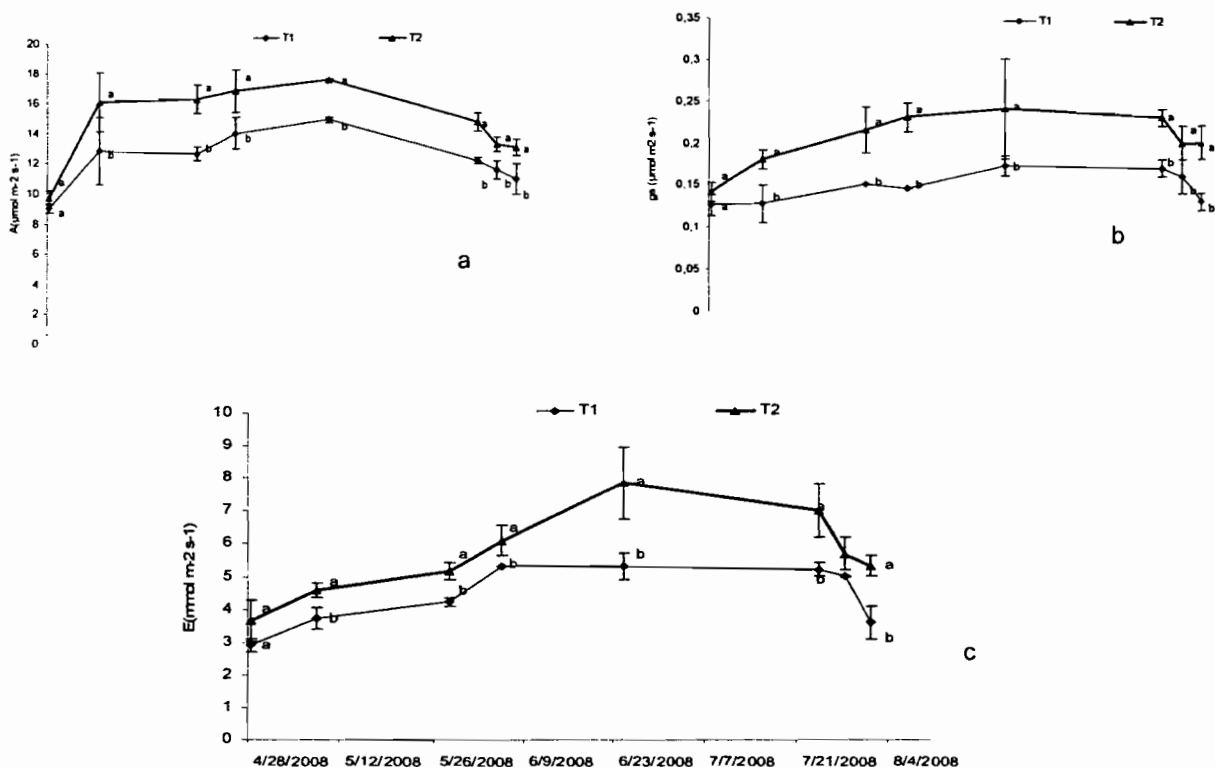


**Figure 4:** Seasonal pattern of leaf gas exchange (a) Photosynthesis, (b) Stomatal conductance (c) Transpiration of irrigation treatments T1 and T2.

#### 5. Discussion

We introduce here and testing physiologically the heat dissipation of sap flow technique as a tool for an automatic irrigation control (AIC) and to calculate the irrigation dose (ID) of olive orchards. Compared to the potential irrigation dose estimated by  $60\%ET_0$ , we can increase by the sap flow technique the irrigation dose by 30% with high agreement with those published by Fernandez et al. (2008b). The reduction of water delivered for irrigation affected soil water content and acted as a drought stress on olive tree physiological behaviour. Similarly Fernandez et al. (2008b) show that the relative extractable water under AIC by HPV decreased by 20 to 55% during the full irrigated season. Consequently, stem water potential decreased markedly in olive trees irrigated by AIC, indicating that the soil water content was too low to prevent a significant increase in the trees water stress. Our results that predawn, leaf and xylem water potentials decreased significantly under AIC irrigated olive tree. But levels of water potentials showed that these olive trees were not stressed during the irrigation season. Mature olive trees irrigated with  $100\%ET_c$  showed higher  $\psi_{pd}$  values than all other water treatments together ( $66$  and  $33\%ET_c$ ). However, minimum values of  $\psi_{pd}$  never exceeded  $-1.5$  and hardly reached  $-2$  MPa, regardless the water treatment (Moriana et al., 2007 ; Tognetti et al., 2009). During 90 days of experimenting, olive trees irrigated daily near to the field capacity stem water potential reached between  $-0.9$  and  $-1.5$  MPa (Lopez et al., 2007 and 2008). The decrease of

irrigation dose and leaf water potential affected directly and significantly photosynthesis, stomatal conductance and transpiration rate during the irrigation season (Ben Ahmed et al., 2008). Hagedimitriou and Pontikis (2005) reported that the high leaf CO<sub>2</sub> assimilation rate value observed during spring were probably due to favourable air temperatures and air humidity. The decrease in A<sub>n</sub> in August is probably due to high temperature and low air humidity. Our results showed that under AIC, photosynthesis decreased only by 15% compared to the fully irrigated olive trees by 60%ET<sub>0</sub> with water saving by about 30%. The water use efficiency estimated in our experiment by photosynthesis and transpiration rate demonstrated the ability to make heat dissipation method to scheduling irrigation on olive orchards. It's very clear that transpiration rate was closed correlated to sap flow ( $R^2 = 0.96$ ), whereas stomatal conductance and photosynthesis were lower correlated ( $R^2 = 0.75$  and  $0.66$ ) (Fig. 6) because of the high active regulation of these two parameters under environmental stress especially water and saline stress (Centritto et al., 2005 ; Fernandez et al., 2006; Tognetti et al., 2009). Compared to irrigation scheduling methods based on the atmospheric demand, such as the crop coefficient method (Allen et al., 1998), plant-based measurements could increase the resolution of the calculated ID, which is certainly an advantage for precise high-frequency irrigation.

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## Estimation of the impact of cultivation practices on desertification risk at olive growing areas in western Crete, Greece

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### Abstract

Land degradation in Mediterranean countries due to topsoil loss can be a significant problem in hilly cultivated land when inappropriate agricultural practices are applied. The predicted increase of extreme meteorological events due to climate change is expected to further accentuate the desertification risk in these areas. In the present work a GIS modeling approach was used for estimating desertification risk, based on specific desertification indicators for olive orchard environment. The selected study area at Kissamos, Crete, Greece, was a hilly landscape with 90% of the land covered with olive orchards. Under the current cultivation management practices 29% of the area is under low to moderate desertification risk. In the worst case scenario, when improper tillage, reduced maintenance of the infrastructure (terraces) and reduced parceling of land were introduced, the area under desertification risk increased to 50%, indicating that good agricultural practices should be followed in the future in order to avoid land degradation and desertification in a significant part of the study area.

**Keywords:** Desertification, Olive groves, Good agricultural practices, GIS.

## Estimation de l'impact des pratiques culturelles sur le risque de désertification à la culture de l'olivier en zones de l'ouest de Crète, Grèce

### Résumé

La dégradation des terres dans les pays méditerranéens en raison de perte de terre arable peut être un problème important dans les collines où les terres cultivées quand des pratiques agricoles inadaptées sont appliquées. L'augmentation prévue de phénomènes météorologiques extrêmes en raison des changements climatiques devrait accentuer encore le risque de désertification de ces régions. Une approche de modélisation des GIS a été appliquée pour estimer le risque de désertification en utilisant des indicateurs, spécifiques, de la désertification pour un oliveraie environnement. La zone d'étude, qui a été choisie, se trouve à Kissamos, Crète, Grèce, est un paysage vallonné avec 90% des terres couvertes des oliveraies. Dans les conditions courants de gestion, 29% des terres cultivables sont sous faible jusqu'à modérée risque de désertification. Dans le pire des cas quand inconvenant travail du sol, maintenance réduite de l'infrastructure actuelle (terrasses) et réduit morcellement des terres ont été introduits, la zone sous risque de désertification a augmentée à 50%, ce qui indique que les bonnes pratiques agricoles doivent être suivies à l'avenir afin d'éviter la dégradation des terres et la désertification dans une partie importante de la zone d'étude

**Mots-clés:** Désertification, Olivier, Bonnes pratiques agricoles, GIS.

### 1. Introduction

Olive farms in the EU countries range from very small (<0.5ha) to very large (>500ha) and from traditional, low-intensity groves to intensive, highly mechanized plantations. Olive groves in Greece occupy an area of about 786,700 hectares with about 154 million trees cultivated by about 686,000 families. Olive oil production in Greece represents about 23% of the European Union production. In the island of Crete 65% of agricultural land (318.394 ha) is covered by olive plantations (NSSG, 2006). Olive groves are found in areas ranging from flat to very steep (slope >35%). In relatively flat areas, intensive cultivation is practiced with dense planting, cultivation of soil and irrigation. On the other hand, the orchard management in hilly areas is closer to the traditional olive cultivation practices, with lower density, no soil cultivation and use of terraces in areas with higher slopes.

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Based on existing classification systems such as the geo-referenced database, classes have been defined for each indicator and presented in a tabulated form, where numbers have been assigned for each class according to its importance on desertification (Stocking and Murnaghan, 2001). The main components of the model were related to the following factors: climate, soil, topography and farm management (Fig. 2).

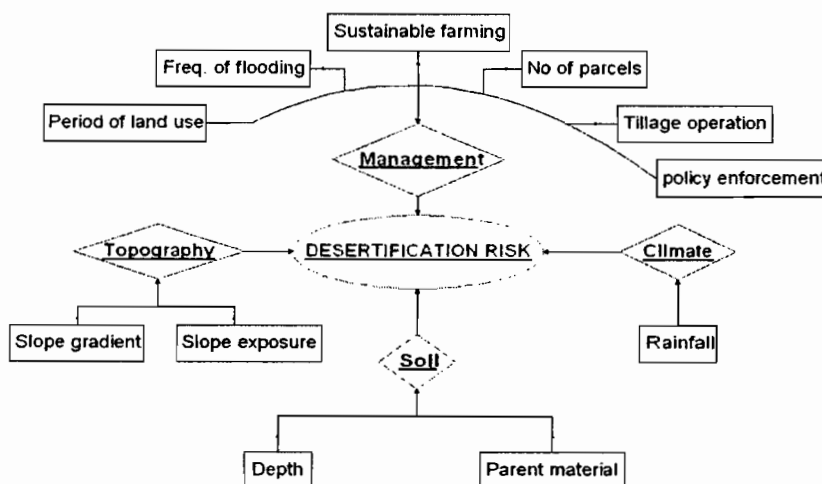


**Figure 2:** Factors of the model .

About 47% of the study area represent steep slopes (>18%) (Table 1). In such areas the main process of land degradation is soil erosion.

The algorithm used for estimating Desertification Risk (DR) for olive cultivated areas was:

$$DR = (4.32) - (0.68 \cdot \text{number of parcels}) + (1.57 \cdot \text{tillage operations}) - (0.68 \cdot \text{period of existing land use}) - (0.56 \cdot \text{soil depth}) + (0.44 \cdot \text{slope gradient}) + (0.19 \cdot \text{parent material}) - (0.79 \cdot \text{rainfall}) + (0.67 \cdot \text{aspect}) + (0.65 \cdot \text{frequency of flooding}) + (0.69 \cdot \text{sustainable farming}) + (0.44 \cdot \text{policy enforcement})$$

Since all the above indicators have spatial – temporal distribution, a grid based model was used in combination with GIS to model the desertification risk at landscape level (Dunio Denti, 2004). For this purpose satellite images interpretation, Digital Terrain Model (DTM) and field-survey techniques were used to recognize these indicators along the study area.

The incorporation of a modeling component to a GIS was required for the prediction of potential outcomes and evaluating alternatives scenarios. The GIS model was created by integrating the mathematical equations of these processes in the ArcGIS software (ESRI, California), which combines GIS tools and spatial modeling. The run of the GIS was individually performed on a one-cell basis (30X30 m) per each indicator in the study site.

**Table 1:** Slope categories of olive orchards in the study area.

| Slope Category | Area (Km <sup>2</sup> ) | Area % |
|----------------|-------------------------|--------|
| <6%            | 3.54                    | 17.3   |
| 6-18%          | 7.32                    | 35.8   |
| 18-35%         | 7.05                    | 34.5   |
| >35%           | 2.53                    | 12.4   |
| Total          | 20.44                   | 100.0  |

The cartography units were identified by interpretation of satellite images in combination with the contours of the area. In an extended olive covered area, like the above described, in which each farm is next to the other, there are a lot of indicators such farm management practices, parent material age of trees and other indicators which could be grouped and analyzed in landscape level. So, we identified cartography units with some common indicators. With the use of DTM the data of slope and aspect of the study area were obtained. A grid with the rainfall pattern in the area was build, using 10-

year-long historical data from 5 meteorological stations within and around the study area. By field survey, the cartography units were validated or modified, and all the other indicators of the model were recorded, giving emphasis on soil depth, number of parcels, tree age, and management practices. All the required information were investigated and integrated to the GIS by field survey with a GPS connected to a laptop. During the field survey, topography, a hydrogeological map and the interpretation of satellite images of the study area were taken into account in order to recognize areas with same values of the indicators. Farmer interviews were used to find out or validate indicators like number of parcels and sustainable farming.

The use of the GIS model provides the ability to investigate easily different scenarios, involving single indicator changes or combinations of several indicator changes. During the present study, apart from the current situation analysis (scenario A: actual case), a hypothetical future situation was also analyzed, including the adoption by farmers of intensive cultivation practices (tillage, etc), the lack of policy enforcement in the area and the reduction of land parceling by public intervention (scenario B: worst case scenario). Therefore, the second scenario involved negative, but possible changes in indicators that could be altered in the future, and might have the most negative effects in increasing the desertification risk in the study area.

### 3. Results and discussion

By testing the two cases it turned out that there is no high risk of desertification for none of them despite the fact that the area was hilly (Table 2). This is probably due to some inherent characteristics of olive growing in the area, including soil depth, no frequent changes in land use, and the limited opportunities for large deviations from good agricultural practices in the future, since the slope of the area does not allow the extensive adoption of practices like, for example, soil cultivation. The analysis of data for scenario A showed that although the area is mainly hilly the desertification risk is low as a result of land management policies (Table 2).

The incorporated changes related to the farming system in scenario B, significantly affected the desertification risk. Therefore, the no risk percentage of 70.9% for scenario A, was reduced to 50.5% in scenario B, thus increasing about 20% the land area that could be subjected to low or moderate desertification risk (Fig. 4).

**Table 2:** Summarized results for desertification risk under different farming conditions.

| Desertification risk | A: Actual case (%) | B: Worst case (%) |
|----------------------|--------------------|-------------------|
| No risk              | 70,9               | 50,5              |
| Low risk             | 23,8               | 34,2              |
| Moderate risk        | 5,3                | 15,3              |
| Total                | 100                | 100               |

The current use of retaining walls (terraces) contributes significantly in limiting soil erosion - land degradation. The present practice of using herbicides instead of cultivating the orchards is another factor that protects soils with extreme gradients. Finally, the existence of small fields in the area, contributes in minimizing the desertification risk. However, although high desertification risk is not evident in the area even under the worst case, about 50% of the area can be subjected to low or moderate risk, if current management practices and policy enforcement are not the appropriate ones in the future. This indicates the importance of retaining or improving the parameters that could be altered by human activities in the study area.

Apart from the above studied cases, a wide range of alternative scenarios may be developed with the GIS model, based on policies of particular relevance to the region, providing a useful tool for evaluating the potential desertification consequences of these policies. As a conclusion, based on the two cases tested through the model, the importance of the adoption of good agricultural practices was evident for protecting from land degradation and reducing the desertification risk.

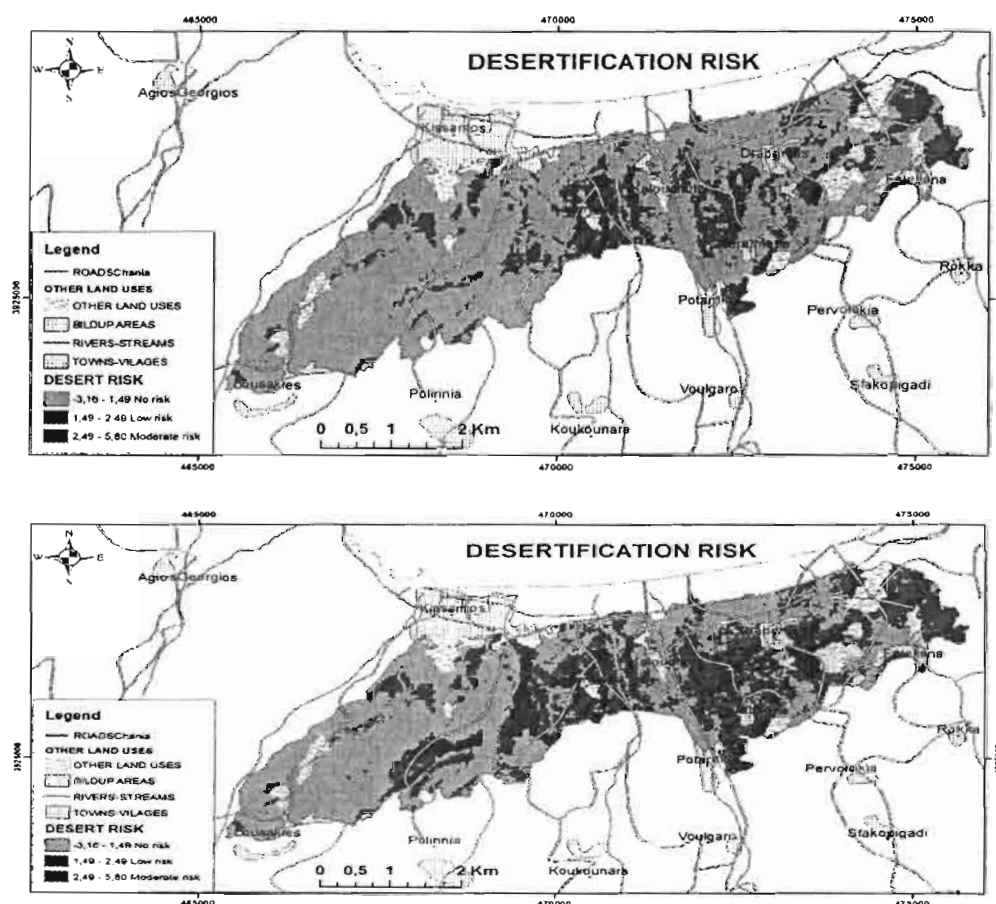


**Figure 4:** The distribution of Desertification Risk (DRI) in the study area under scenarios A and B.

### Acknowledgments

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## Saline water irrigation effects on oil composition and quality of field grown olive cv. Chemlali

Ben Ahmed Chedlia B. Ben Rouina, F. Ben Abdallah & M. Boukhris

### Abstract

Traditionally, olive was cultivated under rain-fed conditions. In recent decades, the olive plantation has been extended to irrigated lands. However, in arid and semi arid regions as those in Tunisia, the limited water availability and the increased need for good water quality for urban and industrial sector use have led to the use of large quantities of marginal water, as saline water, for olive tree irrigation. Fruit development characteristics, yield and oil quality were investigated during two successive crop seasons (2004 / 2006) on fourteen - year - old -Chemlali Sfax olive trees subjected to the following treatments: FW and SS corresponding, respectively to the irrigation with fresh water ( $EC = 1.2 \text{ dS m}^{-1}$ ) and saline water ( $EC = 7.5 \text{ dS m}^{-1}$ ). The irrigation with saline water has led to the decrease of fruit weight, olive yield and oil content. The mean olive yield was of 42.5 and 27 Kg /tree /year, respectively in FW and SS treated plants with a relative reduction of 36% in salt stressed plants. The oil content was of 27.85 and 25.7 % Fw during 2005/2006 crop season in FW and SS irrigated plants, respectively. However, major phenolic compounds (tyrosol, hydroxytyrosol, vanillic, oleuropein, syringic, o-coumaric) and total phenols have increased under saline water irrigation regime. During 2005/2006 crop season, total phenol content was of 198 and 223 mg /Kg in FW and SS treatments, respectively with an increment of 11% under greater salinity conditions. Furthermore, SS treated plants showed higher contents of oleic, linoleic, linolenic and heptadecanoic acids than FW ones. The oil samples of both treatments were classified as "extra virgin". Despite the fact of the decrease of fruit weight and oil content in SS plants, the improvement of their phenolic compounds and total phenols contents assumes that the use of such water quality for olive cultivation could be enhanced, at least in the case of the Chemlali cultivar grown under described experimental conditions.

**Key words:** Irrigation, oil quality, phenolic compounds, saline water, olive yield.

### Effets de l'irrigation à l'eau saline sur la composition chimique et la qualité de l'huile de l'olivier Cv. Chemlali de plein champ

### Résumé

D'une culture traditionnellement pluviale, l'oléiculture irriguée connaît ces dernières décennies une extension notoire, malgré l'insuffisance des quantités d'eaux de qualité, sous les climats semi aride et aride de la Tunisie. Cette extension des oliveraies irriguées est basée essentiellement sur l'utilisation des ressources non conventionnelles d'eaux telles que celles salines. Au cours de cette étude réalisée durant deux saisons (2004 / 2006), le développement des fruits, la production d'olives et la qualité de l'huile produite sont examinés. Deux qualités d'eau sont utilisées pour l'irrigation de l'olivier Chemlali, âgés de 14 ans : FW désigne la parcelle irriguée à l'eau douce ( $C. E = 1,2 \text{ dS / m}$ ) ; alors que SS correspond à celle irriguée à l'eau saline ( $C. E = 7,5 \text{ dS / m}$ ). L'irrigation à l'eau saline a induit une régression du poids moyen d'une olive, de la charge totale de l'arbre en fruits et de la teneur en huile. Les productions moyennes d'olives ont enregistré une régression de 36 % pour passer de 42,5 Kg / arbre / an, chez le traitement FW à seulement 27 Kg / arbre / an chez celui SS. Pour ces deux traitements, le contenu des fruits en huile a respectivement baissé de d'une moyenne de 27,85 % à 25,7 %. Cependant, la concentration des composés phénoliques majeurs (tyrosol, hydroxytyrosol, acide vanillique, euleropeine, acide syringique et o-coumarique) ainsi que celle des phénols totaux se trouvent améliorées par l'irrigation à l'eau saline. Durant la saison 2005/2006, la concentration des phénols totaux dans l'huile de la parcelle SS s'est accrue de 11 % ; puisque égale à 223 mg / Kg en comparaison à 198 mg / Kg chez le témoin (FW). De surcroît, l'huile produite par les arbres SS présente des teneurs en acides oléique, linoléique et heptadécanoïque plus élevées en comparaison à celle des arbres FW. L'huile produite par les arbres des deux traitements est classée dans la catégorie « extra vierge ».

**Mots clés:** irrigation, qualité d'huile, composés phénoliques, eau saline, production d'olives.

## 1. Introduction

Traditionally, olive tree was cultivated under rain-fed conditions. In recent decades, the olive plantation has been extended to irrigated lands. However, in arid and semi arid regions as those in Tunisia, the limited water availability and the increased need for good water quality for urban use restrict the use of fresh water for irrigation. So, large quantities of marginal water, as saline water, are used for olive tree irrigation (33% of irrigated lands are saline water irrigated (16000 ha)).

Most papers dealing with the assessment of olive water needs reported that olive tree is characterized by its limited water requirements and its tolerance to salinity [1, 2, 3]. Existing data on the effects of salinity conditions on yield, quality and phenolic composition of virgin olive oil are few and sometimes contradictory. In 1994, [4] have signalled that salinity alters pollen germination and fruit set, and that it causes the increase of both aliphatic and triterpenic alcohol contents in oil and the percentage of linoleic acid. According to [5], the olive irrigation with saline water ( $EC_e = 6.5 \text{ dSm}^{-1}$ ) increased the fruit dry weight and oil percentage. The same authors stated that no effects were recorded when irrigation was made with water less than  $4.5 \text{ dSm}^{-1}$ . Similarly, [6] did not record any effect of irrigation with brackish water (up to  $4 \text{ g/l}$  of solid residue) on yield and oil percentage of some olive varieties grown intensively in the central part of Tunisia.

Besides, it has been shown that olive yield response to salinity is planting density dependent [8]. The effects of salt treatment at  $EC_e$  of  $4.5 \text{ dSm}^{-1}$  were a 12% increase in oil yield for the 830 trees/ha planting density, and an 18% decrease for the 410 trees/ha one. However, at high salinity ( $EC_e = 7.5 \text{ dSm}^{-1}$ ), the decrease of oil yield was at 89 and 74% of the control in the high and low planting density, respectively. Concerning the oil quality, many studies [8, 9, 10, 11] showed that olive oil produced under high water salinity level has higher amounts of total phenols. In addition, the ratio of unsaturated/saturated fatty acids decreased significantly at moderate and high salinity levels.

Nowadays, the controlled use of marginal water (saline water, treated waste water) to improve the qualitative characteristics of horticultural products is becoming more and more important [8, 9, 10, 11], particularly under actual conditions of limited water resources and rainfall scarcity in arid regions.

The objectives of this study were to determine the effects of saline water used for irrigation on fruit development and the quantitative and qualitative parameters of VOO obtained from trees of the cv. Chemlali grown at a high density orchard. In particular, we are interested in the fatty acid composition and concentrations of phenols of VOO and the evolution of soil moisture and salinity with soil depth and around the irrigation source. Our experimental approach allows us to improve the understanding of the qualitative response (oil quality) of field-grown Chemlali olive tree to saline water irrigation in arid region in Tunisia.

## 2. Materials and Methods

### 2. 1. Plant Material and Treatments

Olive trees (*Olea europaea* L. cv. Chemlali), planted in 1992 in a sandy soil at a density of 625 trees  $\text{ha}^{-1}$  (4 x 4 m layout) at Sfax, Tunisia (34°43N, 10°41E), were used in 2004 and 2005. The sandy soil of the experimental orchard (90.5% sand, 4.5% clay and 5% silt) was characterized by an organic matter of 1.1%, 13.4%  $\text{CaCO}_3$ , 1.3% N, pH of 7.6, a field capacity (measured at 33 KPa) of 11.8% and a wilting point (measured at 1500 KPa) of 5.9%.

In 2004, twenty trees with four replications of 5 trees each were selected to be similar in potential yield and canopy. The Chemlali olive trees were subjected to the following treatments: irrigation with fresh water,  $1.2 \text{ dS m}^{-1}$   $EC_e$  (FW); and saline water,  $7.5 \text{ dS m}^{-1}$   $EC_e$  (salt stress, SS). The water used was either that supplied by the Tunisian National Water Carrier (FW), or saline water (SS) from the local reservoir situated in the area of the Olive Tree Institute in Sfax. The fresh and saline water used with amount of  $4000 \text{ m}^3 / \text{ha} / \text{year}$  were characterized by 145 and  $600 \text{ mg/l Na}^+$ , 326 and  $1169 \text{ mg/l Cl}^-$ , 280 and  $520 \text{ mg/l K}^+$ , 94 and  $261 \text{ mg/l Ca}^{2+}$ , 57 and  $102 \text{ mg/l Mg}^{2+}$ , respectively [1]. The irrigation was delivered using a drip system with 4 drip nozzles (two per side), of  $4 \text{ l h}^{-1}$  per tree set in a line along the rows (at 0.5 m from the trunk). The plants were subjected to the same olive cultivation practices in the area.

## 2.2. Fruit Growth Characteristics and Yield

Control of fruit weight (FrW), fruit diameter (FD), fruit volume (FV) and fruit water content (FWC) of harvested olives were made, during both crop seasons, four times per month from June to December. In every measurement, 60 olives from four plants per treatment (15 olives per plant) were collected for characterization (fresh weight, water content, fruit diameter and volume). For the oil analyses, three samples of 4 Kg of fruits each were harvested for each treatment at maturation. For olive yield determination, 10 trees per treatment were chosen and the harvest was made in mid-December of each year manually to guarantee accuracy.

## 2.3. Oil Quality Indices

Extinction coefficients  $K_{232}$  and  $K_{270}$  were measured at 232 and 270 nm, respectively. Free acidity and peroxide value expressed as milliequivalents of active oxygen per kilogram of oil (meq  $O_2$  / kg) were measured following the analytical method described in the European Regulation EEC 2568/91 [12]. Fatty acid composition was determined based on the European Regulations EEC 2586/91 method. The chlorophyll fraction at 670 nm and the carotenoid fraction at 470 nm were evaluated from the absorption spectrum of each virgin olive oil sample (7.5 g) dissolved in cyclohexane. Oxidative stability is evaluated using a 679 Rancimat apparatus (Metrohm, Switzerland) at 120 °C and 20  $l\ h^{-1}$  air flow. The concentration of total polyphenols was estimated with Folin-Ciocalteu reagent at 725 nm. The different phenolic compounds analysed were determined by HPLC system. The standards used in the quantification of phenolic substances are: Tyrosol, hydroxytyrosol, vanillic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid, o-coumaric acid, oleuropein, glycoside oleuropein and ferulic acid.

## 2. 4. Statistical Analysis

Statistical analyses were performed using the SPSS 10. Windows and treatment means were compared using least significant difference (LSD) test at  $p < 0.05$ . At least three replicates were used for each laboratory test.

## 3. Results and discussions

### 3.1. Soil Salinity

The soil salinity variation was greater in soil irrigated with saline water than in that irrigated with fresh water. For both treatments, there is a slight decrease in ECe through the soil depth. Furthermore, the higher salt accumulation was registered at soil layer of 0-0.3 m. In all soil depths, the salts were more accumulated during summer season (July) and salt distribution through the soil depth was affected by autumn-winter rainfall leaching (December). The seasonal variation of soil salinity in the 1.2 m depth showed that it was lower during autumn-spring period than that of the summer one (Table 1).

**Table 1:** Distribution of soil salinity (E. C dS  $m^{-1}$ ) measured at 0.6 m from nozzle at Different Layers in FW and SS treatments during 2004 and 2005 crop Seasons.

| Treatmt<br>Period            | FW   |      |      |      | SS  |     |     |     |
|------------------------------|------|------|------|------|-----|-----|-----|-----|
|                              | H1   | H2   | H3   | H4   | H1  | H2  | H3  | H4  |
| Soil salinity (dS $m^{-1}$ ) |      |      |      |      |     |     |     |     |
| July 05                      | 1.9  | 1.75 | 1.52 | 1.4  | 7.7 | 6.9 | 6.5 | 6.1 |
| December05                   | 1.30 | 1.25 | 1.23 | 1.10 | 6.7 | 6.0 | 5.8 | 5.5 |

H1, H2, H3 and H4 represent the different soil depths from the surface (30, 60, 90 and 120 cm, respectively). Values are the means of three soil samples measurements ( $n = 3$ ).

### 3.2. Fruit Characteristics and Yield

During both crop seasons, salinity has altered fruit diameter (FD), fruit volume (FV) and fruit weight (FrW). For both salt treatments, FrW averages increased markedly with time. In June 2004, their values were of 0.38 and 0.28 g in FW and SS treated plants, respectively; and they reached 1.33 and 1.05 g, respectively in December. In 2005, these values were of 0.44 and 0.36 g in FW and SS treated plants and reached 1.38 and 1.22 g, respectively for the respective periods. These results showed that the increase was more important under irrigation with fresh water. Furthermore, the fruit fresh weight in SS plants was statistically lower than that in FW ones ( $p = 0.0056$ ). In 2004, FW plants showed higher fruit water content values than the SS ones (53.39 and 50.88% in FW and SS, respectively).



However, in 2005, the two irrigated treatments showed almost similar values (51.43 and 52.27%, respectively) for which differences were not statistically significant. The non significant differences in FWC values between the two crop seasons for both treatments revealed the role of the active root zone of Chemlali olive in the upholding of a suitable hydration level for its tissues.

**Table 2.** Olive Yield (Kg tree<sup>-1</sup>) of olive trees (cv. Chemlali) grown under FW and SS irrigation.

| treatment | FW                     | SS                     | Relative reduction in SS (%) |
|-----------|------------------------|------------------------|------------------------------|
| 2004      | 38±2.9 <sup>a, w</sup> | 22±2.2 <sup>b, w</sup> | -42,1                        |
| 2005      | 14±2.5 <sup>a, x</sup> | 9±2.8 <sup>b, x</sup>  | -35                          |
| Mean      | 27 <sup>a</sup>        | 15.5 <sup>b</sup>      | -42.6                        |

Values represent the means of ten samples ± standard deviations. Different letters (a, b) indicate significant differences ( $P < 0.05$ ) between saline water irrigation treatments within each year. Different letters (w, x) indicate significant differences ( $P < 0.05$ ) between crop seasons within each treatment.

The average olive production of SS plants during the experimental period (15.5 kg tree<sup>-1</sup>) was much lower (42%) than that of FW ones (27 kg tree<sup>-1</sup>). For both treatments, the first crop season was marked by higher olive yield (Table 2). The yield variation in both treatments could be due to the alternate bearing phenomenon characterizing the olive tree production and/or to the effects of climatic conditions characterizing the experimental site.

### 3.3. Oil Quality Indices

During both crop seasons, the irrigation treatments did not affect the oil accumulation in the Chemlali olive tree as no statistically significant differences were observed between total oil content of the two treatments ( $p > 0.05$ ). It was of 27.8 and 30.5 % Fw in FW and of 25.7 and 28.3 % Fw in SS during 2004 and 2005 crop seasons, respectively (Table 3).

**Table 3:** Total oil content, Free Acidity, Peroxide Value, Extinctions Coefficients of total chlorophyll and carotenoids contents of Virgin Olive Oils from Olive Trees (Cv. Chemlali) grown under FW and SS Irrigation.

| Treatment   | Total oil content (% Fw) | Free acidity (%) | Peroxide value (meq O <sub>2</sub> / kg) | K <sub>232</sub> | K <sub>270</sub> | Total chlorophyll l (mg / kg) | Carotenoid s (mg / kg) |
|-------------|--------------------------|------------------|------------------------------------------|------------------|------------------|-------------------------------|------------------------|
| <b>2004</b> |                          |                  |                                          |                  |                  |                               |                        |
| FW          | 27.85±2.6                | 0.25±0.02        | 3.2±0.31                                 | 1.06±0.02        | 0.05±0.01        | 9.5±0.07                      | 0.38±0.07              |
| SS          | 25.7±2.05                | 0.24±0.02        | 2.9±0.12                                 | 1.05±0.04        | 0.05±0.02        | 9.2±0.09                      | 0.41±0.04              |
| <b>2005</b> |                          |                  |                                          |                  |                  |                               |                        |
| FW          | 30.56±3.0                | 0.34±0.04        | 4.6±0.34                                 | 1.62±0.06        | 0.11±0.02        | 10.19±0.05                    | 0.42±0.06              |
| SS          | 28.32±2.2                | 0.32±0.05        | 4.4±0.28                                 | 1.76±0.06        | 0.16±0.018       | 10.22±0.04                    | 0.44±0.075             |

Values are the means of three different VOO samples ( $n = 3$ ) ± standard deviations.

In contrast with previous reports [5, 7, 8, 9, 10, 11], the high saline treatment SS we applied to Chemlali olive tended to decrease oil content relative to FW treated plants (though not significantly so). Furthermore, the non statistically significant differences in oil content between both saline water treatments, in comparison to olive yield, is certainly an advantage for the use of saline water for Chemlali olive cultivation, being extended to saline irrigated lands in arid regions in Tunisia. Chlorophyll concentrations in the virgin oils ranged from 9.5 to 10.2 and from 9.2 to 10.2 mg / kg in FW and SS, respectively (Table 3). Differences between the treatments were not significant ( $p > 0.05$ ) in either season. As well for the chlorophylls contents, during both crop seasons, the carotenoid contents were not influenced by saline irrigation regimes.

During 2004 crop season, the free acidity ranging from 0.24 to 0.25 % and peroxide value, ranging from 2.9 to 3.2 meq O<sub>2</sub> kg<sup>-1</sup> of the different olive oils samples, respectively in SS and FW, were considerably lower than the upper limit of 0.8 % as oleic acid and 20 meq O<sub>2</sub> kg<sup>-1</sup> as the peroxide

value established by the EU legislation for extra virgin olive oil. Moreover, these two quality indices were not influenced by water quality treatment, since no statistically significant differences were observed between both treatments ( $p > 0.05$ ). In the second crop season, the free acidity and peroxide value have increased in both treatments, if compared to those recorded during the first one (Table 4). The free fatty acid level of both olive oil samples we analysed was lower than those recorded in Barnea olive oil subjected to similar salinity treatments [7], and differences between FW and SS treated plants were not significant.

**Table 4:** Fatty Acid Composition (%) of Virgin Olive Oils from Olive Trees (Cv. Chemlali) Grown Under Fresh Water Irrigation (FW) and Saline Water Irrigation (SS) in 2004 and 2005.

| Fatty Acids               | 2004                      |                           | 2005                      |                           |
|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
|                           | FW                        | SS                        | FW                        | SS                        |
| Palmitic acid             | 19.82±0.25 <sup>a,w</sup> | 16.1±0.35 <sup>b,w</sup>  | 16.7±0.32 <sup>a,x</sup>  | 15.51±0.42 <sup>b,w</sup> |
| Palmitoleic acid          | 2.57±0.15 <sup>a,w</sup>  | 2.1±0.17 <sup>a,w</sup>   | 1.76±0.12 <sup>a,x</sup>  | 1.65±0.11 <sup>a,x</sup>  |
| Heptadecanoic acid        | 0.12±0.01 <sup>a,w</sup>  | 0.11±0.02 <sup>a,w</sup>  | 0.14±0.03 <sup>a,w</sup>  | 0.13±0.03 <sup>a,w</sup>  |
| Heptadecenoic acid        | 0.24±0.02 <sup>a,w</sup>  | 0.22±0.03 <sup>a,w</sup>  | 0.27±0.05 <sup>a,w</sup>  | 0.26±0.08 <sup>a,w</sup>  |
| Stearic acid              | 2.19±0.015 <sup>a,w</sup> | 2.01±0.016 <sup>a,w</sup> | 2.83±0.016 <sup>a,x</sup> | 2.25±0.025 <sup>a,x</sup> |
| Oleic acid                | 55.58±1.05 <sup>a,w</sup> | 59.3±2.03 <sup>b,w</sup>  | 60.73±2.45 <sup>a,x</sup> | 64.59±2.56 <sup>b,x</sup> |
| Linoleic acid             | 16.14±0.65 <sup>a,w</sup> | 17.96±0.52 <sup>a,w</sup> | 16.52±1.01 <sup>a,w</sup> | 17.26±0.96 <sup>a,w</sup> |
| Linolenic eicosanoic acid | 0.56±0.08 <sup>a,w</sup>  | 0.63±0.078 <sup>b,w</sup> | 0.56±0.08 <sup>a,w</sup>  | 0.55±0.09 <sup>a,x</sup>  |
| Eicosanoic acid           | 0.34±0.03 <sup>a,w</sup>  | 0.41±0.08 <sup>b,w</sup>  | 0.4±0.06 <sup>a,x</sup>   | 0.43±0.07 <sup>a,w</sup>  |
| Eicosenoic acid           | 0.16±0.02 <sup>a,w</sup>  | 0.14±0.04 <sup>a,w</sup>  | 0.18±0.05 <sup>a,w</sup>  | 0.16±0.07 <sup>a,w</sup>  |
| Uns/ Sat ratio            | 3.34 <sup>a,w</sup>       | 4.31 <sup>b,w</sup>       | 3.98 <sup>a,w</sup>       | 4.61 <sup>b,w</sup>       |
| Mono/ Poly ratio          | 3.5 <sup>a,w</sup>        | 3.32 <sup>a,w</sup>       | 3.68 <sup>a,w</sup>       | 3.74 <sup>a,w</sup>       |

Values are the means of three different VOO samples ( $n = 3$ ) ± standard deviations. Different letters (a, b) indicate significant differences ( $P < 0.05$ ) between saline water irrigation treatments within each year. Different letters (w, x) indicate significant differences ( $P < 0.05$ ) between crop seasons within each treatment.

The comparison of spectrophotometric absorption characteristics in the UV region at 232 and 270 nm between oils from the two saline irrigated treatments did not show significant differences ( $p = 0.433$ ). Taking into account the values of free acidity, peroxide value and  $K_{232}$  and  $K_{270}$ , the oil samples obtained from both treatments met the European Union requirements for the extra virgin olive oil category. The distribution of fatty acid composition of the oil samples of both saline water treatments covers the normal range expected for virgin olive oil (Table 4). For both treatments, the most abundant acid was the oleic one with values recorded in oil obtained from SS treated plants were statistically higher ( $p = 0.0024$ ) than those in oil of FW ones. However, the oil obtained from high saline water irrigated plants would be nutritionally better than that obtained in the case of fresh water irrigated ones.

Table 5 reports the concentrations of the major phenolic, total phenols and oxidative stability of VOO samples in both treatments. Total phenols contents of VOO were significantly influenced by the salinity treatments. During 2004 crop season, the total phenols contents were of 181 and 214 mg / kg, respectively in FW and SS treatments. During the second crop, these values reached 198 and 223 mg / kg, respectively. The use of saline water at 7.5 dS m<sup>-1</sup> has reinforced the phenols accumulation and did not altered the oil quality. The three phenolic compounds in highest concentrations in both oil samples are hydroxytyrosol, tyrosol and glycoside oleuropein.



**Table 5:** Phenolic Composition Concentrations (mg / Kg), Total Phenols Contents (mg/kg of oil) and Oxidative Stability (h) of Olive Oils from Olive Trees (Cv. Chemlali) Grown Under Fresh Water Irrigation (FW) and Saline Water Irrigation (SS) in 2004 and 2005.

|                         | 2004                       |                            | 2005                       |                            |
|-------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
|                         | FW                         | SS                         | FW                         | SS                         |
| Tyrosol                 | 42.5 ± 2.36 <sup>a,w</sup> | 66.4±3.21 <sup>b,w</sup>   | 45±2.56 <sup>a,w</sup>     | 72.5±2.36 <sup>b,x</sup>   |
| Hydroxytyrosol          | 84.5±3.56 <sup>a,w</sup>   | 96.7±2.06 <sup>b,w</sup>   | 88.5±2.47 <sup>a,w</sup>   | 105.4±3.56 <sup>b,x</sup>  |
| Oleuropein              | 10.5±2.84 <sup>a,w</sup>   | 12.7±2.56 <sup>a,w</sup>   | 12.5±3.3 <sup>a,w</sup>    | 16.4±2.84 <sup>b,x</sup>   |
| glycoside oleuropein    | 23.4±2.56 <sup>a,w</sup>   | 34.6±2.47 <sup>b,w</sup>   | 25.5±2.5 <sup>a,w</sup>    | 37.1±2.56 <sup>b,w</sup>   |
| Vanillic                | 13.2±1.56 <sup>a,w</sup>   | 15.6±1.04 <sup>a,w</sup>   | 16.4±2.3 <sup>a,x</sup>    | 17.6±2.45 <sup>a,w</sup>   |
| Caffeic                 | 10.5±1.24 <sup>a,w</sup>   | 13.6±1.03 <sup>b,w</sup>   | 13.2±2.45 <sup>a,x</sup>   | 16.9±2.12 <sup>b,x</sup>   |
| Syringic                | 8.6±1.2 <sup>a,w</sup>     | 10.7±1.24 <sup>b,w</sup>   | 10.7±1.24 <sup>a,x</sup>   | 12.4±1.24 <sup>b,x</sup>   |
| p-coumaric              | 4.2±1.32 <sup>a,w</sup>    | 5.39±1.42 <sup>a,w</sup>   | 6.6±1.2 <sup>a,w</sup>     | 8.2±1.2 <sup>a,x</sup>     |
| o-coumaric              | 3.2±1.02 <sup>a,w</sup>    | 4.6±1.25 <sup>a,w</sup>    | 5.3±1.32 <sup>a,w</sup>    | 7.1±1.32 <sup>b,x</sup>    |
| Ferulic                 | 2.96±1.43 <sup>a,w</sup>   | 4.5±1.65 <sup>b,w</sup>    | 4.25±1.02 <sup>a,x</sup>   | 6.8±1.22 <sup>b,x</sup>    |
| Total phenols (mg/kg)   | 181.46±2.35 <sup>a,w</sup> | 214.17±2.45 <sup>b,w</sup> | 198.08±4.56 <sup>a,x</sup> | 223.67±3.47 <sup>b,x</sup> |
| Oxidative stability (h) | 16.02±1.02 <sup>a,w</sup>  | 18.84±1.45 <sup>b,w</sup>  | 16.43±2.03 <sup>a,w</sup>  | 21.73±2.14 <sup>b,x</sup>  |

**ABBREVIATIONS USED:** VOO, virgin olive oil; FW, fresh water treatment; SS, saline water treatment; Fw, fresh weight; ECe, electrical conductivity; FrW, fruit weight; FD, fruit diameter; FV, fruit volume; FWC, fruit water content; MI, maturation index; LSD, least significant differences; PAL, phenylalanine ammonia-lyase.

**Il n'y a pas de glucoside dans l'huile donc pas d'oléuropéine et pas de glucoside oleuropéine. Il y a de l'oléuropéine aglycone et du ligstroside aglycone parmi les sécoiridoïdes principaux.**

#### 4. Conclusion

In conclusion, saline water (EC = 7,5 dSm<sup>-1</sup>) used for olive tree (cv. Chemlali) irrigation appears to be beneficial not only for water resources management, but also have a direct effects on both quantitative and qualitative characteristics of virgin olive oil in the case of Chemlali olive cultivar tested in this experiment. The changes in VOO fatty acid and phenolic compositions induced perhaps by water deficit, resulting from saline water irrigation as has been confirmed. Recent researches confirmed the protective role of phenols, as natural antioxidants, against cardiovascular diseases and colon, breast and skin cancers. The higher levels of phenolic compounds found in Tunisian Chemlali olive oil would be with more benefits for people. Furthermore, severe studies report different pharmacological activities of olive oil phenols, other than antioxidant potential.

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## Influence of water quality used in irrigation on the sensory properties of olive oil

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### Abstract

This study was conducted to evaluate the influence of three water types applied to irrigate 'Nabali Muhasan' olive (*Olea europaea* L.) trees grown in an orchard in the north-east of Jordan on the sensory attributes of the produced olive oil.

The results of this study revealed the high salinity of the well water used.

On the other hand, the properties of rain water were significantly better than the standards for ground water whereas reclaimed wastewater (TWW) achieved Jordanian standard for TWW except for Na and HCO<sub>3</sub>.

Regarding the soil analysis of the olive orchard used in this study, no marked changes in the parameters analysed for the two years study (2006 & 2007) were found.

Olive oils made from trees that received well and rain water either in 2006 or in 2007 crop seasons were free from any defect and were classified as extra virgin olive oil. On the other hand, the negative attributes fusty, musty, winy and muddy were detected in olive oils made from trees receiving reclaimed wastewater in 2006 crop year; whereas only two negative attributes i.e fusty and musty were detected in 2007 crop season.

The sensory attributes of olive oil samples obtained under the conditions of this study using experimental mill were better than those obtained by commercial press. Such results might be ascribed to the low quantities of olive fruits milled, low malaxation temperature used and the bitter cleanliness degree in case of experimental mill. As a result, it is difficult to conclude that the presence of negative attributes (defects) in the studied olive oils is due to the reclaimed wastewater treatment alone. However, more research is needed to clarify this point.

**Keywords:** Olive oil, treated waste water, rain water, well water, sensory properties, extra virgin

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## **L'importance de l'amélioration des techniques culturales pour assurer la durabilité et la productivité du secteur oléicole dans le sud tunisien**

Mounir Abichou, Ben Rouina Bechir, Labiad Mohamed

### **Résumé**

La filière oléicole dans le sud Tunisien constitue encore une spéculation importante par ses multiples implications sociale, culturelle, économiques et écologiques. Certes, l'aridité climatique et édaphique et la fragilité du milieu constituent une contrainte à la productivité du secteur, mais la région du sud dispose, en outre, de nombreux atouts, dont notamment la biodiversité, le savoir local, et pour cette raison, des efforts d'amélioration et de développement sont souhaitables et nécessaires.

Ce dans ce cadre que l'institut de l'olivier a mené des actions de recherches qui ont pour objectifs d'améliorer la productivité et assurer la durabilité du secteur tel que l'introduction d'autres cultures avec l'olivier (figuier, tomate, luzerne etc....), l'amélioration des techniques culturales relatives aux travaux du sol pour réduire le phénomène d'érosion éolien et la perte en sol et l'épandage de la margine pour améliorer le statut organique des sols sableux initialement très faible.

**Mots clés :** Sud Tunisien, Durabilité, productivité, Techniques culturales.

### **Abstract**

In the south of Tunisia, the olive trees orchards constitute even an important speculation by their multiple social, cultural, economic and ecological implications. Certainly, the aridity of climate and the fragility of this environment constitute a constraint to the productivity of the olive tree sector, but the southern Tunisian region disposes, in addition to numerous assets, of which notably the biodiversity, some local knowledge, and for this reason, many efforts of improvement and development are desirable and necessary.

In this setting, the olive tree institute led several research actions in order to improve the productivity and to assure the durability of the sector as the introduction of others culture with the olive tree (fig-tree, tomato, alfalfa etc.), the improvement of the technical culture relative to soil tilling in order to reduce the wind erosion phenomenon and the soil loss and the Mulching of the "margine", the Olive Mill Waste water, to improve the organic matter content of the sandy soils initially very weak.

### **1. Introduction et problématique**

Dans le Sud Est tunisien, à bioclimat aride (100-200 mm de précipitation par an), on assiste à l'extension des cultures aux dépens de pâturage. Parmi ces cultures, celle de l'olivier qui tient une place prépondérante. Dans le gouvernorat de Médenine, on comptait déjà en 2007 un effectif d'environ 4,5 millions pieds (CRDA Médenine). Toutefois, dans cette région, l'oléiculture se heurte à des problèmes sérieux d'économie d'eau et d'érosion. Les techniques culturales relatives aux travaux du sol adoptées actuellement pour l'olivier, comme le labour conventionnel, se basent sur des passages fréquents au cours de toute l'année. Les agriculteurs considèrent la complète éradication de la végétation naturelle dans les interlignes nécessaire pour éliminer la concurrence en eau avec les arbres. En même temps, ils espèrent rompre la capillarité (effet mulch), augmenter l'infiltration des eaux de pluies et aérer le sol. Néanmoins, ces techniques sont à l'origine d'une exposition du sol aux agents érosifs du climat (vent et pluie) ce qui engendre une érosion accrue et un déchaussement des racines de la culture. Le travail fréquent par des outils non adéquat détruit la partie la plus superficielle du système racinaire des oliviers, la partie nécessaire à optimiser l'emploi de l'eau des précipitations

peu abondantes. Le passage des outils de travail crée des semelles de labour qui réduisent sensiblement le taux d'infiltration de l'eau de pluie en profondeur entravant ainsi la recharge d'eau de profil. L'eau de pluie demeure ainsi près de la surface, exposée à une évaporation intense et non exploitable par les racines qui ont été coupée par les façons culturales. En plus, le passage de tracteurs est à l'origine de la formation de zones de compaction superficielles.

Aussi la réduction de la couverture végétale, la perte de sa capacité de régénération et l'ameublissement des horizons de surface conduisent à la disparition de l'horizon sableux superficiel apte à stocker les eaux de pluie. Le sol est progressivement tronqué de ses horizons meubles de surface pouvant conduire à la mise à nu du substrat non altéré et quasiment imperméable. Dans les situations les plus fragiles, la mise en œuvre de ces pratiques entraîne un fort accroissement de l'érosion tant éolienne qu'hydrique. Dans les plaines sableuses, les vents entraînent le départ et le transport des éléments les plus fins du sol et peut mettre en suspension de très fines particules et les transporter sur des grandes distances. Les particules moyennes et fines peuvent être soulevées et redéposées alors que les grosses particules peuvent être soufflées en surface (saltation). L'abrasion qui en résulte peut réduire la dimension des particules de sol et augmente d'autant sa susceptibilité à l'érosion.

En outre l'activité oléicole est peu rentable, la faible productivité des plantations oléicoles, qui est liée à l'effet de plusieurs facteurs dont la sécheresse, la sénescence, la qualité des sols et le manque d'entretien des plantations affecte sensiblement la rentabilité économique de cette activité (B Karray ; A Mounir, 2007).

L'institut de l'olivier conscient de ces problèmes à développer un programme de gestion technique du sol et des plantations d'olivier dans les zones aride pour améliorer la productivité et assure la durabilité du secteur. Et a mené certaines actions de recherches (l'étude du flux d'érosion pour deux parcelles labouré par deux outils différents, l'étude de l'effet de l'épandage de la margine sur le sol sableux, l'introduction du figuier (*Ficus carica* L.) Ces actions ont pour objectifs :

- Résoudre les problèmes liés à la mécanisation du travail du sol et cherché les outils adéquats pour l'ameublissement superficiel du sol tout en le protégeant contre l'érosion éolienne.
- L'amendement organique pour l'amélioration du statut du sol et l'amélioration des ses propriétés physiques structurales.
- Introduction du figuier (*Ficus carica* L.) dans le système oléicole des zones arides : un moyen de protection de l'environnement et d'amélioration de la productivité

## **2. Action 1: Etude de flux horizontal pour deux cas de labour**

Le travail du sol jugé nécessaire pour la croissance et la production des cultures doit tenir compte des caractéristiques du sol et du climat afin d'éviter les risques d'érosion, le bon choix de l'outil adéquat présente beaucoup d'importance pour cette opération. C'est dans ce cadre que s'insère ce travail de recherche qui consiste à évaluer et étudier l'effet du travail du sol sur l'érosion éolien. Pour réaliser ce travail, on a calculé et mesurer le flux d'érosion pour deux parcelles labouré, une par les socs de scarifiage (queues d'hirondelle) habituellement très utilisés par les agriculteurs et l'autre par la charrue polysocs (jamoussi) dont l'objectif d'estimer les flux de saltation dans les zones arides tunisienne, où le phénomène de l'érosion éolienne est très actif, et par la suite comparer les flux horizontaux d'érosion pour les différents outils utilisés.

### **2.1. Mesure du flux horizontal d'érosion**

En pratique, la mesure du flux d'érosion est effectuée moyennant un réseau de piège à sable de type BSNE (Big Spring Number Eight) installés sur des supports menus d'un système girouette. Chaque BSNE a une section rectangulaire bien déterminé mesurée moyennant un pied à coulisse. Pour suivre la variation du flux avec la hauteur, ces mini mâts ont été équipés de sept BSNE installés à des hauteurs comprises entre 5 cm et 100 cm par rapport au niveau du sol afin de couvrir la hauteur de la couche de saltation qui est de l'ordre de 80 cm dans la nature. En outre, le flux de saltation a été collecté après chaque événement érosif et la mesure a été réalisée sur deux parcelles soumises à deux techniques différentes du travail du sol afin de dégager les techniques les plus agressives.

## 2.2. Calcul du flux

Le flux horizontal  $q(z)$  ( $\text{g.cm}^{-2} \cdot \text{s}^{-1}$ ) à une hauteur donnée  $z$ , exprime la concentration des particules en saltation à cette hauteur. Il est défini par la masse de matériel traversant par unité de temps une section verticale de surface unité, perpendiculaire à la direction du vent. Cette concentration est très forte au niveau des 10 premiers centimètres de la surface du sol Bagnold, (1941) ; Fryrear et al, (1991) et diminue avec la hauteur suivant une loi exponentielle ou en puissance. Fryrear et saleh, (1993) ont développé l'expression suivante pour décrire la variation du flux horizontal avec la hauteur :  $q(z)=a \exp(-bz)$

Où  $a$  et  $b$  sont des coefficients d'ajustement exprimés respectivement en  $\text{g}^{-1}$  et  $\text{g.cm}^{-2}$ . L'intégration du flux sur la hauteur de la couche de saltation donne le flux horizontal d'érosion ( $Q$ ).

## 2.3. Résultats

### 2.3.1. Charrue à dents

**Tableau 1:** flux mesuré et ajustement des paramètres du flux calculé pour la parcelle traitée avec l'outil à dents (queues d'hirondelle).

|                    | hauteur               | poids<br>Sable (g) | section<br>des pièges | flux mesuré | hauteur<br>bas piège | h section |
|--------------------|-----------------------|--------------------|-----------------------|-------------|----------------------|-----------|
| piège 1bas         | 14                    | 6,05               | 2,30                  | 2,63        | 13,47                | 1,06      |
| piège 1 haut       | 22,63                 | 8,95               | 10,12                 | 0,68        | 20,12                | 5,02      |
| piège 2            | 35,085                | 5,18               | 9,96                  | 0,52        | 32,62                | 4,93      |
| piège 3            | 48,185                | 3,96               | 10,01                 | 0,40        | 45,72                | 4,93      |
| piège 4            | 61,155                | 2,43               | 10,31                 | 0,24        | 58,68                | 4,97      |
| piège 5            | 79,455                | 2,06               | 9,69                  | 0,21        | 76,97                | 4,97      |
| piège 6            | 119,195               | 1,32               | 9,38                  | 0,14        | 116,72               | 4,95      |
|                    |                       | a                  | b                     |             |                      |           |
| $q(z)=a \exp(-bz)$ | paramètres régression | 9,223              | 0,092                 |             |                      |           |

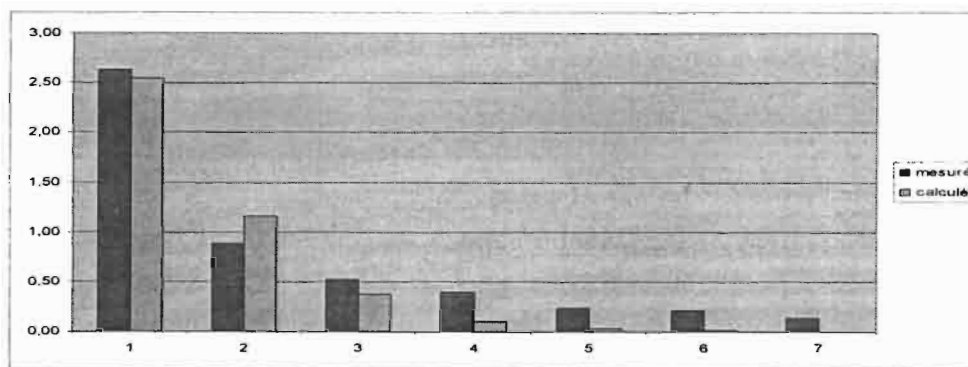


**Figure 1:** comparaison entre flux mesuré et calculé.

### 2.3.2. Charrue à socs

**Tableau 2:** flux mesuré et ajustement des paramètres du flux calculé pour la parcelle traitée avec la charrue à soc (jamousi).

|                    | hauteur               | Poids<br>Sable (g) | section<br>des pièges | flux<br>mesuré | hauteur bas<br>piège | h section |
|--------------------|-----------------------|--------------------|-----------------------|----------------|----------------------|-----------|
| piège 1bas         | 11,62                 | 3,15               | 2,01                  | 1,57           | 11,11                | 1,02      |
| piège 1 haut       | 20,33                 | 4,62               | 9,95                  | 0,46           | 17,91                | 4,84      |
| piège 2            | 31,69                 | 2,78               | 11,21                 | 0,25           | 29,01                | 5,36      |
| piège 3            | 44,53                 | 1,44               | 10,69                 | 0,13           | 41,96                | 5,14      |
| piège 4            | 60,35                 | 1,11               | 10,60                 | 0,10           | 57,61                | 5,07      |
| piège 5            | 77,15                 | 0,72               | 10,44                 | 0,07           | 74,61                | 5,08      |
| piège 6            | 117,10                | 0,49               | 10,02                 | 0,05           | 114,61               | 4,97      |
|                    |                       | a                  | b                     |                |                      |           |
| $q(z)=a \exp(-bz)$ | paramètres régression | 6,087              | -0,118                |                |                      |           |

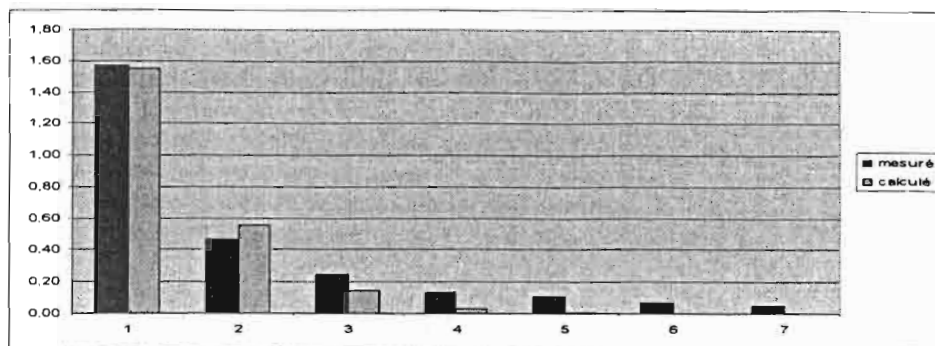


Figure 2: comparaison entre flux mesuré et calculé.

Dès lors que les paramètres de régression a et b sont connus, l'intégration du flux sur une hauteur comprise entre la surface du sol et 80 cm (50 cm) donne le flux horizontal d'érosion (Q) ( $\text{g.cm}^{-1} \cdot \text{s}^{-1}$ ) pour chaque parcelle.

|                 |      |        |                        |
|-----------------|------|--------|------------------------|
| Charrue à dents |      |        | flux intégré sur 50 cm |
|                 | a    | b      |                        |
|                 | 9,22 | -0,092 | 99,34587482            |

|                |      |        |                        |
|----------------|------|--------|------------------------|
| Charrue à socs |      |        | flux intégré sur 50 cm |
|                | a    | b      |                        |
|                | 6,09 | -0,118 | 51,52351811            |

La comparaison entre ces deux flux nous a permis d'avoir une idée sur l'ampleur qui prend le phénomène de l'érosion éolienne dans les zones arides tunisiennes, puisque les flux ont été collectés événement par événement, et puis de trancher entre deux outils de travail du sol afin de réduire au maximum la perte en sol dans la parcelle. Par ailleurs le soc semble être l'outil le plus approprié pour les sols sableux car il réduit à moitié les flux de saltation et permet le retournement du sol lors de la saison pluvieuse sur une profondeur allant de 15 à 25 cm. Elle crée par la suite, des conditions physiques favorables pour le développement et l'activité racinaire, bonne porosité et perméabilité suffisante. Alors que les queues d'hirondelle suite à des passages cet outil conduit à la formation de semelle de labour.

### 3. Action 2: L'amendement organique pour l'amélioration des propriétés physiques structurales du sol

Le manque de la matière organique en zone aride en quantité suffisante contribue à la baisse de productivité des sols, compromettant ainsi les niveaux de rendement et la qualité des récoltes. Et pour le maintenir à un seuil optimal cela constitue une contrainte pour tout programme de conservation d'amélioration des sols. Aujourd'hui, des sources nouvelles de matières organiques peuvent être utilisées pour amender les sols tel que les margines qui s'accumulent d'une année à l'autre et constituent un véritable problème environnementale face au tonnage grandissant produit par les huileries qui dépassent les 700000 tonnes/an en Tunisie (source Direction Générale de Production Agricole) surtout après l'introduction du matériel d'extraction chaîne continue (3 phases) ce matériel nécessite des quantités importantes d'eau et génère des quantités importantes des margines (pour l'extraction d'une tonne d'olive il faut entre 1000 et 1200 litres d'eau). La valorisation de ces effluents par épandage dans les oliveraies du sud Tunisien caractérisé par un sol sableux est synonyme d'une meilleure gestion de l'environnement d'une part et d'amélioration de la cohésion du sol d'autre part. Ces constats ainsi réalisés, il nous a semblé nécessaire de faire le lien entre les différents doses des margines épandues apportés au sol (50,100, 200 m<sup>3</sup>/ha), et la stabilité de la structure d'un sol sableux connu comme instable. Cela nous a conduits à engager ce travail de recherche présenté ici et qui se propose :

\* D'évaluer les effets des différents doses de margines sur l'évolution du taux de la matière organique, la stabilité structurale, la végétation naturelle et la perte en sol.



### 3.1. Evolution de teneur en matière organique dans le sol après 10 ans d'épandages:

La teneur en matière organique est déterminée selon la méthode de Walkley et Black qui consiste en une oxydation à froid par le bichromate de potassium ( $K_2Cr_2O_7$ ) en milieu acide et titrage par le sulfate ferreux ( $FeSO_4 \cdot 7H_2O$ ). La matière organique est déterminé par l'équation  $MO \% = C \% \times 1.725$

**Table 1:** Evolution du taux de la matière organique après 10 ans d'épandages de la margine.

| Doses   | T0               | T1                | T2               | T3               |
|---------|------------------|-------------------|------------------|------------------|
| Moyenne | 0,35 ± 0,12<br>a | 0,49 ± 0,13<br>ab | 0,60 ± 0,21<br>b | 1,05 ± 0,28<br>c |

L'analyse de la variance montre une nette amélioration du taux de la matière organique dans le sol de 0,35 % à 1,05 % (tableau 2). L'augmentation des taux de matière organique dans le sol est proportionnelle à la dose appliquée. Les différences observées pourraient être attribuées à la cinétique de minéralisation de la matière organique dépendant de l'importance des micro-organismes dans le sol traité (nombre et qualité) et les conditions climatiques. Ces résultats sont analogues à ceux de Cabrera et al, (1996) qui mentionnent que l'apport annuel sur un sol sableux contenant initialement 0,45 % de MO de 37 ou de 61 l/m<sup>2</sup> de margines pendant trois années de suite, engendre un accroissement de la matière organique (1,62 % et 1,98% respectivement).

### 3.2. Effet de la margine sur l'agrégation du sol et la stabilité structurale

La stabilité des agrégats a été étudiée en se basant sur le concept du diamètre moyen pesé, exprimé dans la littérature anglo-saxonne comme « the mean weight diameter », qui a été introduit par Van Bavel, (1949), et son changement, 'the change in mean weight diameter », selon la méthode dite de tamisage sous eau de (De Leenheer et De Boodt, 1959).

Cette méthode consiste à comparer la distribution des différences fractions des agrégats du sol issue du tamisage à sec (état initial) à celle obtenue après un tamisage sous eau (état final). Le diamètre moyen pesé (DMP) à l'état initial et final peut être ainsi déterminé Sillanpää, M, (1958) ; Baver et al, (1972) par :

$$DMP = \frac{\sum m_i d_i}{\sum m_i} \quad (II.1)$$

Avec :  $m_i$  = masse de la fraction i (g)  
 $d_i$  = diamètre moyen de la fraction i (mm)  
 $n$  = nombre total des fractions

le changement du diamètre moyen pesé qui correspond à l'indice d'instabilité (II) est alors obtenu par :

$$II = (DMP)_s - (DMP)_h \quad (II.2)$$

Où s et h désignant respectivement le tamisage à sec et sous eau.

L'inverse de cet indice n'est autre que l'indice de stabilité (IS) :

$$IS = \frac{1}{II} \quad (II.3)$$

### 3.3. Résultats

Concernant le témoin sans margine les particules inférieure à 2mm est 100 %, La distribution granulométrique obtenue par 50m<sup>3</sup>/ha caractérisée par une très forte proportion des particules les plus fines, avec 93% de la distribution inférieure à 2mm. Il s'agit bien d'un sol peu stable. Lorsque les agrégats ont été préalablement traités à la dose de 100m<sup>3</sup>/ha, la distribution granulométrique se trouve décalé : le taux d'éléments fin ou particule inférieure à 2mm passe à 87%, alors que le taux d'éléments grossiers (ou agrégats) supérieure à 2mm passe de 7 à 13% avec une augmentation peu significative. Aux doses supérieures, ces taux d'agrégats supérieurs à 2 mm augmentent très fortement atteignant 34% avec le traitement de 200m<sup>3</sup>/ha. Une réduction de toutes les classes inférieures à 2mm s'effectue simultanément. L'amélioration de liaison entre les particules a été obtenu avec la dose la plus élevée mais les agrégats de grande taille résiste moins à la désagrégation mécanique puisque l'indice de stabilité le plus bas est obtenu par la dose la plus élevée, les indices de stabilité sont respectivement 3,83, 2,94 et 2,31 pour les doses 50, 100, 200 m<sup>3</sup>/ha (tableau 1). Ces résultats peuvent être comparé à celle de Mellouli, (1996) qui a conclu qu'il existe une amélioration de la stabilité structurale pour les sols instable (les sables limoneux) suite à l'épandage de 50m<sup>3</sup>/ha.

3.3.1 Effet de l'épandage de la marge sur la végétation naturelle

Il s'agit du nombre d'individus par unité de surface, dans notre cas nous avons utilisé un cerceau d'un mètre carré puis nous avons compté le nombre de plantes annuelles existantes dans la surface délimitée par le cerceau. La végétation spontanée stimulé par l'épandage de marge représente une autre source d'apport de matière organique après son incorporation dans le sol, aussi la présence de végétation est connue pour être le principal facteur limitant l'érosion éolienne.

3.3.2. Résultats

Tableau 2: Densité de végétation naturelle.

| Doses | T0               | T1                  | T2                 | T3                   |
|-------|------------------|---------------------|--------------------|----------------------|
| Moyen | 2,66 ± 3,06<br>a | 100,5 ± 21,67<br>ab | 143,2 ± 21,31<br>b | 1185,2 ± 121,68<br>c |

On a remarqué que la densité de l'herbement naturelle est très élevés (1185) au niveau de la parcelle qui a reçu la forte dose de la marge (200 m3/ha) cette végétation constitue le couvert végétal qui assure la protection de la surface par la limitation de l'érosion et l'opposition à l'écoulement de l'air. Cette végétation est très utile pendant la saison du printemps, période caractérisée par un vent très actif.

3.4. Effet de l'épandage de la marge sur la perte en sol

Pour un agrégat de sol, quand la force aérodynamique est supérieure à la somme de la force de gravité et la force de cohésion inter particulaire, l'agrégat commence à bouger. Deux vitesses sont importantes dans le processus de saltation. La vitesse seuil d'érosion  $U_{*i}$  et la vitesse de friction  $U_{*}$ . Pour chaque classe de taille de particule ( $D_p$ ) il y a une vitesse seuil d'érosion. La vitesse de friction dépend de la vitesse de vent, de la densité moyenne des particules dans le sol  $\rho_p$ , de la présence d'éléments non érodables (végétaux, cailloux, roches) et l'humidité du sol et du taille des billons et sillons La quantification de la perte en sol déterminé par le flux mesuré dans la soufflerie et calculé en utilisant le modèle LISA élaboré par Gilles Bergametti.

$$Q = a.U_{*}(U_{*} - U_{*i}) \text{ (g.cm}^{-1}.s^{-1}\text{)}$$

A = Coefficient d'érodabilité

$$U_{*} = \text{vitesse de friction : } U_{*} = \frac{U(Z).K}{L_n = \frac{Z}{Z_0}}$$

$U_{*t}$  = vitesse de friction seuil

$Z_0$  = Rugosité de la surface

Z = Hauteur au dessus de la surface du sol.

K= Constante de Von Karman (~0.4)

3.4.1. Résultats

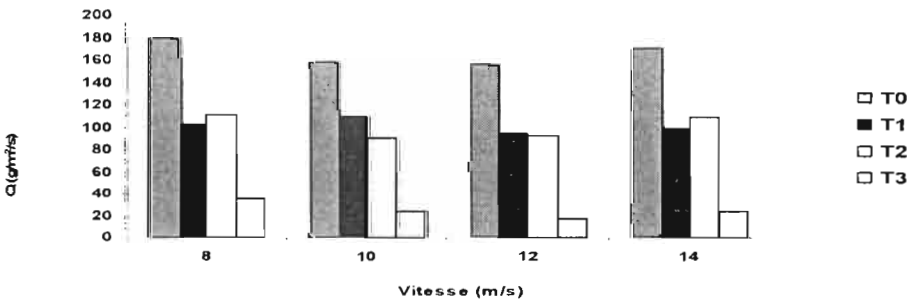


Figure 1: Flux mesuré dans la soufflerie a différentes vitesses.

Tableau 3: Flux calculé par le modèle LISA

| Traitement                 | T0                    | T1                   | T2                    | T3                    |
|----------------------------|-----------------------|----------------------|-----------------------|-----------------------|
| Flux (g/cm.s) en15 minutes | 9.92 10 <sup>-2</sup> | 9.9 10 <sup>-2</sup> | 9.65 10 <sup>-2</sup> | 8.63 10 <sup>-2</sup> |

La perte en sol démunie avec l'épandage de la margine cette remarque est plus claire avec la dose la plus forte ????

### **3.5. Action 3: Introduction du figuier (*Ficus carica* L.) dans le système oléicole des zones arides : un moyen de protection de l'environnement et d'amélioration de la productivité (en cour de réalisation)**

Une méthode possible de remédiation et d'amélioration des situations dégradées est la transformation de l'agro système oléicole actuel des zones arides par l'introduction du figuier (*figus carica* L).

Pourquoi le figuier ?

- Facile à installer
- bien adapté à la région
- écoulement de production prometteuse
- conservation facile

#### **3.5.1. Objectifs**

L'objectif de ce programme de recherche est d'étudier les effets de l'introduction du figuier en intercalaire avec l'olivier sur :

L'état de surface du sol (éléments non érodables, croûtes, distribution granulométrique du sol, etc...), Flux d'érosion éolien, L'activité microbiologique du sol, L'ombrage et l'évapotranspiration, La productivité et rentabilité par hectare

#### **3.5.2. Les effets attendus**

- Rentabiliser les systèmes de productions oléicoles dans les zones arides
- Trouver un champ d'activité plus large pour la femme rurale à travers la création des unités de séchages de figuier.
- Ralentir l'effet de l'érosion éolien par l'implantation de figuier entre les lignes de l'olivier qui sont déjà très éloignés l'une à l'autre 24 m / 24 m.
- Amélioration de la biodiversité dans les zones arides et diversification des produits destinés à l'exportation.
- Passer d'un système de production monoculture à un système intégré (réhabilitation de l'écosystème).

#### **3.5.3. Etudes préliminaires:**

Concernant l'étude de l'effet de l'ombrage sur l'activité microbiologique du sol on a quantifié le nombre des bactéries pour le sol exposé totalement au soleil et pour le sol couvert par l'ombrage du figuier

#### **3.5.4. Dénombrement de la microflore bactérienne totale**

Il s'agit de la flore aérobie mésophile viable.

##### **3.5.4.1. Préparation de la suspension du sol**

\* 10g de chaque échantillon nous ajoutons 90ml d'eau distillée stérile. Cette suspension est mise sous agitation à 250 tours/min pendant 30 minutes, Ensuite nous réalisons des dilutions jusqu'à  $10^{-4}$ .

##### **4.5.4.2. Ensemencement des boîtes de Pétri**

\* Le milieu utilisé est le nutriment Agar (28g de gélose nutritive et 5g d'agar) prêt à l'emploi. Pour cela, 0,1ml de chaque dilution est ensemencé en surface puis étalé dans trois boîtes de pétri. Ces boîtes sont incubées à 25°C avec alternance d'obscurité et de lumière pendant 48h.

Le comptage est effectué par la méthode du nombre le plus probable (MPN), ceci permet de déterminer les micro-organismes viables. Elle consiste en une approche statistique basée sur une série de dilutions successives. Les résultats sont exprimés en unités formant des colonies par g de sol (UFC).

### 3.5.5. Résultats

Le nombre des bactéries dans un échantillon de sol exposé totalement au soleil :  $52.66 \cdot 10^4$  CFU/g de sol. Le nombre des bactéries dans un échantillon de sol couvert par l'ombrage :  $65.33 \cdot 10^4$  CFU/g de sol. Le décompte de la flore montre que l'ombrage a permis d'augmenter le nombre de micro-organismes dans le sol. D'une manière générale, la croissance de ces bactéries est contrôlée par plusieurs paramètres. En effets, la plupart des espèces ont une croissance optimale pour un pH entre 7,5 et 8, une température de l'ordre de 25 à 30 °C (dans la zone d'étude la température du sol à 20 cm dépasse 36°C en été) et une concentration en oxygène dissous entre 0,5 et 4mg/l Féray C., (2000)

## 4. Conclusion

L'amélioration des techniques culturales relatives aux travaux du sol améliore sa structure (bilan hydrique, aération et réduction du lessivage des éléments nutritifs) et ainsi garantir leur productivité. L'épandage de la margine Permet de limiter la déflation éolienne, phénomène très répandu dans les oliveraies du Sud Est suite au maintien de la couverture végétale, la création de mottes et l'apport d'amendement ce qui améliorer le statut organique des sols sableux initialement très faible et réduire le phénomène d'érosions éoliennes. Aussi Introduction du figuier (*Ficus carica* L.) dans le système oléicole des zones arides permet d'avoir un agro-système qui respecte la bonne santé des sols et leurs biodiversité, une meilleure résilience par rapport au changement climatique, réduction de l'érosion éolienne et les services en rapport avec la vie humaine (alimentation, nutrition, santé, revenus, identité culturelle, esthétique, zones créatives, et qualité de vie) par conséquence développement durable.

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## **Olive, Asparagus and animals: an agroforestry model for temperate climate in developed countries**

Rosati A., Caporali S., Paoletti Q.

### **Abstract**

Due to the high harvesting cost, traditional olive cultivation is rapidly becoming anti-economical in developed countries. This might result in abandonment of millions of hectares of agricultural landscape. Converting such a landscape to an olive-based agroforestry system, intercropping olives with other economically viable crops, can provide economic sustainability, allowing maintenance of a traditional agricultural landscape, which is also functional to tourism activities. Here, the cultivation of a wild asparagus species (*Asparagus acutifolius*) as an understory crop in the olive orchard is proposed. The possible inclusion of animal raising in the olive-asparagus policulture are discussed. Olives, wild asparagus and animals could be managed together as a productive agroforestry system, where each crop benefits the others, while producing more income on the same land.

**Key words:** olive, asparagus, animal, agroforestry, policulture.

## **Olivier, Asperge et animaux: un modèle de l'Agroforêtierie pour les pays développés avec climat tempéré**

### **Résumé**

À cause des coûts élevés de la récolte, la culture traditionnelle de l'olivier dans les pays développés est en train de devenir rapidement antiéconomique. Il est probable que ceci porte à l'abandon de millions d'hectares du paysage agricole. Convertir tel paysage en un système "agroforestier" basé sur l'olivier, en associant l'olivier avec d'autres cultures économiquement viables, il peut offrir durabilité économique, en permettant de conserver un paysage agricole traditionnel qui est ouvert aussi aux activités touristiques. Ce travail propose la culture d'une espèce d'asperge sauvage (*Asparagus acutifolius*) comme une culture de sous-bois dans l'olivieraie. Il est également proposé de faire du pastoralisme avec des animaux d'élevage dans la polyculture olivier-asperge. Oliviers, asperges et animaux pourraient être gérés comme un système "agroforestier", où chaque culture apporte bénéfice aux autres et en même temps en produisant plus des revenus dans le même terrain et en particulier autour des villes.

**Mots clés:** olivier, asperge, animal, agroforêtierie, polyculture.

### **1. Introduction**

Due to the high harvesting cost and the cost of other field practices that require much labor, traditional olive cultivation is rapidly becoming anti-economical in developed countries where hand labor is very expensive. Decoupling of agricultural funding from the production (i.e. 100% decoupling for olive in Italy) and the probable end of agricultural subsidies after 2013, might result in the abandonment of millions of hectares of agricultural landscape in Europe. This might negatively affect not only the agricultural activities and income, but also the landscape of several European countries where olive has been historically important and very much part of the landscape. In some of these countries, olive trees are protected and cannot be removed, even if the orchard is abandoned or unproductive. In this context, new ways of deriving an income from olive orchards would be highly desirable.

One way to do this, is to grow other valuable crops under the olive orchard canopy, creating an olive policulture; that is, an agroforestry system where the olive is the tree component. The additional income deriving from such crops would justify maintenance of the olive landscape, while growing more

crops on the same land would generate scale economy, lowering the cost of olive production, hence making it more competitive. Maintenance of traditional landscapes, like the olive landscape, is important also from an economical point of view in that situation where the beauty of the landscape is functional to tourism activity. Italy, for instance, has over 20 thousand "agri-turism" enterprises (on-farm hospitality): if each of them benefits from 50 hectares of surrounding landscape, this results in one million hectares that contribute to the tourism business.

Old agriculture was based on policulture or agroforestry, but in modern times the agricultural design in western countries has been simplified in an attempt to transform farming in a process more similar to industry, where scale economy can be achieved and labor reduced. In this process the environmental costs associated with modern farming have not been accounted for. The recently increasing interest for more sustainable farming provides an opportunity and a challenge to design new agricultural systems, which are ecologically sustainable but also economically viable (i.e. high yields and low labor). To design such systems, a better understanding of the needs and functions of plants and animals is necessary so that different species can be integrated in a single system where they give some yield while providing ecological functions that help reducing outside inputs and labor.

The theoretical basis of such an approach has been amply discussed, and there are many successful practical examples. However, most of these examples regard farming in developing countries where labor is still relatively inexpensive and usually provided within the family, farm size is small, and expected income is low. In developed countries successful examples are less frequent because agriculture has come further away from being ecologically designed: hand labor is expensive and replaced by machines which usually require large farm size and monocultures, which in turn, lead to heavy use of chemicals. Redesigning such systems with an agroecological approach might imply radical changes, which are difficult to implement, but also hard to accept culturally. However, some recent examples exist. Within an European agroforestry research project, the "SAFE" (Silvo-Arable Forestry for Europe) project, cereals have been grown among large rows of trees, both crops being completely mechanized. This system has shown increased yield compared to the monocultures, while providing environmental benefits. Other positive examples of successful agroforestry in western countries are related to the economical advantages associated with the environmental benefits of the presence of trees, as in windbreaks, protection of riparian vegetation, prevention of erosion and consequent economic losses, like the reduction of water holding capacities of filled-up water reservoirs.

In the present paper, the cultivation of a wild asparagus species (*Asparagus acutifolius*) as an understory crop in the olive orchard is proposed as a possible way to increase both income and sustainability of olive growing. The potential yield and the market of such asparagus is discussed as well as the ecological design that justifies its inclusion in the olive orchard and possible strategies for the field management of the proposed agroforestry system are suggested.

## 2. Cultivation and market of *Asparagus acutifolius*

Wild asparagus (*Asparagus acutifolius*), was known and probably cultivated in ancient times (Aliotta et al., 2004), but not cultivated in modern times until very recently (Rosati, 2001). This species is one of the eight wild asparagus species present in Italy and the Mediterranean area (Bozzini, 1959) where it has always been enjoyed in traditional cooking (Venezia et al., 1993; Rosati, 2001; Fiori et al., 2001; Adam, 2004; Pieroni, 2005; Della et al., 2006). The spears are gathered from plants grown in the wild and sold in local markets. Given the high price of these spears in the existing market, recently some farmers have attempted to cultivate this species (Rosati 2001; Adam, 2004). Cultivation may supply this market which, up to now, has been limited by the availability of produce from the wild, and allow its expansion. However, little information on suitable field techniques is available to support farmers in the cultivation of this potential new crop. Such techniques differ from those of the cultivated asparagus (*Asparagus officinalis*) since the two species have quite different ecological requirements and yields (Aliotta et al., 2004; Rosati et al., 2005). Unlike *Asparagus officinalis*, the wild *A. acutifolius* is evergreen and prostrate, requiring different strategies for weed management and needing less light, water and nutrients, in view of the lower biomass produced, which results in lower yields (i.e. about 10-15% of the yields of cultivated asparagus). The lower yield is mostly due to the small size and weight of spears: about 5g per spear of 0.3m (Rosati et al., 2005; Benincasa et al., 2007). The low productivity makes this new crop suitable for marginal rural areas where high quality and price, rather than high yields, is the strategy for market crops.

The frugality of the wild asparagus, currently cultivated using seeds from local, non-selected wild plants, allows for a crop virtually free of pests and diseases, perfectly suited for organic or any other natural farming techniques.

The two species differ not only botanically and in ecophysiological requirements but also in their use: *A. officinalis* is used as a vegetable and therefore consumed in large quantities, while *A. acutifolius*, having a much stronger flavour, is used as a condiment in small quantities. This allows for a higher price of the spears, which ranges usually between 7 and 25 € per kg (i.e. two to four times the price of cultivated asparagus).

The most important problem that has limited success in growing *A. acutifolius*, has been that its seeds have a strong dormancy and do not germinate easily (Venezia et al, 1993). A suitable technique for production of transplants has been proposed (Rosati and Falavigna, 2000). More recent information has been published on field practices, yield potential, and labor requirements for cultivation and harvest (Rosati et al., 2005; Benincasa et al., 2007). In the following section some of the main results are summarized.

Wild asparagus (*A. acutifolius*) bare root transplants, obtained as described by Rosati and Falavigna (2000) have been transplanted in rows 1m apart and 0.33m within the row (Rosati et al., 2005), or rows 0.8m apart and either 0.4 or 0.25 within the row (Benincasa et al., 2007). In this wild asparagus the crown, although large, does not rise with time as with the cultivated asparagus, so planting must not be as deep. Harvest usually begins two or three years after transplanting, depending on transplant size, nutrient and water availability, soil type and climate. The spears are harvested in the spring when they first emerge, as soon as they reach sufficient height (0.3-0.5m). Harvest is protracted for 4 to 8 weeks or until spear diameter starts decreasing. The prickly vegetation can be removed before harvest, allowing for easier and faster harvest, but this might affect negatively plant vigor and yield in the following years (Rosati et al., 2005).

Harvest efficiency (i.e. kg of spears harvested per hour of labor) ranges between 7.2 kg/h and 3.0 with vegetation removal and between 3.8 kg/h and 1.2 without vegetation removal (Rosati et al., 2005; Benincasa et al., 2007).

Yield ranges between 1 and 1.5 tons per hectare but can easily reach 2 tons per hectare with good field practices and good quality transplants (Rosati et al., 2005; Benincasa et al., 2007). Given the high prices of the spears, which may range between 10 and 30 euro per kg, the wild asparagus appears to be an economically interesting crop, even though harvesting is a very expensive practice. In fact, hypothesizing 500 hours per hectare (i.e. cutting the vegetation before harvest), a sale price of 10 € per kg of spears and a cost of 10 € per hour of labour, harvest cost would represent one third of the crop's gross income. Even with more conservative numbers, the crop should easily pay for the labour, which might represent an opportunity of self-employment in small farms and/or in rural marginal areas. Alternatively, the spears could be harvested directly by the consumers in pick-your-own operations associated with tourism. Market potentials must be carefully assessed before planting.

### 3. The olive-asparagus agroforestry

Despite the fact that the literature hitherto available reports data for the cultivation of wild asparagus as a monoculture, the use of *Asparagus acutifolius* as an understory crop in policultures, including an olive-based agroforestry, has long been suggested (Rosati et al., 2001). Even though no data are available, there are several reasons to believe that such policulture could be ecologically and economically beneficial. Below I will discuss some such reasons.

The wild asparagus here proposed grows spontaneously in the Mediterranean area, sharing similar ecological needs as the olive, though the asparagus range is wider than that of the olive. In fact, both species are drought and heat tolerant, can grow on deep as well as shallow soils as long as there is no water logging, and tolerate well rocky soils. In addition, the asparagus species proposed, is also shade tolerant, unlike the olive, thus it can easily grow in the thin shade of the olive tree. In a recent study, Villalobos and co-workers (2006) have demonstrated that the olive maximum yield is obtained when the olive trees intercept 55% of the incoming light. With greater canopy densities the trees produce less oil, having to invest in vegetative growth to reach light. Therefore, about 45% of the available light cannot be used by the olive orchard. This radiation, however, can be used by the asparagus here studied which, as mentioned, is shade adapted. The productivity of the olive orchard can therefore be increased by producing not more oil, but a second crop.



The fact that this asparagus cannot produce large biomasses and its productivity is only about 10-15% of that of the cultivated asparagus, already suggests that this species needs not only little light, but also less nutrients to achieve its maximum potential yield, making its monoculture an ecological nonsense (i.e. a waste of light and nutrients that cannot be exploited by this crop with limited biomass and yield potential). Instead, the amount of light that is transmitted by the olive orchard is probably sufficient for the little biomass produced by the asparagus, while the light intercepted by its canopy, below the olive orchards, does not affect the light absorption by the olive canopy above. Similarly, the nutrients available in a marginal soil should be sufficient and there is no need for a very fertile soil as there is for the cultivated *Asparagus officinalis* with much greater yields. In conclusion, the low yield potential and the frugality of the wild asparagus, together with its shade and drought tolerance, suggest that its cultivation under the canopy of the olive orchard represents an optimal ecological niche, allowing two crops to be obtained on the same land with no detriment on the yield of either crop.

The overlapping of the natural distribution of olives and wild asparagus represents a guarantee that the asparagus will grow well and with little pest and disease problems in the olive orchard. The species is rather tolerant to the few pests and diseases that affect it, like the asparagus beetle (*Crioceris asparagi*) and the spotted asparagus beetle (*Crioceris duodecimpunctata*) among the pests and the asparagus rust (*Puccinia asparagi*) and purple spot, otherwise called fern spot (*Stemphylium vesicarium*) among the diseases.

Another good reason to grow wild asparagus under the olive canopy is that the asparagus is perennial and does not require tilling. Most olive orchards are now managed with green mulch and no tilling, in order to increase soil organic matter and water storage and to prevent soil erosion. An understory crop that requires soil cultivation would interfere with such practices and lead to reduced benefits. The wild asparagus is a long lasting perennial and, once established, does not require tilling, but only mowing of the grass, which is also needed by the olive orchard.



**Figure 1:** This is how a modern olive orchard could look like.

Mowing the weeds represents both a cost and an ecological toll, like any use of machinery. In olive orchards, animals have been used recently to obtain weed control without machinery, while obtaining a further animal crop. A selective weed control by the animals, leaving the olive trees intact, is obtained using animals that do not eat olive leaves or animals that cannot reach them. As for the asparagus, given that the vegetation is prickly, most animals, like sheep, tend not to eat it. Other animals like geese could be used as well, as they are used for weeding many crops in the USA (Geiger et al., 2006). The animals must be kept out of the orchards during the harvest period, both to avoid damage to the emerging spears as well as for sanitary reasons. Similarly, organic fertilization or phytosanitary compounds should not be administered before and during spear harvest.

One possible problem of the proposed policulture could be the interference of the prickly vegetation during olive harvest. This vegetation could easily get caught in the screens used for olive harvest if these are laid directly to the ground. Removing the asparagus vegetation during olive harvest in the fall is not advisable as this would probably affect asparagus plant vigor by removing the source of photosynthesis (i.e. the evergreen vegetation) for the whole winter and the following spring during harvest, thus depleting plant reserves too much. However, with mechanical harvesting as trunk shakers equipped with an umbrella or with over-the-row harvesters used for the super high density orchards, this should not be a problem. Other systems to prevent such problems could be probably developed.

#### 4. Conclusions

The wild asparagus (*Asparagus acutifolius*), for its ecological needs and its frugality appears suitable as an understory crop in the olive orchards, with the potential to provide additional income on the same land, while increasing diversity in the field and with no negative effects on the olive yield. Although management of the asparagus-olive policulture, as well as the assessment of the market

potential, must be carefully evaluated, this agroforestry system could allow an increase of income and sustainability of olive orchards, especially in marginal areas. This agricultural model, together with others that could be developed, could contribute to maintaining the olive landscape, which is both historically and economically important in many areas of Europe and the world.

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## **Thème 5**

**Bonnes pratiques de fabrication de l'huile d'olive et d'élaboration  
des olives de table**

## Essais de désamérisation biologique des olives à l'échelle industrielle

I. Janati Idrissi & M. Rahmani

### Résumé

Des essais de fermentation des olives vertes désamérisées par trois souches sélectionnées de *Lactobacillus plantarum*, résistantes à l'oleuropéine (F<sub>1</sub>, H<sub>24</sub> et L<sub>15</sub>), et par voie chimique ont été menés au sein d'une unité industrielle de production des olives de table à Fès/ Maroc. Les analyses physico-chimiques et microbiologiques des saumures montrent que les olives désamérisées chimiquement, fermentent (pH=4,0) au bout de 60 jours. Cependant, pour les olives désamérisées par voie biologique, le pH atteint des valeurs de 4,0 au bout de 40 jours de saumurage, mais l'amertume n'a disparu qu'au bout de 120 jours. Par ailleurs, l'évaluation organoleptique des olives fermentées a montré une meilleure qualité sensorielle des olives désamérisées par voie biologique.

**Mots clés :** Olives vertes, fermentation lactique, oleuropéine, *Lactobacillus plantarum*, désamérisation.

## Trials on biological debittering of olives at an industrial scale

### Abstract

Trials on debittering of green olives were conducted by three strains of *Lactobacillus plantarum*, resistant to oleuropein (F<sub>1</sub>, H<sub>24</sub> & L<sub>15</sub>), and by chemical means in an industrial unit specializing in table olives, at Fes/ Morocco. Physico-chemical and microbiological analyses carried on the olive brines showed that olives debittered chemically completed fermentation (to pH 4.0) after 60 days. However, for the biological debittering process, fermentation was shortened to 40 days but the bitterness disappeared only after 120 days. Organoleptic evaluation of fermented olives showed better sensorial characteristics in olives debittered with the biological process.

**Key words:** Green olives, lactic fermentation, oleuropein, *Lactobacillus plantarum*, debittering.

### 1. Introduction

Les olives de table vertes « style espagnol » constituent une part importante dans la production mondiale en olives de table. Ce procédé consiste en un traitement alcalin des olives vertes, afin de dégrader l'oleuropéine qui est un composé phénolique responsable de leur amertume (Shasha et Leibowitz, 1961), pour donner de nouveaux composés phénoliques non amers (le dihydroxy-phényl éthanol et l'acide élénolique) (Figure 1). Cette opération est suivie de phases de rinçage et de saumurage (Fernandez Diez, 1971). Ensuite, les olives sont fermentées par les bactéries lactiques, en particulier les *Lactobacillus plantarum*. La désamérisation chimique des olives engendre des rejets alcalins très polluants et contribue à l'altération de la qualité nutritionnelle et sensorielle du produit. Ainsi, l'introduction d'une désamérisation biologique des olives de table, comme alternative au procédé classique, s'avère nécessaire en vue de produire des olives « Bio » tout en respectant l'environnement.

En effet, de nombreux travaux (Ciafardini et al., 1994 ; Marsilio et al., 1996, 1999 ; Marsilio et Lanza, 1998) ont été réalisés dans le but d'étudier la possibilité d'hydrolyser l'oleuropéine par voie biologique par des souches de *Lactobacillus plantarum*, isolées partir des saumures en pleine fermentation. Les résultats de ces études ont montré que les souches testées hydrolysent l'oleuropéine *in vitro* par l'action de la  $\beta$ -glucosidase dans une première étape pour donner l'oleuropéine aglycone, puis par une estérase pour donner l'hydroxytyrosol et l'acide élénolique, composés non amers (Fig. 1).

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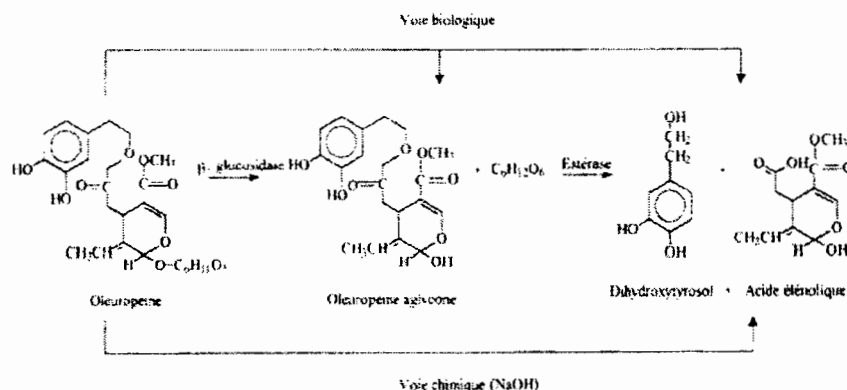


**Figure 1:** Voies de dégradation de l'oleuropéine, d'après Marsilio et Lanza (1989) .

La désamérisation biologique des olives a été testée à un stade expérimental et n'a pas été entamée à un échelon industriel. Aussi, le présent travail a pour but de sélectionner des souches de *Lactobacillus plantarum* ayant une forte activité oleuropéinolytique et d'expérimenter, à échelle industrielle, la désamérisation biologique des olives.

## 2. Matériel et méthodes

### 2.1. Sélection des souches de bactéries lactiques résistantes à l'oleuropéine

Des souches pures de bactéries lactiques ont été isolées sur un milieu MRS Agar à partir de saumures industrielles, du laboratoire, des feuilles d'olivier « *Picholine marocaine* » des margines et des grignons. Ces souches ont été identifiées comme étant des bacilles Gram + non sporulés.

Les souches isolées ont été inoculées sur un milieu MRS Agar contenant 1% (m/v) d'oleuropéine (Extrasynthèse, Genay, France). Les boîtes de Pétri inoculées ont été incubées pendant trois jours à 30°C. Le milieu MRS Agar à composition normale (sans oleuropéine) a été ensemencé et incubé dans les mêmes conditions pour servir comme témoin.

### 2.2. Sélection des souches à forte activité oleuropéinolytique

Les souches résistantes à l'oleuropéine ont été inoculées dans les tubes contenant 9 ml de MRS liquide additionné d'un ml d'une solution d'oleuropéine à 1% (m/v). Ensuite, les tubes ont été incubés à 30°C pendant 96 heures. Après 48 heures d'incubation et à la fin de celle-ci, 2 ml d'échantillon de chaque tube ont été prélevés et centrifugés à 12.000 x g pendant 10 minutes. Le surnageant a été extrait avec 3 ml d'un mélange chloroforme-méthanol (2/1, v/v). Les extraits ont été agités à l'aide d'un vortex pendant 1 minute et centrifugés à 12.000 x g pendant 10 minutes. Le surnageant a été extrait avec 3 ml d'un mélange chloroforme-méthanol (2/1, v/v). Les extraits ont été agités à l'aide d'un vortex pendant 1 minute et centrifugés à 3.500 x g pendant 5 minutes. Le surnageant a été évaporé à sec et le résidu a été récupéré dans l'acétonitrile (Ciafardini et al. 1994). Le même substrat a été utilisé pour l'étude de l'activité estérasique.

L'oleuropéine résiduelle a été dosée par CLHP suivant la méthode de Brenes et al. (1998), en utilisant une colonne sphérisorb 5 ODS (250 x 4,6 mm) et un détecteur UV, réglé à 280 nm avec un gradient eau-acétonitrile (ajusté à pH 2,3 avec l'acide phosphorique). Ce gradient est composé de deux stades ; le premier allant de 95% à 75% en eau pendant 20 minutes et le deuxième allant de 75% à 50% en eau pendant 10 minutes. Le débit a été réglé à 1 ml/min.

### 2.3. Identification des bactéries oleuropéinolytiques

Les bactéries oleuropéinolytiques ont été identifiées par un ensemble de tests physiologiques et biochimiques (catalase, acidification, tests d'incubation à 30°C et 45°C, production de gaz ; la fermentation des sucres a été évaluée par les galeries API-50CHL).

## 2.4. Etude de l'hydrolyse de l'oleuropéine

### 2.4.1. Activité $\beta$ -glucosidase

L'activité  $\beta$ -glucosidase a été étudiée en utilisant comme substrat le 5-bromo-4-chloro-3-indoly- $\beta$ -D-glucopyranoside (X-gluc) (Sigma Chemical Co., Saint-Louis, Mo.). Dans les boîtes de Pétri contenant le MRS solide modifié, on a ajouté 0,2 ml d'une solution N,N diméthyl formamide contenant 0,3 % de X-Gluc (m/v). Les boîtes de Pétri ont été ensuite séchées pendant 1 heure à l'obscurité. Ces boîtes ont été inoculées par les colonies bactériennes et incubées à 30°C pendant 48 heures. Les colonies produisant la  $\beta$ -glucosidase se colorent en bleu (Ciafardini et al., 1994).

### 2.4.2. Activité estérase

Le substrat est le même que celui utilisé pour la sélection des souches oleuropéinolytiques.

Le surnageant a été évaporé à sec sous un courant d'azote puis converti en dérivés triméthylsilyls après un chauffage à reflux à 40°C pendant 30 minutes en présence de l'agent de silylation composé du mélange pyridine-hexaméthylidisilazane-tri-méthylchlorosilane (2 : 1 : 1, v/v) (Marsilio et al., 1996).

Le CG-SM a été réalisée avec un chromatographe Carlo Erba GC 8000, équipé d'un détecteur sélectif de masse tisons Trio 1000. La colonne utilisée est une colonne capillaire DB5 de 30 m de longueur et de 0,32 mm de diamètre. Le gaz vecteur est l'hélium (20 Kpa). La programmation de la température et la suivante : de 50°C à 150°C à 5°C/min et de 150°C à 300°C à 10°C/min. La température de l'injecteur est de 200°C ; la quantité injectée est de 1  $\mu$ l.

Le spectre de masse de l'hydroxytyrosol a montré un pic moléculaire [M+] 370 (36) et un pic de base 267 (100).

### 2.4.3. Conduite de l'essai de fermentation

L'essai de fermentation a été mené au sein d'une unité industrielle de production d'olives de table à Fès. Les olives utilisées pour cet essai proviennent d'un même lot, de la variété «*Picholine marocaine*». Six fûts en plastique d'une capacité de 140 kg d'olive, lavés et désinfectés, ont été remplis d'olives de calibre 260/290 puis de saumure fraîche à 10° Bé. Ces fûts ont été ensuite inoculés par la biomasse déjà préparée à partir des trois souches sélectionnées. Pour chaque souche, deux fûts ont été inoculés, à raison de 10<sup>7</sup> UFC/ml de saumure. Pour l'essai témoin, deux fûts lavés et désinfectés ont été remplis d'olives du même lot ayant subi une désamérisation à la soude à 3 Bé pendant 8 heures, suivie de deux lavages, le premier ayant duré 3 heures et le deuxième 6 heures, et un saumurage à 10° Bé.

### 2.4.4. Analyses physico-chimiques

Il s'agit des déterminations de pH, de l'acidité libre, et des teneurs en NaCl et en sucres réducteurs.

### 2.4.5. Analyses microbiologiques:

Il s'agit de dénombrements de bactéries lactiques, de levures et de la flore mésophile aérobie totale (FMAT).

### 2.4.6. Evaluation organoleptique des olives de table

Une note globale variant de 1 à 9 a été attribuée pour la description des différents attributs sensoriels (odeur, saveur, texture, apparence externe).

## 3. Résultats et discussion

Les souches F<sub>1</sub>, H<sub>24</sub> et L<sub>15</sub> ont été sélectionnées pour leur activité oleuropéinolytique prononcée (>90%) et ont été isolées respectivement à partir des feuilles d'olivier, de saumures d'olives tournantes et de saumures d'olives vertes. Les résultats des tests d'identification ont montré qu'il s'agit de souches de *Lactobacillus plantarum*.

### 3.1. Hydrolyse de l'oleuropéine

Le test d'incubation en présence de X-Gluc a montré que les souches étudiées ont une activité  $\beta$ -glucosidasique. En effet, selon Marsilio et al. (1988), le premier composé intermédiaire de la dégradation enzymatique de l'oleuropéine est l'oleuropéine aglycone, grâce à l'action de la  $\beta$ -glucosidase.

L'analyse chromatographique des dérivés triméthylsilyls des extraits du MRS liquide de l'oleuropéine, inoculé par les souches de bactéries lactiques sélectionnées, montre que le produit d'hydrolyse de l'oleuropéine est l'hydroxytryrosol, identifié par son spectre de masse, tandis que l'acide élénolique n'a pas été identifié. Ces résultats sont en accord avec ceux trouvés par Marsilio et al. (1996) qui suggèrent que ces produits d'hydrolyse de l'oleuropéine sont obtenus sous l'action d'une activité estérasique.

### 3.2. Suivi des paramètres physico-chimiques au cours de la fermentation:

Le suivi des paramètres physico-chimiques tout au long de la fermentation nous a permis de conclure au bon déroulement de ce processus pour les essais non désamérisés, saumurés à 10° Bé, puis inoculés avec les souches F<sub>1</sub>, L<sub>15</sub> et H<sub>24</sub> ainsi que pour l'essai témoin désamérisé par une solution de soude à 3° Bé, puis saumuré à 10° Bé.

En effet, le pH a montré une diminution remarquable (de 7,0 à 5,0) pour les essais inoculés et ce, dès le troisième jour après saumurage. Les valeurs de pH atteintes à la fin de la fermentation étaient de l'ordre de 4, au bout de 41 jours de fermentation (Fig. 2).

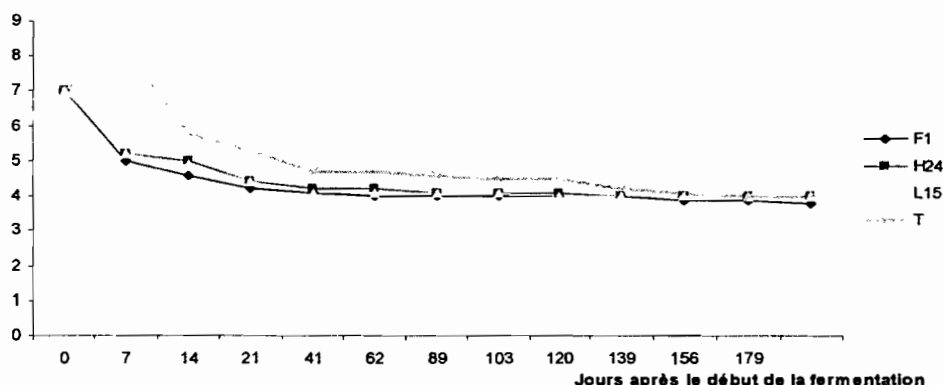


Figure 2: Evolution du pH dans les saumures de fermentation.

Concernant l'acidité libre (exprimée en grammes d'acide lactique pour 100 ml de saumure), les valeurs obtenues à la fin de la fermentation sont de l'ordre de 0,8 % pour tous les essais. Ces valeurs témoignent des bonnes conditions de fermentation, en particulier la température et l'anaérobiose. Concernant l'essai témoin, les valeurs de l'acidité au cours de la première semaine restent faibles par rapport à celle dans les olives non désamérisées. Ces valeurs sont corrélées avec celles du pH lors de la même période.

La teneur en NaCl a baissé sous l'effet des échanges diffusifs. En effet, par gradient de concentration, le sel passe de la saumure vers la pulpe des olives jusqu'à l'établissement de l'équilibre à des valeurs de 7° Bé pour les essais inoculés et 6° Bé pour l'essai témoin. Cette différence est due probablement au passage plus facile du sel de la saumure vers les olives à travers la paroi qui est attendrie par le traitement alcalin.

Les concentrations des sucres réducteurs dans les saumures ont atteint leur maximum à 0,7% ; 0,8% et 0,4%, respectivement pour les essais inoculés et l'essai témoin. Tout au long de la fermentation, ces concentrations étaient plus élevées dans les saumures correspondant aux essais inoculés, ce qui peut être expliqué par la perte des sucres réducteurs causée par le traitement alcalin et le lavage dans l'essai témoin. Cette perte peut atteindre 50% environ de la teneur initiale de ces composés dans l'olive (Marsilio et al., 1998).



### 3.3. Analyses microbiologiques

Les résultats du dénombrement des micro-organismes ont mis en évidence le développement d'une flore importante, surtout pendant les premières semaines de fermentation. Pendant cette période, on a noté un nombre important de bactéries lactiques ( $10,5 \cdot 10^6$  UFC/ml pour les essais biologiques et  $10^6$  UFC/ml pour l'essai inoculé par la souche L15 ; contre  $2,6 \cdot 10^6$  UFC/ml pour l'essai témoin).

On dénote ensuite une diminution des bactéries dont la population se maintient à un niveau plus au moins stable jusqu'à la fin de la fermentation ( $3,2 \cdot 10^5$  UFC/ml pour les essais biologiques et  $6,5 \cdot 10^5$  UFC/ml pour l'essai témoin).

On remarque aussi une diminution de la FMAT (de l'ordre  $5 \cdot 10^2$  UFC/ml pour tous les essais) et des levures (de l'ordre de  $5 \cdot 10^3$  UFC/ml pour les essais, qui est due à l'acidification du milieu).

### 3.4. Evaluation organoleptique des olives biologiques

Les résultats de l'évaluation organoleptique des olives biologiques nous ont permis de classer le produit dans la catégorie "très bonne".

### 3.5. Conclusion

A la lumière des résultats obtenus au terme de la présente étude, on peut confirmer que les olives fermentées par voie biologique présentent toutes les qualités pour qu'elles soient commercialisées. Dans le but de faciliter l'introduction de ce nouveau procédé à une échelle industrielle. On se propose de développer les cultures « starter » et d'optimiser les conditions de fermentation (teneur en NaCl de la saumure, température, teneur des substances fermentescibles, anaérobiose et la concentration de la charge bactérienne du "starter").

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## Infection par des bactériophages au cours de la fermentation de l'olive de table

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### Résumé

Une infection de phages pendant la transformation des olives de table fermentées pourrait se traduire dans une substantielle diminution et, dans certains cas, même dans une cessation totale de la production d'acide lactique par les ferments lactiques. Donc la présence éventuelle de phages a été recherchée pendant la fermentation des olives. Les olives ont été traitées comme des olives de table verte selon le style traditionnel grec, et divisé en neuf lots. À quatre saumures on a ajouté, comme cultures de demurrage, souches sélectionnées de *Lactobacillus plantarum*; les cinq autres saumures ont été laissés à fermenter spontanément. Après une semaine on a observé l'inhibition de croissance des souches inoculées, nous amenant à soupçonner une éventuelle attaque de phages. Pour vérifier cette hypothèse on a effectué un test de turbidité. La présence de phage a été démontrée par l'inhibition de la croissance de souches testées cultivées en présence d'une aliquote de la saumure privée de cellules microbiennes par filtration stérilisante. Les phages ont également été isolés à partir de saumures fermentées naturellement. La diminution de la turbidité du milieu a indiqué la présence présumée de phages capables de lyser les cellules bactériennes sensibles. La présence de bactériophages a été confirmée par microscopie électronique (SEM). La présence démontrée de phages lytiques active contre *Lactobacillus plantarum* pourrait représenter un sérieux obstacle à l'obtention d'un régulier processus d'acidification. Rechercher des souches résistantes aux phages pourrait aider à surmonter ce problème

### Phage infection during fermentation of table olives

### Abstract

A phage infection in fermented table olive processing results in a serious decrease and, in some cases, even a total failure in the production of lactic acid by the starter cultures. Olives were processed as green table olives according to the traditional Greek-style, with the addition of *Lactobacillus plantarum* B1 as starter culture. After one week, growth inhibition of inoculated strain was observed, leading us to suspect a possible phage attack. To verify this hypothesis, a turbidity test was applied. Phage presence was demonstrated by the growth inhibition of tested strains grown in the presence of an aliquot of the brine, deprived of microbial cells by sterilizing filtration. The decrease in the turbidity of the medium indicated the presumptive presence of phage particles able to lyse sensitive bacterial cells. The presence of bacteriophages was confirmed by scanning electron microscopy. The demonstrated presence of lytic phages active against *L. plantarum* could represent a serious obstacle on the regular acidification process. Search for phage resistant strains could help to overcome this problem.

**Key-words:** table olives, *Lactobacillus plantarum*, phage infection, fermentation, lactic acid bacteria.

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## **Extraction de l'huile d'olive vierge par le système de centrifugation continu à deux phases : influence de différentes variables du processus, sur certains paramètres de qualité de l'huile**

Mohamed Ayadi, Meriem Gharssellaoui, Taieb Hamdi

### **Résumé**

Dans le but de préserver à l'huile d'olive ses meilleures caractéristiques physico-chimiques et organoleptiques, de nouveaux procédés sont apparus, dont notamment la chaîne continue à deux phases.

L'extraction de l'huile par la chaîne continue intervient par effet de la force centrifuge qui accentue la différence entre les poids spécifiques des liquides non miscibles et des matières solides et permet donc la séparation continue et simultanée de l'huile, de l'eau (margines) et des grignons.

L'évolution et le progrès technologique, qu'ont connu les procédés d'extraction de l'huile d'olive, a fait que les systèmes utilisant la centrifugation de pâte ont pris de la place au dépend des autres. Ce pendant, leur utilisation est loin maîtrisée.

Dans le cadre de rationalisation du système continu (chaîne continue à 2 phases) nous avons voulu connaître l'effet de la température, du temps de malaxage, et du débit de la pâte injecté au décanteur sur les caractéristiques physico-chimiques de l'huile.

Le contenu en polyphénols, chlorophylle, carotène et la stabilité étaient en relation avec les variations de température, le temps de malaxage et le débit de la pâte.

**Mots clés :** parameters de qualité

### **Assessment of a two-phase extraction system on olive oil quality parameters**

#### **Abstract**

The new methods for olive oil extraction use industrial decanters to separate all the phases by centrifugation. The efficiency of centrifugation is achieved thanks to the differences between specific weights of non miscible liquids and solid materials. Thus, it permits simultaneous and continuous separation of oil, water (olive mill wastewater) and husk. Several technological advances were realized in order to obtain better olive oil characteristics such as physical, chemical and organoleptical traits. Among these novel progresses, appeared the two-phase oil decanter. Yet, satisfactory industrial and technical management of this apparatus is not reached. The aim of this work is the assessment of the effects of the temperature and the duration of mixing and paste injection flow in the decanter on olive oil quality parameters. Several correlations were found between the polyphenol, chlorophyll and carotene contents as well as the oil stability and the studied parameters of the extraction process.

**Key words:** quality parameters

## Phenolic and volatile compounds from oils of three olive cultivars introduced in the north of Tunisia

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### Abstract

The behaviour of three European olive varieties, AscolanaTenera, Koroneiki and Picholine, cultivated in the north of Tunisia, was studied of the quality indices and the minor compounds of their oils.  $\alpha$ -Tocopherol showed high values varied from 337 for Ascolana to 424 mg/Kg for Koroneiki whereas total phenols showed the highest value for Picholine oils. Significant differences were observed for the aroma composition between the oils from the foreign cultivars. The major volatile component was the C-6 aldehyde fraction whose content varied greatly between the different varieties studied: The *E*-2-hexenal content ranged from 1.6 mg/kg of oil in the AscolanaTenera variety to >5 mg/kg for the Picholine and Koroneiki cultivars. Therefore, results showed good adaptation for Koroneiki and Picholine to the Tunisian cultivation conditions.

**Keywords:** Aroma profile / Introduced European cultivars / Olive oil / Phenolic compounds.

### 1. Introduction

Oleiculture has great economic and social importance in the countries of the Mediterranean region. While Tunisia is a relatively major producer country after the European Union and it is ranked forth. However, the predominated Tunisian cultivar, *Chemlali* variety, is characterized by high levels of palmitic and linoleic acids and sometimes by a low level of oleic acid. A major effort has been made recently for improving the quality of olive oil produced in Tunisia through intervarietal controlled crossings (Rjiba *et al.*, 2009; Dabbou *et al.*, 2006) and the introduction of new cultivars (Dabbou *et al.*, 2009) constitutes means for providing cultivars having a better oil quality. The aim of this work was to study the behavior of three European cultivars introduced in the north of Tunisia.

### 2. Materials and methods

**Plant material and growing areas selected.** Olive fruits (*Olea europaea* L.) of the *Ascolana Tenera* (Italy), *Koroneiki* (Greece) and *Picholine* (France) varieties were collected in November 2006 at a known ripeness index from trees all located in the same orchard (a National Collection, 20 km away from Tunis, in North-East Tunisia) and which benefited from the same cultural practices. The experimental field in Béjaoua is characterised by a rainfall of 500 mm/year and a temperature varying from a minimum of 18°C to a maximum of 32°C. Olives were transformed into oil within 24h. Only undamaged and uninfected fruits were processed at laboratory scale system (Abencor analyser). Finally, the oils were decanted and immediately stored in dark bottles until analysis. **Analytical methods.** **Quality parameters** (Free Fatty acids, peroxide value and UV absorption characteristics) were carried out following the analytical methods described in the EUC (1991). **Phenolic fractions** were extracted by liquid-liquid extraction (Montedoro *et al.*, 1992) and analysed by HPLC as reported by Selvaggini *et al.* (2006), using an Agilent Technologies system model 1100 (Agilent Technologies, Palo Alto, CA, USA).  $\alpha$ -tocopherol was measured by the method reported by Psomiadou *et al.* (1998). **Volatile compounds** were determined by headspace solid phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC/MS), as previously reported by Servili *et al.* (2001) using the 65-mm Carbowax/divinylbenzene fibre (Supelco, Bellefonte, PA, USA) and a GC Varian 4000 equipped with a 1078 split/splitless injector coupled with a Varian Saturn 3 mass spectrometer. The volatile compounds were identified as reported by Dabbou *et al.* (2009). **Statistical analysis.** Significant differences among varieties studied were determined by an analysis of variance which applied a Duncan test with a 95% significant level ( $P < 0.05$ ), using the SPSS programme, release 11.0 for windows (SPSS, Chicago, IL, USA). All analyses were carried out in triplicate and the results were presented as means of three repetitions.

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### 3. Results and discussion

**Analytical parameters of olive oils.** Physico-chemical characteristics of virgin olive oils obtained from olives of three varieties were presented in Table 1. All the oils produced and analyzed were completely within the limits established by IOOC (2008) as required for the 'extra virgin' category, except for the *Ascolana Tenera* variety having a K232 value of 2.7 (Table 1). This value can be attributed to the oxidation of the sample during the processing technology, because the raw material was carefully selected, picked and processed. In fact, cultivar or origin area had no significant influence on these analytical parameters, which were basically affected by factors causing damage to the fruits, e.g., olive fly attacks or improper systems of harvesting, transport and storage of olives (Kiritsakis, 1998; Ranalli & Angerosa, 1996; Ben Temine *et al.*, 2006).

**Table 1:** Physico-chemical characteristics of the virgin olive oils from the different European olive cultivars grown in the studied area.

| Characteristics  | Ascolana Tenera            | Koroneiki                | Picholine                |
|------------------|----------------------------|--------------------------|--------------------------|
| Free fatty acids | 0.36±0.06 <sup>a</sup>     | 0.27±0.06 <sup>a</sup>   | 0.26±0.03 <sup>a</sup>   |
| Peroxide value   | 16.78 ± 1.07 <sup>a</sup>  | 7.48 ± 0.7 <sup>b</sup>  | 3.42±0.12 <sup>c</sup>   |
| K232             | 2.78 ± 0.09 <sup>a</sup>   | 2.01 ± 0.17 <sup>b</sup> | 1.59 ± 0.08 <sup>c</sup> |
| K270             | 0.15 ± 0.01 <sup>a,b</sup> | 0.18 ± 0.03 <sup>a</sup> | 0.12 ± 0.03 <sup>b</sup> |

Values are the means of the three different VOO samples (n=3) ± standard deviations. Different letters indicate significant differences (P<0.05) between cultivars.

Phenolic compounds (Table 2) are of fundamental importance in virgin olive oil for their nutritional properties, sensory characteristics, and the shelf life of the product (Morello *et al.*, 2004; Servili *et al.*, 2008). In particular, the phenolic compounds are potent antioxidants, and can confer a marked bitter taste or a sweet taste typical of some virgin olive oil (Artajo *et al.*, 2006; Esti *et al.*, 2009). In addition, they are thought to play an important role in human diets as preventative agents against several diseases (Owen *et al.*, 2000, 2004) and are divided in two groups, hydrophilic (phenols) and lipophilic compounds (tocopherols).

**Table 2:** Phenolic composition (mg kg<sup>-1</sup>) evaluated in the virgin olive oil samples from the different European olive cultivars grown in the studied area.

| lthanol                  | Ascolana Tenera          | Koroneiki               | Picholine               |
|--------------------------|--------------------------|-------------------------|-------------------------|
| □-Tocopherol             | 337.4±1.3 <sup>c</sup>   | 424±1.0 <sup>a</sup>    | 392±0.9 <sup>b</sup>    |
| 3,4-DHPEA                | 1.60±0.9 <sup>b</sup>    | 5.50±0.0 <sup>a</sup>   | 1.10±0.0 <sup>b</sup>   |
| p-HPEA                   | 34.03±0.45 <sup>b</sup>  | 19.93±0.55 <sup>c</sup> | 43.57±0.06 <sup>a</sup> |
| 3,4-DHPEA-EDA            | 54.53±0.15 <sup>c</sup>  | 58.20±0.6 <sup>b</sup>  | 116.20±0.1 <sup>a</sup> |
| p-HPEA-EDA               | 22.90±0.0 <sup>c</sup>   | 37.23±0.85 <sup>b</sup> | 43.80±0.1 <sup>a</sup>  |
| (+)-1-Acetoxypinoresinol | 3.00±0.0 <sup>c</sup>    | 6.57±0.06 <sup>b</sup>  | 9.40±0.0 <sup>a</sup>   |
| (+)-1-Pinoresinol        | 25.70±0.4 <sup>a</sup>   | 9.53±0.06 <sup>b</sup>  | 6.27±0.06 <sup>c</sup>  |
| 3,4-DHPEA-EA             | 33.90±0.1 <sup>c</sup>   | 73.83±1.35 <sup>a</sup> | 54.13±0.06 <sup>b</sup> |
| Total Phenols (HPLC)     | 174.63±0.15 <sup>c</sup> | 210.80±3.5 <sup>b</sup> | 274.50±0.0 <sup>a</sup> |

Values are the means of the three different VOO samples (n=3) ± standard deviations. Different letters indicate significant differences (P<0.05) between cultivars.

**Phenols:** The total phenol concentrations (Table 2), established by HPLC, showed significant differences (p<0.05) between different studied olive oils where the ranges were from 174.63 to 274.5 mg kg<sup>-1</sup>. However, the highest amount of the total phenols was present in *Picholine* olive oil followed by *Koroneiki* oil. These differences were approximately the same for the chromatographic profiles of the different oils. Furthermore, the chromatographic profile showed that the most important phenolic compounds that have been identified in the studied olive oils were phenolic alcohols (hydroxytyrosol, 3,4-DHPEA and tyrosol, p-HPEA), secoiridoid derivatives (the dialdehydic form of elenolic acid linked to tyrosol (p-HPEA-EDA), the dialdehydic form of elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EDA) and oleuropein aglycone (3,4-DHPEA-EA)) and lignans (pinoresinol and acetoxypinoresinol). These results proved previous works (Bendini *et al.*, 2007; Servili *et al.*, 2004; Hrnčirik & Fritsche, 2004).

Table 2 showed that the concentrations of lignans varied inversely (Table 2). In fact, concentration of (+)-1-acetoxypinoresinol ranged from a minimum for *Ascolana Tenera* ( $3 \text{ mg kg}^{-1}$ ) to a maximum for *Picholine* olive oil ( $9 \text{ mg kg}^{-1}$ ). However, the concentration of (+)-1-pinoresinol was the highest for *Picholine* olive oil ( $25 \text{ mg kg}^{-1}$ ) and the lowest for *Ascolana Tenera* ( $6 \text{ mg kg}^{-1}$ ). For the simple phenols, the level of tyrosol ranged from 20 to  $44 \text{ mg kg}^{-1}$ , and was generally higher than that of hydroxytyrosol which ranged from 1 to  $6 \text{ mg kg}^{-1}$  and was found in all samples analyzed. These results were in accordance with those of several authors for olive oil varieties (Hrncirik & Fritsche, 2004). Moreover, a clear variation can also be observed on the basis of the oils' phenol content (Table 2). In particular, extra virgin olive oil having a low degradation level, estimated by quality parameters, had a high content of the secoiridoid derivative 3,4-DHPEA-EDA and low contents of the simple phenolic compounds 3,4-DHPEA and *p*-HPEA, which was the case of *Picholine* olive oil the most abundant in 3,4-DHPEA-EDA and *p*-HPEA-EDA ( $116.2$  and  $43.8 \text{ mg kg}^{-1}$ , respectively). On the other hand, oils having an intermediate or an advanced degradation level showed a lower content of 3,4-DHPEA-EDA and higher contents of 3,4-DHPEA and *p*-HPEA. These data are in agreement with the results of Lavelli (2002). Consequently, the concentration and composition of phenolic compounds were strongly affected by olive cultivar (Tura *et al.*, 2007) since agronomical and technological factors were the same for the varieties studied.

$\alpha$ -tocopherol, the main tocopherol species of virgin olive oil (Table 2) seem to be higher in the studied varieties where contents ranged from  $337.4 \text{ mg kg}^{-1}$  for *Ascolana tenera* to  $424 \text{ mg kg}^{-1}$  for *Koroneiki* olive oils. *Picholine* oil showed a moderate concentration ( $392 \text{ mg kg}^{-1}$ ) but higher than the amount found by Sakouhi *et al.* (2008). In addition, the three olive cultivars, which grown in the same area, differed significantly in  $\alpha$ -tocopherol content. This difference probably linked to genotype characteristic and metabolic behavior of each cultivar (Sakouhi *et al.*, 2008).

*Volatile compounds*, being responsible for most sensory properties of virgin olive oils, play a significant role in the evaluation of the overall oil quality and in the generation of preferences among consumers (Angerosa, 2002) since they are the only parameters that consumers can appraise directly, while other quality features (e.g. chemical composition) are not always labelled on the bottle (Tura *et al.*, 2008). For a better understanding of the aromatic profile of each cultivar, the total content of volatile compounds with chemical affinity or similar biosynthetic pathway was taken in account (Table 3). The total volatile compounds ranged from  $17.2 \text{ mg kg}^{-1}$  (*Ascolana tenera*) to  $23.9 \text{ mg kg}^{-1}$  (*Koroneiki*).

Results of SPME–GC analysis of the oil headspace volatile composition (Table 3) showed a large number of substances (23 compounds) consisting mainly of alcohols, esters, as well as flavour active aldehydes and ketones. In fact, the identified compounds were mainly alcohols (such as 1-hexanol, (Z)-2-hexen-1-ol and (E)-3-hexen-1-ol) which ranged from  $0.1 \text{ mg kg}^{-1}$  to  $12.1 \text{ mg kg}^{-1}$ . Furthermore, the quantities of aldehydes differed according to the cultivar with a wide variability from  $0.1$  to  $5.5 \text{ mg kg}^{-1}$ , including (E)-2-Heptenal and (E,E)-2,4-Hexadienal that are not originated from lipoxygenase pathway (LOX). However, ketones and esters were also present in small amounts. In addition, (Z)-Hexenyl acetate was present in the aroma of *Picholine* and *Ascolana Tenera* oils in low amounts, thus indicating a low presence of alcohol acyl transferase (AAT) whereas it was completely absent in *Koroneiki* oil. Comparing volatile compounds of the three virgin olive oils collected in the same period and planted in the same area, significant differences were observed indicating that olive oil aroma compounds accumulate differently according to the cultivar (Angerosa *et al.*, 1999). Then, *Koroneiki* oil seems to be the richest in alcohols, followed by *Picholine* and *Ascolana Tenera* ( $17.1$ ,  $16.3$  and  $13.2 \text{ mg kg}^{-1}$ ; respectively), whereas *Picholine* was the richest in aldehydes ( $6.3 \text{ mg kg}^{-1}$ ).

The LOX pathway is a biochemical reaction scheme that accounts for most of the aroma fraction of olive oil containing C6 aldehydes, alcohols and esters (Angerosa, 2002). In fact, the amount of compounds produced from the oxidation of linolenic acid (73.7 and 85.7% of the total of C6 compounds for *Ascolana Tenera* and *Koroneiki* varieties, respectively) was greater than the amount of those biogenerated from the oxidation of linoleic acid (Table 3). Thus, hexanal, the main volatile oxidation product derived from the 13-hydroperoxide, which is one of the major hydroperoxides formed by autooxidation of linoleic acid (García-Martínez *et al.*, 2009), was approximately absent in the studied olive oils. In addition to LOX pathway compounds, the aroma of the studied VOO contained reasonable amounts of pentene dimers and C5 compounds.

**Table 3:** Volatile composition (mg kg<sup>-1</sup>) of virgin olive oil, identified by HS-SPME-GC/MS, of the different European olive cultivars grown in the studied area.

| Compounds                             | Ascolana Tenera         | Koroneiki               | Picholine               |
|---------------------------------------|-------------------------|-------------------------|-------------------------|
| 6-Methyl-5-hepten-2-one               | tr <sup>b</sup>         | tr <sup>b</sup>         | tr <sup>a</sup>         |
| (E)-2-Heptenal                        | tr <sup>a</sup>         | nd <sup>b</sup>         | 0.1 ± 0.1 <sup>a</sup>  |
| (E,E)-2,4-Hexadienal                  | 0.1 ± 0.1 <sup>c</sup>  | 0.1 ± 0.1 <sup>b</sup>  | 0.2 ± 0.1 <sup>a</sup>  |
| (E,E)-2,4-Hexadienal                  | 0.2 ± 0.1 <sup>b</sup>  | 0.2 ± 0.1 <sup>b</sup>  | 0.4 ± 0.1 <sup>a</sup>  |
| 2-Methyl-1-propanol                   | 0.5 ± 0.1 <sup>a</sup>  | 0.1 ± 0.1 <sup>c</sup>  | 0.3 ± 0.1 <sup>b</sup>  |
| 2-Methyl-1-butanol/3-Methyl-1-butanol | 0.1 ± 0.1 <sup>a</sup>  | 0.1 ± 0.1 <sup>b</sup>  | 0.1 ± 0.1 <sup>c</sup>  |
| Benzyl alcohol                        | 0.1 ± 0.1 <sup>b</sup>  | 0.1 ± 0.1 <sup>a</sup>  | 0.1 ± 0.1 <sup>b</sup>  |
| Phenylethyl alcohol                   | 0.4 ± 0.1 <sup>a</sup>  | 0.4 ± 0.1 <sup>a</sup>  | 0.4 ± 0.1 <sup>a</sup>  |
| Phenol                                | tr <sup>a</sup>         | tr <sup>a,b</sup>       | tr <sup>b</sup>         |
| 3-Pentanone                           | 0.6 ± 0.1 <sup>c</sup>  | 1.2 ± 0.1 <sup>a</sup>  | 0.7 ± 0.1 <sup>b</sup>  |
| 1-Penten-3-one                        | nd <sup>b</sup>         | nd <sup>b</sup>         | nd <sup>a</sup>         |
| (E)-2-Pentenal                        | nd <sup>b</sup>         | nd <sup>a</sup>         | 0.1 ± 0.1 <sup>a</sup>  |
| 1-Penten-3-ol                         | 0.2 ± 0.1 <sup>a</sup>  | 0.3 ± 0.1 <sup>b</sup>  | 0.3 ± 0.1 <sup>b</sup>  |
| 1-Pentanol                            | 0.1 ± 0.1 <sup>c</sup>  | 0.1 ± 0.1 <sup>a</sup>  | 0.1 ± 0.1 <sup>b</sup>  |
| ΣC5 compounds                         | 1.0 ± 0.1 <sup>a</sup>  | 1.6 ± 0.1 <sup>c</sup>  | 1.2 ± 0.1 <sup>b</sup>  |
| Ethyl acetate                         | 0.8 ± 0.1 <sup>b</sup>  | nd <sup>c</sup>         | 0.1 ± 0.1 <sup>a</sup>  |
| Hexanal                               | tr <sup>b</sup>         | tr <sup>c</sup>         | tr <sup>a</sup>         |
| 1-Hexanol                             | 3.4 ± 0.1 <sup>a</sup>  | 3.0 ± 0.1 <sup>b</sup>  | 4.4 ± 0.1 <sup>b</sup>  |
| Hexyl acetate                         | 0.2 ± 0.1 <sup>b</sup>  | nd <sup>c</sup>         | nd <sup>a</sup>         |
| ΣC6 compounds from LA                 | 4.4 ± 0.1 <sup>b</sup>  | 3.0 ± 0.1 <sup>c</sup>  | 4.6 ± 0.1 <sup>a</sup>  |
| (E)-2-Hexenal                         | 1.6 ± 0.1 <sup>c</sup>  | 5.3 ± 0.1 <sup>b</sup>  | 5.5 ± 0.1 <sup>a</sup>  |
| (E)-2-Hexen-1-ol                      | 3.7 ± 0.1 <sup>c</sup>  | 12.1 ± 0.1 <sup>a</sup> | 9.3 ± 0.1 <sup>b</sup>  |
| (E)-3-Hexen-1-ol                      | 0.2 ± 0.1 <sup>a</sup>  | 0.1 ± 0.1 <sup>c</sup>  | 0.1 ± 0.1 <sup>b</sup>  |
| (Z)-3-Hexen-1-ol                      | 4.5 ± 0.1 <sup>a</sup>  | 0.8 ± 0.1 <sup>c</sup>  | 1.2 ± 0.1 <sup>b</sup>  |
| (Z)-3-Hexenyl-acetate                 | 0.3 ± 0.1 <sup>a</sup>  | nd <sup>c</sup>         | tr <sup>b</sup>         |
| ΣC6 compounds from LnA                | 10.3 ± 0.1 <sup>c</sup> | 18.2 ± 0.2 <sup>a</sup> | 16.2 ± 0.1 <sup>b</sup> |
| Total LOX products                    | 14.7 ± 0.2 <sup>c</sup> | 21.2 ± 0.2 <sup>a</sup> | 20.8 ± 0.1 <sup>b</sup> |
| Total esters                          | 1.3 ± 0.1 <sup>a</sup>  | -                       | 0.1 ± 0.1 <sup>b</sup>  |
| Total Ketones                         | 0.6 ± 0.1 <sup>c</sup>  | 1.2 ± 0.1 <sup>a</sup>  | 0.7 ± 0.1 <sup>b</sup>  |
| Total aldehydes                       | 1.9 ± 0.1 <sup>c</sup>  | 5.6 ± 0.1 <sup>b</sup>  | 6.3 ± 0.1 <sup>a</sup>  |
| Total alcohols                        | 13.2 ± 0.1 <sup>c</sup> | 17.1 ± 0.1 <sup>a</sup> | 16.3 ± 0.1 <sup>b</sup> |
| Total volatile compounds              | 17.2 ± 0.1 <sup>c</sup> | 23.9 ± 0.2 <sup>a</sup> | 23.6 ± 0.1 <sup>b</sup> |

Values are the means of the three different VOO samples (n=3) ± standard deviations. Different letters indicate significant differences (P<0.05) between cultivars.

The detection of these compounds indicated the existence of an additional branch of the LOX pathway leading to the production of C5 compounds through the alkoxy radical. Consequently, the production of C6 and C5 compounds through the enzymatic oxidation of linolenic and linoleic acids is affected by the cultivar since the degree of ripeness of fruits and their processing conditions were the same (Angerosa *et al.*, 1999).

#### 4. Conclusion

According to results of volatile compounds, phenols and α-tocopherol, we can conclude that virgin olive oils produced from three European cultivars introduced in Tunisia contain a higher number of volatiles. Also, the total phenol content was higher. Furthermore, from the nutritional aspect based on measurements of total antioxidant and volatiles, virgin olive oils produced are to be preferred due to their abundance in aldehydes. In contrast to volatiles and phenols, tocopherol content of these oils appeared to be very consistent between the different olive varieties. These introduced European olive cultivars could be used for blending their oils with autochthonous olive oils, or used for crossings. Finally, the geographic area appears to have a significant effect on the qualitative characteristics and the chemical composition of olive oil.



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## **Chemical composition of Arbequina, Chemlali, Chetoui virgin olive oil in relation to extraction system; storage conditions and preferences of consumers**

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### **Abstract**

Virgin olive oil is characterized by particular aroma, taste and color that distinguish it from other vegetable oils. Virgin olive oil consumption is currently increasing thanks to its excellent organoleptic and nutritive qualities. Our work was carried out to assess the effect of cultivar, extraction method and the packaging on olive oil. Three olive varieties cultivated in different regions on Tunisia have been used: Chetoui (south), Chemlali (centre), and Arbequina the Spanish variety introduced in the south.

Each variety was processed by three extraction systems: two-phase centrifugation, three-phase centrifugation and press system and stored in four types of packaging (opaque glass, transparent glass, metal and polyethylene terephthalate PET) during six months. Analysis revealed significant statistical differences in some parameters mainly in phenol contents, oxidative stability, chlorophyll and carotenoid compound. Most of the quality indices showed significant variations among olive varieties. The study showed that the best packaging which preserves the quality of olive oil is opaque glass. Finally, the sensorial analysis demonstrates that oils extracted by centrifugation system are more preferred by consumers than oils extracted by pressure system.

**Key words:** olive oil, packaging, sensorial analysis.

## **Composition chimique d'huile d'olive vierge d'Arbequina, Chemlali, Chetoui en relation avec le système d'extraction, les conditions de stockage et les préférences des consommateurs**

### **Résumé**

L'huile d'olive est très appréciée pour sa flaveur caractéristique et sa valeur biologique et nutritionnelle. Ces caractéristiques sont fortement liées à la qualité qui, elle-même, est influencée par plusieurs paramètres tel que la maturité des olives, la variété, les techniques culturales, les modes d'extraction...

Parmi les facteurs, nous nous sommes intéressés dans le présent travail à l'étude de l'impact de la variété, du système d'extraction et du matériau d'emballage utilisé pour la conservation sur la qualité finale de l'huile d'olive. Les huiles utilisées pour cette étude appartiennent à trois variétés cultivées dans différentes régions : chetoui du Nord, chemlali du Centre et arbequina du Nord ; chaque variété a été triturée par deux systèmes d'extraction : le système par centrifugation (deux phases, trois phase) le système par presse. Chaque huile a été conditionnée dans quatre types d'emballage différents : le PET, le verre opaque, le verre transparent et le métal et conservée pendant deux mois.

Les résultats trouvés ont permis de mettre en évidence que les principaux critères de qualité de l'huile d'olive tels que l'acidité, la teneur en carotène et en chlorophylle, la teneur en polyphénols totaux ainsi que la stabilité oxydative sont fortement influencés par le système d'extraction et la variété.

D'autres paramètres comme l'extinction spécifique à 232 nm et 270 nm ainsi que l'indice de peroxyde ne présentent de différence significative que pour l'effet variétal.

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L'influence des interactions entre ces deux paramètres se trouve significative pour l'acidité, l'IP et les phénols totaux.

Concernant la nature de l'emballage, elle n'est pas significative sur l'acidité et l'IP, par contre elle présente une différence significative pour le K232 et le K270.

Ainsi, l'interaction entre système d'extraction-contenant, variété-contenant et terme-contenant n'ont aucun effet significatif sur les critères de qualité.

De même, l'analyse sensorielle a montré qu'il y a une différence significative entre les systèmes d'extraction et les attributs positifs de l'huile ainsi qu'entre le système d'extraction et les notes de préférence des consommateurs.

**Mots clés :** huile d'olive, emballage, stabilité oxydative, évaluation sensorielle.

## 1. Introduction

Olive Oil Extra Virgin (HOVE) is - undoubtedly - the more natural it is a good protector and controller of the balance of our health. Its properties beneficial to health and nutrition than justify its price. It represents one of the fat the most popular Mediterranean populations. It is well known for its balanced composition of fatty acids and especially for its high oleic acid composition, in natural antioxidants such as polyphenols and tocopherols as well as for its delicious flavor. The quality of olive oil varies not only depending on the variety, soil and climatic conditions but also many factors in the production cycle, the extraction process and conservation and the material of packaging used. However, like other vegetable oils, virgin olive oils undergo oxidation during storage and therefore it is necessary to limit degradation by the good preservation and good choice of container packaging (Grati Kamoun, 2006). Thus, the objective of this work is to study, initially, the influence of the variety of retrieval system (centrifuge and pressure) and certain storage conditions, specifically the type of packaging (transparent glass, opaque glass, metal and polyethylene tetrathalate (PET)) on the final quality of olive oil. In a second step, we propose to study the preferences of the oil through a consumer panel.

## 2. Materials and methods

### 2.1. Oil extraction and storage conditions

The study focused on different oils from three varieties which were obtained by two extraction systems: system by centrifugation (two and three phases) and pressure system. Oils were packaged in transparent glass of 90 ml, opaque glass bottles of 90 ml and metal cans of 160 ml and polyethylene tetraphthalate (PET) bottles of 90 ml. Then the bottles were completely filled to avoid the air space and were stored at room temperature for two months. Monitoring of quality criteria has been performed on these samples every 15 days.

### 2.2. Oil analyses

#### 2.2.1. Analytical parameters

Free fatty acid (FFA), peroxide value (PV) and Ultra Violet absorption were determined according to the European Communities official methods (EEC Reg. 2568, 1991).

#### 2.2.2. Fatty acid composition

Fatty acid composition was carried out according to the COI norms (COI/T.20/Document: 24 (2001)). Fatty acid methyl esters (FAMES) were prepared by dissolving 0.1 g of olive oil in 2 mL of heptanes and a methanolic solution of KOH (0.2 N). FAMES were separated and quantified by gas chromatography using a model 5890 series II instrument (Hewlett-Packard Co, Palo Alto, CA, USA) equipped with a flame ionisation detector, and a fused silica capillary column HP – INNOWAX (30 m length- 0.25 mm i.d. and 0.25  $\mu$ m of film thickness). Iodine values were calculated from FA percentages using the formula elaborated by Torres & Maestri, (2006). Oxidative susceptibility was calculated from FA composition according to the formula established by Cert et al. (1999).

### 2.2.3. Total phenol content

Total phenols were extracted following the method proposed by Gutfinger (1981). Olive oil (2.5 g) was put into a test tube with 5 mL hexane and 5 mL methanol/water (60:40/v/v) added. Aliquot from the methanol/water layer were added to Folin Ciocalteu reagent and 1 mL sodium carbonate. The phenol content was determined spectrophotometrically at 725 nm and the concentration was expressed as mg of gallic acid per kg of virgin oil.

### 2.2.4. Pigments content

Beta-carotenes and chlorophylls (mg/ kg) were determined at 470 and 670 nm, respectively, in cyclohexane using the specific extinction values, according to the method of Minguez-Mosquera et al. (1991).

### 2.2.5. Evaluation of oxidative stability

The oxidative index was determined by the Rancimat Model 743 (Metrohm, Switzerland). Three grams of the sample were introduced in the test tube and heated at 120 °C, and air was bubbled through the oil at a flow rate of 20 L h<sup>-1</sup>.

### 2.2.6. Sensory analysis

The sensory evaluation was performed by two different tests: the analytical test which determine the sensory characteristics of the oil and ranking it according to its sensory profile; this analysis is performed by an expert jury corresponding a group of subjects selected according to the guidelines of international standards. Panel members are trained to recognize the different flavor characteristics and measuring their intensities using the standard COI/T.20/Doc No 15/Rév.2 (2007). The hedonic tests are conducted by a panel of 200 consumers through a questionnaire. The tasters choose, for each sample, the category that corresponds to their degree of appreciation. The different samples were coded and presented to consumers by randomization and repetition. All varieties were tasted by the same panel of consumers, not trained in order to know the olive oil most appreciated by consumers.

## 2.3. Statistical analyses

The values of the parameters studied were expressed in average, the number of repeat is 3; the comparison between samples was performed by analysis of variance (ANOVA) followed by test "t" of Student for averages inappropriate. These tests were made through the software Statgraphics version 5.0, the significance level  $\alpha$  is 0.05.

## 3. Results and discussion

### 3.1. Analytical parameters

Analysis of the acidity function of the retrieval system shows acidity values between 0.2 and 1.3%. All acidity results from the centrifuge system (two and three phases) to meet the standard COI (2009) (acidity <0.8 for the extra virgin), while the pressure system generates an oil more acidic (0.5 to 1.3%). These results are confirmed by the work of Torres and Maestri (2006) and Chimi (2006). The largest change of acidity was observed in oils from the pressure system. From the box diagram of the acidity we can see that the average acidity of different samples obtained by the SP system is shifted to the median which highlights the tendency of these oils with an acidity important than the oils obtained by centrifugation system with two and three phases. These results are consistent with results found by Torres and Maestri (2006) and Chimi (2006).

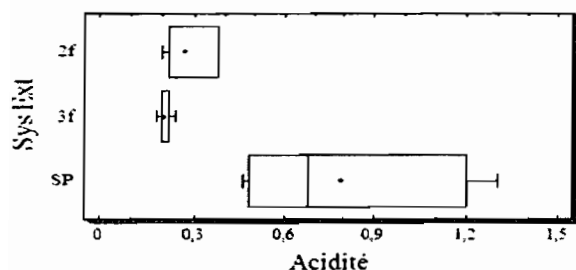


Figure 1: Diagram box acidity.

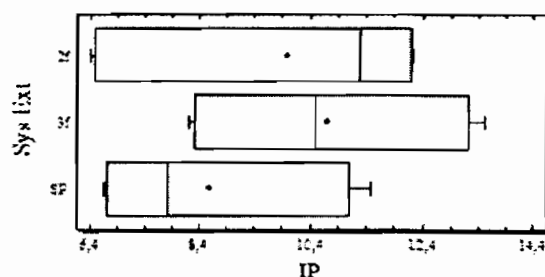


Figure 2: Diagram box of peroxide index.

The results for the specific extinction show that all values are in the  $K_{232}$  standard (COI, 2008) which sets a limit of  $K_{232} < 2.5$ . The lowest value was recorded in oil from the system three phases, it is of the order of 2.136. The highest is observed in oil from the system by pressure. Regarding the  $K_{270}$ , all samples meet the standards of the COI ( $K_{270} < 0.22$ ). The lowest value is observed in oil from the system in three phases, it is of the order of 0.142. The highest value (0.162) was observed in oil from the system by pressure.

All mean values of the peroxide index (PI) of different samples meet the standard (COI, 2009) which sets a value of  $PI < 20$  méq d'O<sub>2</sub> / Kg. Statistical analysis showed that the influence of the extraction system on the peroxide value is not significant. This result is confirmed by the work of Gimeno et al. (2002). The lowest index is recorded in the oil obtained by pressure system (8,594 méq O<sub>2</sub> / kg) while the highest (10.704 méq O<sub>2</sub> / kg) was observed in oil from the system three phases.

### 3.2. Evaluation of oxidative stability

The statistical study showed that the influence of the extraction system on the oxidative stability was significant ( $p < 0.05$ ). The highest average is observed in the oil from the system in two phases (10.96 h) while the lowest average value is observed in the oil from the system by pressure (8.56778 h). This allows us to conclude that oil from the two phase system is more resistant to oxidation and extraction centrifugal systems generate oil more stable. These results are consistent with those found by Salvador et al. (2003).

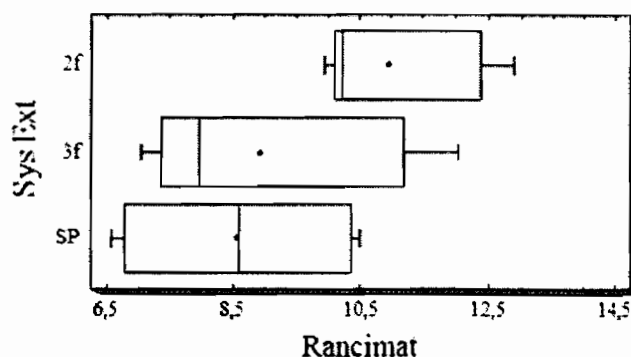


Figure3: Diagram box oxidative stability.

### 3.3. Chlorophylls and B-Carotenes content

The study of the influence of the extraction system on the chlorophyll content of different samples showed a significant difference that mainly depends on the extraction system ( $P < 0.05$ ). This result is in compliance with the work of Torres and Maestri (2006) who reported that there was no significant difference between the extraction system and the chlorophyll content. The oils from the two-phase system have the highest values (8.16 ppm). However, the lowest value is observed in oils from the pressure system (6.09 ppm).

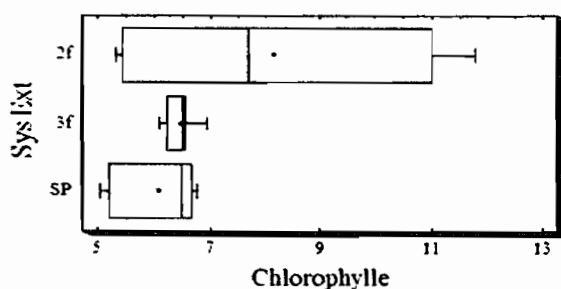


Figure 4: Diagram box of chlorophylls.

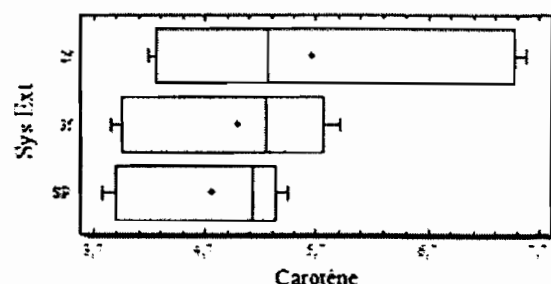


Figure 5: Diagram box of Carotenes.

The highest content (5.66) is observed in oils extracted from the two-phase system while the lowest (4.77) is recorded in oils crushed by pressure. Taking into account that the carotenoids, especially the  $\beta$ -carotene are potent antioxidants thanks to their ability to trap oxygen species (Morello et al., 2004). Therefore, it is possible to say that the oils with the highest values  $\beta$ -carotene (oils obtained from the two-phase system) are more resistant to oxidation.

### 3.4. Total phenol content

The highest content is observed in the oil obtained from the two-phase system; the lowest level is observed in the sample from the three-phase extraction system.

In fact, the phenols of the olive paste are soluble in water and oil; the addition of water during the mixing operation reduces the concentration of phenols in the oil phase. So the two-phase centrifugation system better preserves the amount of polyphenols, as the addition of water is very low during the mixing. This result is consistent with the work of Gimeno et al. (2002).

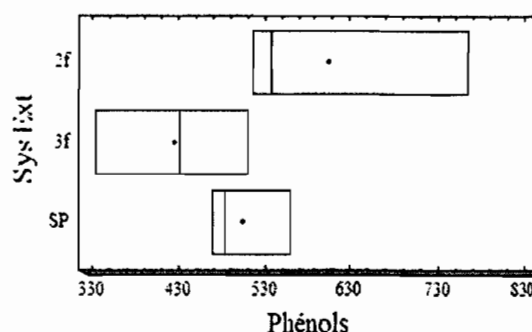


Figure 6: Diagram box of chlorophylls.

### 3.5. Sensory analysis

#### 3.5.1. Sensory analysis by the expert panel

##### 3.5.1.1. Effect of the extraction system on the attribute notes

The statistical study shows that there is a significant effect of the extraction system on the bitter attribute. The highest score was observed in the two-phase system. The lowest grade was observed in the pressure system. Statistical analysis shows that there is a significant difference between the extraction systems and fruitiness. The two-phase system gives a fruitier oil than the three-phase and pressure systems.

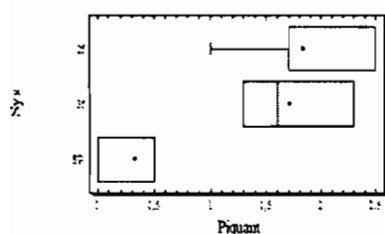


Figure 7: Diagram box of the astringency attribute.

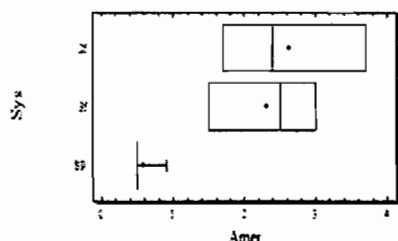


Figure 8: Diagram box of the bitter attribute.

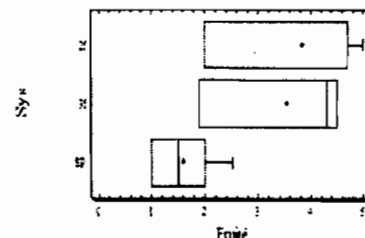


Figure 9: Diagram box of the fruity attribute.

#### 3.5.2. Sensory analysis by consumers

Statistical analysis reveals a significant effect of the extraction system on the grades given by consumers. Those under the age of 30 preferred oils from the three-phase system than the oil obtained from the two-phase and the pressure systems. Yet, consumers whose age is above 30 preferred oils from the two-phase system rather than the three-phase and the pressure systems.

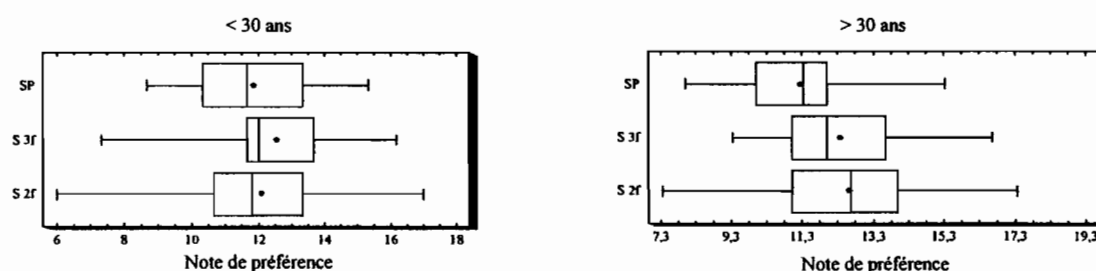


Figure 10: Diagram box of the grades of consumer preferences.



### 3.6. Effect of packaging on the quality of the oil acidity

By analyzing the diagram box of acidity, we note that the values of acidity meet all the standards (COI, 2009). The oil stored in a glass container, either opaque or transparent, has the lowest acidity content, while the oil stored in a PET packaging has the highest content. So the glass is the best type of packaging material for the preservation of oil followed by the metal packaging in the second rank and eventually the PET in the third rank.

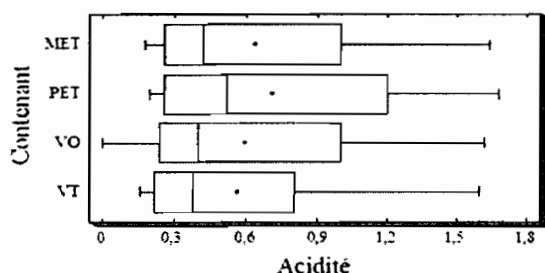


Figure 11: Diagram box of Acidity.

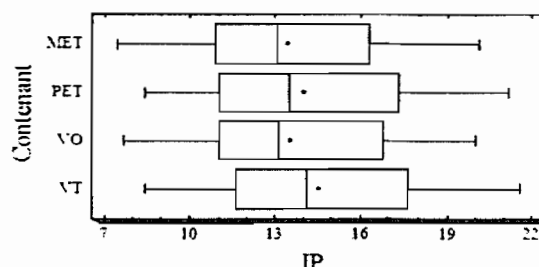


Figure 12: Diagram box of peroxide index.

VT: clear glass; VO: opaque glass, PET: polyethylene terephthalate; MET: metal; PI: peroxide index

The analysis diagram of the PI box shows that all values are classified according to the standard (COI, 2009). The oil stored in opaque glass shows the lowest value of PI, the one stored in PET has the highest value. But statistical analysis by ANOVA doesn't show a significant effect of container on the PI.

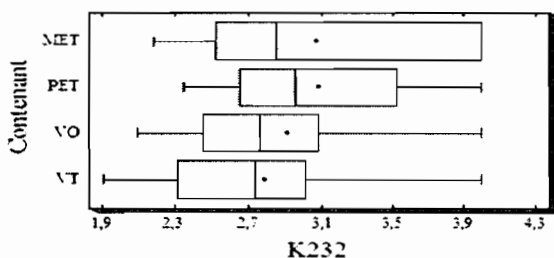


Figure 13: Diagram box of K232.

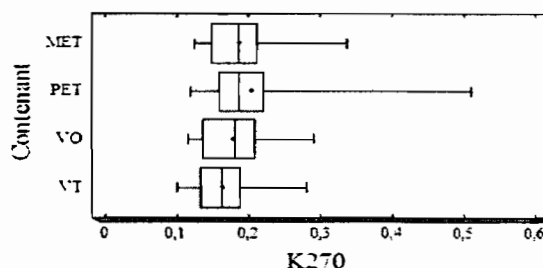


Figure 14: Diagram box of K270.

VT: clear glass; VO: opaque glass, PET: polyethylene terephthalate; MET: metal

Statistical analysis shows that there is a significant influence of packaging on the K232 ( $P < 0.05$ ). Oils packaged in PET at room temperature have values significantly higher than those packaged in glass. So PET promotes primary oxidation, due to its permeability to oxygen. These results are identical to those found by Ben Tekaya (2007a). Similarly for K270, statistical analysis shows that there is a significant effect of packaging on the K270. The highest value is observed in oils packaged in PET. The lowest is observed in oils packaged in glass; result consistent with that of Ben Tekaya *et al.*, (2007b).

### 4. Conclusion

Upon completion of this work, we have noted that the main quality index essentially acidity, peroxide value, the K232, K270 and the oxidative stability are influenced by variety and retrieval system type of packaging used. This study has therefore concluded that: Variety "Chetoui" is the most stable point of view resistance to oxidation than the varieties 'Chemlali' and 'Arbequina' due to its richness in antioxidants (polyphenols, chlorophyll and carotene) and C18: 1 and C18:2. The extraction system by two-phase centrifugation allows for better oil quality (physico-chemical and sensory) than the system by centrifugation three phases and pressure, which can give the best oils to improve the condition grinding conditions: splitting the amount of water added during mixing, low temperature of 25 ° C and mixing time between 45 and 60 min. The opaque glass is the most appropriate packaging for packaging olive oil because it preserves more nutritional quality and taste and do not let light through.

Consumers with an age <30 years preferred oils of the variety "Chemlali" from the extraction system by centrifugation three phases. By consumers aged over 30 prefer the variety of oils "Chetoui" from the extraction system by centrifugation two phases.

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## Composition and Antioxidant Activity of Olive Leaf Extracts from Greek Olive Cultivars

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### Abstract

The phenolic composition of olive leaves of the Greek cultivars *koroneiki*, *megaritiki* and *kalamon* grown in North Greece was determined using LC/MS. Furthermore, the antioxidant activity of olive leaf extracts from the above three cultivars, obtained using solvents of increasing polarity (petroleum ether, dichloromethane, methanol and methanol/water 60%) was studied. The antioxidant activity of the olive leaf extracts was determined using the stable free radical diphenylpicryl-hydrazyl (DPPH) test, as well as the oxidative stability index (OSI) in comparison to the synthetic antioxidant TBHQ and commercial oleoresin (rosemary extract). The ability of phenolic compounds to inhibit the lipoxygenase (LOX) activity was also investigated. The ten main components determined in the olive tree leaf extracts for the cultivars *koroneiki* and *kalamon* were: secologanoside, dimethyloleuropein, oleuropein diglucoside, luteolin-7-O-glucoside, rutin, oleuropein, oleuroside, quercetrin, ligstroside and verbascoside.

Respective compounds for the cultivar *megaritiki* were: secologanoside, dimethyloleuropein, oleuropein diglucoside, luteolin-7-O-glucoside, oleuropein, oleuroside, quercetrin and ligstroside. In all three cultivars, oleuropein represented the main phenolic component. When methanol/water (60%) was used, as solvent, more phenolic compounds were determined. The total phenols determined in the extracts, obtained by successive extractions using the above solvents, were 6094, 5579 and 6196 mg/Kg (mg gallic acid/kg dried olive leaves) for the cultivars *megaritiki*, *kalamon* and *koroneiki*, respectively. Among all extracts, methanol/water extracts exhibited the highest antioxidant activity applying the DPPH and OSI methods. The antioxidant activity of olive oil samples containing the above additives during the OSI method followed the sequence: synthetic antioxidant TBHQ > commercial oleoresin > olive tree leaf extracts > control. Likewise, methanol/water extracts significantly inhibited soybean lipoxygenase, although some small differences in the activity among the extracts of the different cultivars of olive leaves were observed. A positive correlation was shown between the antioxidant activity of leaf extracts and the total phenol content.

**Keywords:** olive leaves, antioxidant activity, phenolic components, lipoxygenase activity, DPPH

### 1. Introduction

Lipid oxidation has been one of the main interests of the scientific community for centuries. Researchers are continuously seeking those natural antioxidants that will sufficiently protect fats and oils from oxidation. Synthetic antioxidants are very effective and stable under usual processing and storage conditions of oils (Kiritsakis et al. 2003). They have however certain disadvantages, such as relatively high cost. Moreover, there are scientific reports on the possible toxicological effects of synthetic antioxidants, such as BHA, BHT and TBHQ (Gharavi et al. 2007). Although there are no conclusive results on the safety of these substances, world-wide interest has arisen for the recovery and utilization of antioxidants from natural sources (Loliger et al. 1996). Research has focused on antioxidant compounds derived from leaves and fruit of olive trees, numerous fruits and vegetables, as well as aromatic plants and spices (Gamel and Kiritsakis 1999; Papagrigoriou et al. 2007; Gkanatsiou 2007).

Recently, research has focused on the antioxidant properties of olive tree leaf extracts (Kähkönen 2000). Salta et al. (2007) enriched commercially available oils (olive oil, sunflower oil, palm oil and a vegetable shortening) in polyphenols by adding olive leaf extract. Results showed that both antioxidant capacity and oxidative stability were substantially improved for all oils studied. A level at 400 ppm of free phenolics, extracted from olive leaves exhibited high antioxidant activity and was superior to that of butylated hydroxy toluene (BHT) in retarding sunflower oil oxidative rancidity (Farag et al. 2003).

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Aliquots of concentrated crude olive leaf juice were added to sunflower oil and heated to 180°C. The samples exhibited remarkable antioxidant activity and at a concentration of 800 ppm were superior to that of BHT in increasing sunflower oil stability (Farag et al. 2007). A phenol extract of high hydroxytyrosol content obtained from olive leaves (*Olea europaea* L.) increased the oxidative stability of different food lipids (butter, lard and cod liver oil) (De Leonardis, 2008). The enrichment of refined olive and husk oils with olive leaf extract and its hydrolysate extract by Bouaziz et al. (2008) resulted in an increased resistance to oxidative deterioration due to its phenolic antioxidants content. The authors suggested that both hydrolysate and leaf extracts are excellent antioxidants and may serve as substitutes for synthetic antioxidants.

Due to the increasing interest in the use of natural antioxidants, the present study was carried out to evaluate the phenolic composition of selected olive leaves of three Greek cultivars and to determine the antioxidant activity of olive leaf extracts obtained by different solvents of increasing polarity.

## 2. Materials and Methods

## 2.1. Materials

Olive leaves were collected from an olive orchard located in the area Trilofos of Thessaloniki in the fall of 2007. Four trees of each of the cultivar *koroneiki*, *megaritiki* and *kalamon* were selected. Samples were taken from each tree including different parts of the tree and were mixed. Leaves were left to dry at room temperature for one week before use. 1,1-Diphenyl 2-picryl hydrazyl (DPPH), Folin-Ciocalteu reagent, lipoxygenase (Kirtsakis 1983; Bouaziz et al. 2008; De Leonardis et al. 2008; Zheng and Wang 2001) type I-B (soybean) and linoleic acid (sodium salt), 99% purity, were purchased from Sigma (St Louis, MO, USA). TBHQ was obtained from Eastman Co, while the commercial oleoresin was purchased from (Kalsec. Co., MI, USA). All chemical reagents were of analytical grade. Extra virgin olive oil from the island of Crete, with acidity of 0.5% and peroxide value of 7 meqO<sub>2</sub>/kg oil, was used.

## 2.2. Phenol extraction from the olive leaves

Phenols were extracted from the olive leaves by successive extractions, using solvents of increasing polarity (*petroleum ether, dichloromethane, methanol and methanol/water 60%*). 10 g of dried leaves from each cultivar were mechanically milled and placed in Erlenmeyer flasks. 200ml of petroleum ether were added into each flask and left for 24 h at room temperature followed by filtration using Whatman 47mm x 0.45 Micron filters. The filtrates were evaporated in a rotary evaporator. The dry residues of the olive leaves were returned in the flasks, where 200 mL dichloromethane were added and contents were kept for another 24 h. The procedure was repeated as shown in Fig. 1, with methanol and methanol/water 60%. All extracts were evaporated under mild conditions of temperature (40–45°C), in order to avoid the decomposition of phenolic compounds.

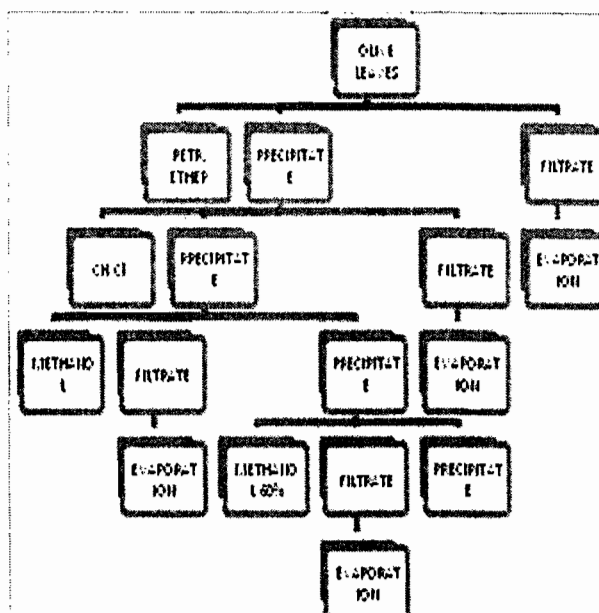


**Figure 1: Flow diagram for the preparation of olive leaf extracts with successive extractions.**

Dry residues obtained from each cultivar and solvent treatment were analyzed for their phenol content.

### 2.3. Determination of Total Phenol Content

The total phenol content of the obtained fractions was determined using the method of Zheng and Wang (2001) with few modifications: 5 mg of dry residue from each solvent extraction was dissolved in 1 ml DMSO. 100  $\mu$ l of this solution were transferred to a volumetric flask to which 500  $\mu$ L of Folin-

Ciocalteu phenol reagent and 400  $\mu$ L of 7.5% sodium carbonate solution were added. The mixture was shaken thoroughly and kept for 1.5 h at 30°C, in the absence of light. The absorbance of the blue colour formed was measured at 765 nm. The concentration of total phenol compounds for each extract was calculated on the basis of a standard curve obtained using gallic acid as the standard (twelve serial-2 fold dilutions to give a range of 0.01-0.001 mg/ml in triplicate). Results were expressed as mg of gallic acid per 100 g of dried weight.

#### 2.4. Evaluation of the antioxidant activity with OSI apparatus

Given amounts of the dry residues from each cultivar, the synthetic antioxidant TBHQ and the commercial oleoresin (rosemary extract) were dissolved in DMSO. The stock samples were of such concentration that by adding 1 mL of each solution to 4 g of olive oil, mixtures of oil containing 100 ppm phenols were prepared. A control sample, containing 4 g of olive oil and 1 mL DMSO, was also prepared. The flow rate of air in the OSI apparatus was set at 16L/h and the temperature at 110°C. The OSI values, which correspond to the beginning of the propagation period or the end of the initiation period, were automatically recorded.

#### 2.5. Interaction with DPPH stable free radical

The DPPH method (Kontogiorgis and Hadjipavlou-Litina, 2005) was used to determine antioxidant activity of olive leaf extracts. 20  $\mu$ L from the stock solution of the sample (approximately 2.5 mg in 1 mL DMSO) were dissolved in absolute ethanol to a final volume of 1 mL and then added to 1 mL DPPH (0.1 mM, in absolute ethanol). The reaction mixture was kept at room temperature. The optical density (OD) of the solution was measured at 517 nm, after 20 and 60 min. The optical densities of the samples in the absence of DPPH were subtracted from the corresponding OD with DPPH. The % reduction values were determined and compared to appropriate standards.

$$\% \text{Reduction} = \frac{\text{control OD (mean)} - \text{sample OD (mean)}}{\text{control OD (mean)}} \times 100$$

#### 2.6. Soybean lipoxygenase inhibition

All extract samples were initially dissolved in DMSO (approximately 2.5 mg in 1 mL DMSO). 10  $\mu$ L or 1  $\mu$ L of the solution were mixed with 100  $\mu$ L of sodium linoleate (0.1 mM) and 0.2 mL of the enzyme solution ( $1/9 \times 10^{-2}\%$  w/v salt solution pH=9). Samples were incubated at room temperature for 3 min. The conversion of sodium linoleate to 13-hydroperoxylinoic acid was recorded at 234 nm and compared to an appropriate standard inhibitor (caffeic acid  $IC_{50}$ =600  $\mu$ M) (Zheng and Wang, 2001; Kontogiorgis and Hadjipavlou-Litina, 2005).

#### 2.7. Analysis of the phenolic compounds in olive leaf extracts

LC-MS analysis was performed in a Finnigan LCQ Deca ion-trap mass spectrometer (Finnigan MAT, San Jose, CA, USA) coupled with a Thermo Separation series liquid chromatographic system (Thermo products, San Jose, CA, USA) consisting of UV3000, AS3000, P4000, SCM1000, a membrane degasser and an injection valve (100 $\mu$ L loop). An XTerraR RP 18, 3.5 $\mu$ m, 4.6x150mm (Waters, Ireland), with an XTerraR RP 18, 3.5 $\mu$ m, 4.6x10mm guard column. Elution was performed at a flow rate of 1.0 mL/min, using as mobile phase a mixture of water/acetic acid (99.9:0.1 v/v) (solvent A) and acetonitrile/acetic acid (99.9:0.1 v/v) (solvent B). Both eluents were of HPLC grade and filtered through a 0.20 $\mu$ m filter disk (for solvent A) and a 0.45 $\mu$ m filter disk (for solvent B). The injection volume was 20 $\mu$ L. The solvent gradient changed according to the following conditions: from 96% (A)-4% (B) to 76% (A)-24% (B) in 35min, 53% (A)-47% (B) in 15min, 100% (B) in 10min and 96% (A)-4% (B) in 10min followed by 10min of conditioning the column with the initial conditions. Chromatograms were acquired at 232nm, 280nm and 370nm. The HPLC data were collected and processed by Chromeleon data system.

The MS analyses were carried out using an electrospray (ESI) interface operating in both positive and negative mode using the following conditions: Helium gas was used as a sheath gas at a flow rate of 28 arb. The electrospray voltage was 3.70kV and the heated capillary temperature and voltage were maintained at 270°C and -37.00V respectively for negative mode. The tube lens offset voltage was set at -55 V. For positive mode scanning, the electrospray voltage was 4.70kV, the heated capillary

temperature and voltage were maintained at 270°C and 15.00V respectively, while the tube lens offset was set to 45V. The molecular ions were scanned from 100.0 to 2000.0 ( $m/z$ ) in such a scanning order that full-scan mass spectrum was followed by a tandem mass spectrum (MS/MS). The MS data were collected and processed by Xcalibur data system. The system was optimized for oleuropein on the  $m/z$  ratios of 539 and 541 corresponding to the negative and positive ion of oleuropein respectively.

### 3. Results and Discussion

#### 3.1. Analysis of phenolic compounds

Figure 2 shows the phenolic compounds isolated from the olive cultivars megaritiki, koroneiki and kalamon. Nine compounds namely: demethyleuropein, oleuropein diglucoside, luteolin-7-O-glucoside, rutin, oleuropein, oleuroside, quercetrin, ligstroside, verbascoside were identified in the cultivar megaritiki, while ten compounds (secologanoside, demethyleuropein, oleuropein diglucoside, luteolin-7-O-glucoside, rutin, oleuropein, oleuroside, quercetrin, ligstroside, verbascoside) were identified in kalamon cultivar and the same apart from secologanoside and ligstroside were identified in koroneiki cultivar. Oleuropein was by far the main constituent in all three cultivars. The compounds identified were in accordance with the related literature (Savourin, 2001).

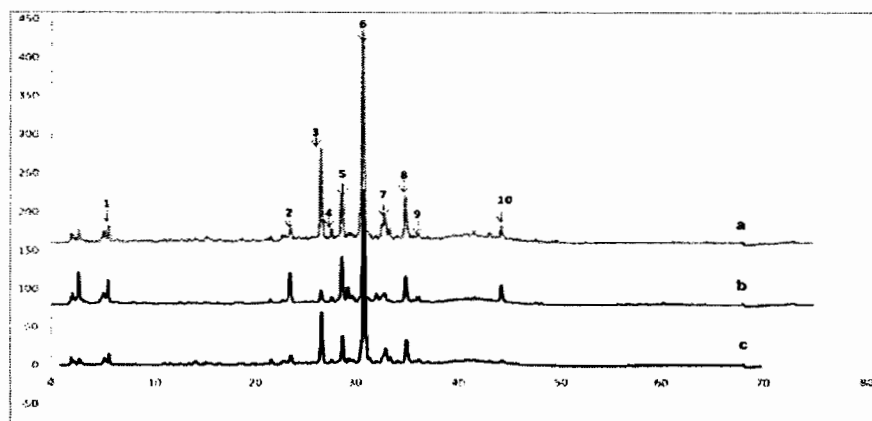


**Figure 2:** RP-HPLC chromatograms of the three cultivars extract at 280nm UV absorbance (1: Secologanoside, 2: Demethyleuropein, 3: Oleuropein diglucoside, 4: Luteolin-7-O-glucoside, 5: Rutin, 6: Oleuropein, 7: Oleuroside, 8: Quercetrin, 9: Ligstroside, 10: Verbascoside). (a: Kalamon, b: Koroneiki, c: Megaritiki).

Two oleuropein derivatives (Fig. 3) were identified during MS analysis. The two peaks represent oleuropein (Fig. 4, retention time 31.96 min) and oleuroside (Fig. 4, retention time 34.01 min) which have the same mass. The only difference in structure between the two compounds is the position of the olefinic double bond in the elenolic acid moiety so the identification based on MS was not possible.

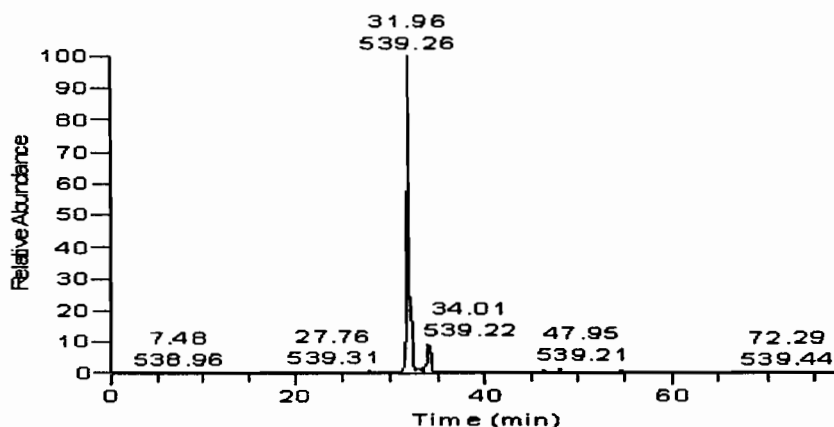


**Figure 3:** Oleuropein derivatives identified during MS ion scanning (Oleuropein, retention time 31.96 and Oleuroside (retention time 34.01)).





**Table 3:** Phenol concentration when petroleum ether was used as solvent.

| Cultivar   | mg gallic acid in 100 g sample | mg phenols in dry residues |
|------------|--------------------------------|----------------------------|
| Megaritiki | 470                            | 0.227                      |
| Kalamon    | 440                            | 0.238                      |
| Koroneiki  | 280                            | 0.137                      |

**Table 4:** Phenol concentration when dichloromethane was used as solvent

| Cultivar   | mg gallic acid in 100 g sample | mg phenols in dry residues |
|------------|--------------------------------|----------------------------|
| Megaritiki | 86                             | 0.305                      |
| Kalamon    | 47                             | 0.137                      |
| Koroneiki  | 88                             | 0.267                      |

**Table 5:** Phenol concentration when methanol was used as solvent

| Cultivar   | mg gallic acid in 100 g sample | mg phenols in dry residues |
|------------|--------------------------------|----------------------------|
| Megaritiki | 2250                           | 25.925                     |
| Kalamon    | 2220                           | 21.052                     |
| Koroneiki  | 2300                           | 27.533                     |

**Table 6:** Phenol concentration when methanol/water (60%) was used as solvent

| Cultivar   | mg gallic acid in 100 g sample | mg phenols in dry residues |
|------------|--------------------------------|----------------------------|
| Megaritiki | 2400                           | 34.469                     |
| Kalamon    | 2400                           | 34.358                     |
| Koroneiki  | 2300                           | 34.024                     |

The amount of olive leaves used was 10g. Thus, the phenol concentration in mg/Kg for the cultivars, megaritiki, kalamon and koroneiki was 6093, 5579 and 6196 respectively. Amounts of phenols determined in the present work are substantially lower than those reported by Skerget et al. (2004). Differences observed may be related to the use of different cultivars and different procedures of extraction.

### 3.3. Evaluation of the antioxidant activity with OSI apparatus

As Table 7 shows, all olive leaf phenolic extracts extended the initiation period of oxidation compared to the control. They enhanced the oxidative stability by 5.5 to 6.5 hrs or 30% compared to the control. TBHQ showed the strongest effect, increasing the oxidative stability by 10 hrs or 47%. Commercial oleoresin also increased antioxidant activity, extending the initiation period by 9,2 hrs or 42%. TBHQ seems to be the strongest antioxidant in other studies as well (Wanasundara and Shahidi, 1994).

### 3.4. Interaction of olive leaf extracts with the stable free radical of DPPH (1,1-diphenyl-2-picrylhydrazyl)

Figure 5 shows the results of the interaction of the olive leaf extracts from three different cultivars with the stable free radical of DPPH. The % interaction was determined after 20 and 60 min.

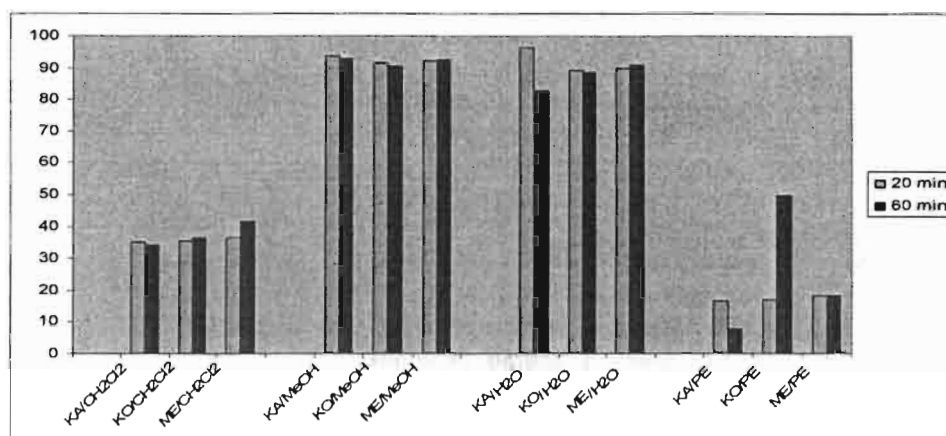


**Figure 5:** Interaction of olive leaves extracts with DPPH for 20 and 60 min. (Cultivars: ME: Megaritiki, KO: Koroneiki, KA: Kalamon, Solvents: CH<sub>2</sub>Cl<sub>2</sub>: Dichloromethane, MeOH: Methanol, H<sub>2</sub>O: MeOH 60%, PE: Petroleum ether).

Almost all petroleum ether and dichloromethane extracts were found to be less active against DPPH. Between the two, dichloromethane extracts presented higher reducing activity than petroleum ether extracts, except in the case of cultivar koroneiki after 60 min. The interaction with DPPH does not increase with time. Only in the case of koroneiki cultivar/petroleum ether, there was a significant increase in reducing activity with time. On the contrary, the reducing activity of the kalamon cultivar/petroleum ether seemed to decrease with time, whereas for megaritiki it remained constant. Almost all methanol and methanol/water (60:40) extracts presented the same antioxidant activity. Their activity did not seem to be time dependent. Only in kalamon cultivar/methanol-water extract, the reducing activity decreased after 60 min.

All of the olive leaf extracts presented antioxidant activity. However there was no statistically significant difference ( $p < 0.05$ ) among the antioxidant activities of the cultivars. The petroleum ether extracts of koroneiki and kalamon cultivars were the least potent, due to their low polyphenol content.

### 3.5. Determination of soybean lipoxygenase inhibitory activity induced by the extracts from the olive tree leaves of four greek cultivars

The soybean lipoxygenase assay was used as an indication of the antioxidant activity of the extracts with the higher DPPH interaction values. Linoleic acid was used as substrate for soybean lipoxygenase. Linoleic acid is enzymatically converted to a conjugated diene, which results to a continuous increase in absorbance at 234 nm. A mixture of DMSO and buffer served as control (no enzyme inhibition), while the reported value for caffeic acid was used as a positive control.

The inhibitory activity of the extracts of olive tree leaves from the three different Greek cultivars was tested at two different concentrations (10  $\mu$ L and 100  $\mu$ L). Almost all the extracts presented the same inhibitory activity at the higher concentration (Fig. 6). Significant differences were observed at the lower concentration (10  $\mu$ L) of the extracts. The inhibitory activity was concentration dependent.

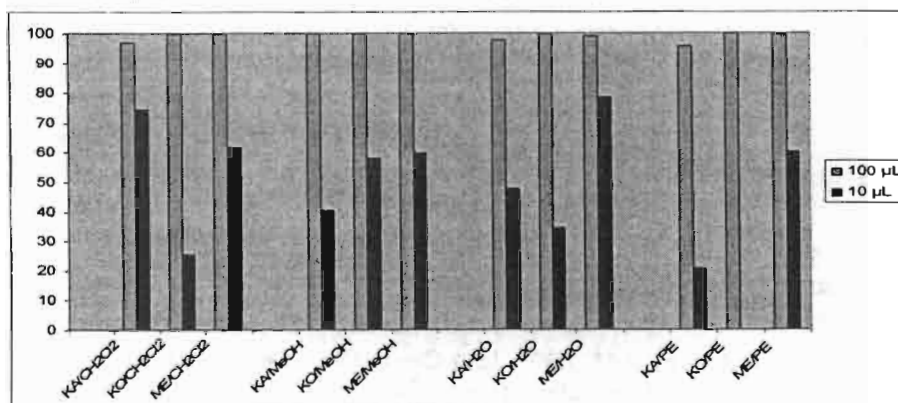


**Figure 6:** Soybean lipoxygenase inhibition by 10 and 100  $\mu$ L olive leaf extracts (Cultivars: ME: Megaritiki, KO: Koroneiki, KA: Kalamon, Solvents: CH<sub>2</sub>Cl<sub>2</sub>: Dichloromethane, MeOH: Methanol, H<sub>2</sub>O: MeOH 60%, PE: Petroleum ether).

There was a statistically significant difference ( $p > 0.05$ ) among the extracts and the cultivars respectively. Almost in all cases the methanol and methanol/water (60:40) extracts were the most potent against lipoxygenase. The dichloromethane extracts of kalamon and megaritiki cultivars presented a higher inhibitory activity against LOX as compared to the koroneiki cultivar. Most of the LOX inhibitors are antioxidants or free radical scavengers (Skerget et al. 2004), since lipoxygenation occurs via a carbon-centered radical. Based on present results it is obvious that the % DPPH scavenging activity is correlated to the LOX % inhibition (Fig. 6). Certain studies (Muller 1994) suggest a relationship between LOX inhibition and the ability of the inhibitors to reduce  $\text{Fe}^{+3}$  at the active site to the catalytically inactive  $\text{Fe}^{+2}$ . Many flavonoids and other phenolic derivatives inhibit soybean lipoxygenase through the above proposed mechanism (Muller 1994). Thus the presence of compounds with a free -OH group could account for the inhibition effect shown in tested samples. It has been suggested that olive leaves are a source of antioxidants acting as radical scavengers (Papoti and Tsimidou, 2009).

#### 4. Conclusion

The present study showed that due to the diversity and complexity of the natural mixtures of phenolic compounds in the olive leaf extracts of various cultivars it is rather difficult to compare their antioxidant activities. There were also some antioxidant activities in olive leaves that may be attributed to other unidentified compounds or to synergistic interactions.

The concentration of phenols obtained from the three different Greek cultivars (koroneiki, megaritiki and kalamon) varied with type and polarity of solvent used for the extraction. The highest amount of phenols was extracted when methanol/water 60% was used. Among the cultivars, koroneiki seemed to have the highest concentration of phenols. Many different phenolic compounds were determined in the leaves of the three olive cultivars. However, oleuropein was found in the highest concentration in all three cultivars.

When the OSI method was applied, using virgin olive oil as the substrate, the antioxidant activity of additives followed the order: Synthetic antioxidant TBHQ > Commercial oleoresin > Olive tree leaf extracts > Control.

The LOX inhibitory activity was affected by the solvent polarity used. Results obtained for this enzyme, using petroleum ether as solvent, confirmed the results of the DPPH method and gave an indication for the higher antioxidant activity of the phenolic compounds present in the cultivar koroneiki.

The olive leaf extracts present significant antioxidant activity which renders such products useful for the enhancement of the oxidative stability of edible oils.

#### Acknowledgments

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## Études organoleptique, chimique et spectrale d'huiles d'olives vierges d'AOC françaises

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### Résumé

Les caractéristiques sensorielles, chimiques (acides gras et triglycérides) et spectrales (Moyen infrarouge) d'huiles d'olive vierges de six AOC françaises (Aix-en-Provence, Haute-Provence, Nice, Nîmes, Nyons and Vallée des Baux de Provence) (n= 600) ont été déterminées sur des huiles provenant de six années de récolte consécutives. L'évaluation du fruité, de l'amertume et du piquant n'est pas suffisante pour décrire les AOC. Aussi, il est nécessaire de décrire les huiles par des descripteurs analogiques qui sont estimés par les dégustateurs selon des critères définis. Une Analyse en Composante Principale utilisant 34 paramètres (acides gras, triglycérides et squalène) permet de différencier les six AOC. Toutefois les AOC Aix-en-Provence et Vallée des Baux de Provence ne sont pas complètement résolues car elles possèdent toutes les deux, deux variétés principales en commun (*Salonenque* et *Aglandau*). L'utilisation conjointe de la spectrométrie infrarouge et de l'analyse multivariée (PLS-DA) constitue une approche originale pour étudier les relations entre les AOC, leur composition et leur origine géographique. L'interprétation spectroscopique des premiers vecteurs de régression indique que chaque AOC est caractérisée par un acide gras ou un triglycéride spécifique. De bons résultats ont été obtenus malgré la similitude de composition des deux AOC Aix-en-Provence et Vallée des Baux de Provence. La spectroscopie infrarouge constitue une méthode alternative à l'authentification des huiles d'olive vierges de six AOC françaises.

**Mots clés :** Huile d'olive vierge, AOC, analyse sensorielle, analyse chimique, analyse infrarouge, chimiométrie, authentification.

### Abstract

The sensory and chemical (fatty acid and triacylglycerol compositions) characteristics of six registered designations of origin (RDOs) of French virgin olive oils (Aix-en-Provence, Haute-Provence, Nice, Nîmes, Nyons and Vallée des Baux de Provence) (n= 600) were determined over five consecutive year harvest periods. The evaluation of the fruity, bitter and pungent attributes was insufficient for describing the RDOs, so it was necessary to complete the oil descriptions with descriptive attributes (analogical descriptors) estimated by the tasters. Principal Component Analysis, using 34 parameters, has ensured to differentiate the six RDOs. The Aix-en-Provence and Vallée des Baux de Provence RDOs are not completely differentiated because the two RDOs have two principal varieties in common : *Salonenque* and *Aglandau*. The morphograms of fatty acid and triacylglycerol compositions are genuine fingerprints of the six RDOs. The combination of mid infrared spectroscopy with multivariate analysis (PLS-DA) provides an original approach to study profile of virgin olive oils (VOOs) in relation to composition and geographical origin. Spectroscopic interpretation of regression vectors has shown that each RDO is correlated to one specific component of VOO according to their cultivar compositions. The results are satisfactory, in spite of the similarity of cultivar compositions between two denominations of origin (Aix-en-Provence and Vallée des Baux de Provence). Chemometric treatment of MIR spectra makes it possible to obtain similar results to those obtained by time-consuming analytical techniques such as GC and HPLC, and constitute a fast and robust tool for authentication of these French VOOs.

**Keywords:** Virgin olive oil, RDO, sensory, chemical analysis, infrared analysis, chemometry, authenticity

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## **1. Introduction**

La détermination de la traçabilité des produits de l'industrie agroalimentaire est devenue essentielle en raison des problèmes de santé publique qu'ils peuvent présenter ainsi que pour assurer la loyauté des échanges commerciaux. La traçabilité nécessite de disposer d'outils non subjectifs pour connaître l'ensemble des éléments permettant de suivre le cheminement d'un produit de son origine jusqu'à son utilisation par les consommateurs. La filière oléicole n'échappe pas à cette nécessité de traçabilité.

À ce jour, environ 200 variétés différentes d'oliviers ont été dénombrées en France parmi lesquelles une quinzaine sont des variétés principales pour la production d'huiles d'olive vierges (HOV) (Moutier et al., 2004). 30% environ de la production française sont commercialisés et valorisés soit sous forme d'huiles monovariétales soit sous forme d'assemblages avec le label Appellation d'Origine Contrôlée (AOC) (Aix-en-Provence, Corse, Haute-Provence, Nice, Nîmes, Nyons, Provence et Vallée des Baux de Provence). Diverses approches ont été proposées pour répondre à la demande de traçabilité des HOV en utilisant des caractéristiques chimique ou organoleptique. Toutefois, la détermination de la traçabilité constitue un véritable défi analytique qui nécessite la mise en œuvre de méthodes permettant la détermination de paramètres multiples pour répondre à cette demande émergente.

Cette étude porte sur les huiles de 6 AOC françaises (Aix-en-Provence, Haute-Provence, Nice, Nîmes, Nyons et Vallée des Baux de Provence), pour lesquelles les caractéristiques organoleptique, chimique (composition en acides gras et en triglycérides) et spectrales seront déterminées et utilisées pour la reconnaissance de leur origine.

## **2. Matériels et méthodes**

### **2.1. Analyse sensorielle**

Les intensités de fruité, d'amer et de piquant ont été évaluées selon la méthode d'analyse organoleptique décrite dans le Règlement Européen n°2568/91. Les analyses ont été réalisées sur des huiles des campagnes oléicoles 1996/1997 à 2005/2006 (Aix-en-Provence, n= 27 ; Haute-Provence, n=31 ; Nice, n=38 ; Nîmes, n=18 ; Nyons, n= 19 ; Vallée des Baux de Provence, n=48). Les dégustations ont eu lieu deux mois et quatorze mois après la date de production. Pendant cette période, les échantillons sont conservés à 12°C à l'abri de la lumière. Ces échantillons d'huiles ont fait parallèlement l'objet d'une dégustation analogique selon la méthode présentée par Pinatel et al (2004) qui fournit une classification des descripteurs analogiques selon leur importance, à la fois en intensité et en reconnaissance par les divers membres du jury. Le nombre maximal de descripteurs pris en compte est de 14.

### **2.2. Analyses chromatographiques**

#### **2.2.1. Echantillons**

600 échantillons provenant du Centre Technique de l'Olivier, (CTO), Aix-en-Provence, France et sont représentatifs de lots commerciaux produits dans les moulins français. Ils sont issus de cinq campagnes oléicoles successives (2000/2001-2004/2005). Les AOC Haute-Provence (n=92), Nice (n=102), Nyons (n=104) sont constituées d'une seule variété principale. Les AOC Aix-en-Provence (n=130) et Vallée des Baux de Provence (n=131) peuvent être constituées de trois ou quatre variétés principales dont deux sont obligatoires sans que leurs proportions ne soient imposées. L'AOC Nîmes (n=41) doit être constituée d'au moins 60% de la variété Picholine.

#### **2.2.2. Préparation et analyse des esters méthyliques d'acides gras**

La préparation et l'analyse des esters méthyliques ont été précédemment décrites par Ollivier et al. (2006).

#### **2.2.3. Analyse des triglycérides**

L'analyse des triglycérides a été précédemment décrite par Ollivier et al. (2006).

### 2.3. Analyses spectrales

Les spectres (100 accumulations) sont enregistrés entre 4000 et 600  $\text{cm}^{-1}$  avec une résolution spectrale de 4  $\text{cm}^{-1}$  sur un spectromètre Thermo Nicolet Avatar à transformée de Fourier (source Ever-Glo, détecteur DTGS). Tous les échantillons sont enregistrés en utilisant un accessoire de réflexion totale atténuée (ATR) munie d'un cristal de diamant. Chaque spectre est la moyenne de 3 spectres indépendants.

### 2.4. Chimiométrie

Cette étude utilise une méthode de prédiction, la régression PLS appliquée conjointement aux données spectrales et aux données d'origine codée en binaire. Cette méthode est une alternative à la régression linéaire multiple classique. La régression PLS est une méthode d'analyse de données initiée par Wold (1966) qui a connu un fort développement par la suite (Geladi & Kowalski, 1986 ; Tenenhaus, 1998). Ses premières applications à l'analyse quantitative remontent aux années 1980 (Mardia et al, 1981). Les applications chimiométriques sont réalisées à l'aide du logiciel Unscrambler 9.6, distribué par la société CAMO (Computer Aided Modelling, Trondheim, Norway).

### 2.5. Morphogramme

Une représentation graphique radiale (morphogramme), réalisée à partir d'un tableau est fondée sur l'écart des variables (acides gras et triglycérides) par rapport à une moyenne déterminant l'origine (0%) des mêmes variables issues d'une base de données (FATG-BD02). Cette base de données a été établie à partir des compositions en acides gras et en triglycérides provenant d'HOV de 46 variétés françaises, de cinq AOC françaises et d'HOV provenant de six pays méditerranéens (Espagne, Italie, Grèce, Tunisie, Maroc, Turquie) totalisant 1400 échantillons d'huiles. Sur chaque morphogramme, qui correspond à une AOC donnée, chaque axe donne pour une variable donnée, la position de la moyenne centrée à 50 % des valeurs centrales pour cette variable. Cette moyenne est établie à partir des valeurs comprises entre le premier et le troisième quartile, bornes qui sont aussi représentées sur le graphique par le tracé en pointillés. Ces trois points sont positionnés sur chaque axe en pourcentage par rapport à la variation maximale observée pour la variable correspondante sur l'ensemble des huiles de la base de données.

## 3. Résultats et discussions

### 3.1. Analyse sensorielle

La description des six AOC françaises, selon le Règlement Européen n°2568/91, avec les trois descripteurs positifs : fruité, amer et piquant obtenus ne permettent pas de les différencier. On ne peut en effet distinguer certaines appellations que par l'amertume qui permet de séparer les huiles de Nîmes, des huiles de trois autres appellations, Nice, Nyons et Vallée des Baux de Provence. Ce sont les descripteurs analogiques qui peuvent permettre à l'analyse sensorielle d'obtenir une discrimination plus efficace. Le tableau 1 donne l'ordre d'importance des différents descripteurs relevés pour chacune des appellations obtenus à partir des profils analogiques des mêmes huiles.

**Tableau 1:** Principaux descripteurs analogiques des huiles d'olive vierges de six AOC françaises.

| AOC                         | Premier descripteur              | Deuxième descripteur   |
|-----------------------------|----------------------------------|------------------------|
| Aix-en-Provence             | Artichaut cru (2,0) <sup>a</sup> | Feuille/herbe (3,0)    |
| Haute-Provence              | Artichaut cru (1,0)              | Banane verte (2,0)     |
| Nice                        | Amande fraîche (1,0)             | Artichaut cru (3,0)    |
| Nîmes                       | Prune (1,0)                      | Foin frais (2,5)       |
| Nyons                       | Pomme (2,0)                      | Noisette fraîche (4,0) |
| Vallée des Baux de Provence | Artichaut cru (1,0)              | Amande fraîche (2,0)   |

<sup>a</sup> La valeur entre parenthèse correspond à la médiane des positions du descripteur au classement par ordre d'importance.

Ces résultats constituent une première étape pour la recherche de descripteurs en vue de construire des moyens d'identification et de contrôle des huiles d'olives en appellation. Les résultats les plus intéressants, de ce point de vue, sont ceux qui fournissent, pour une appellation donnée, un



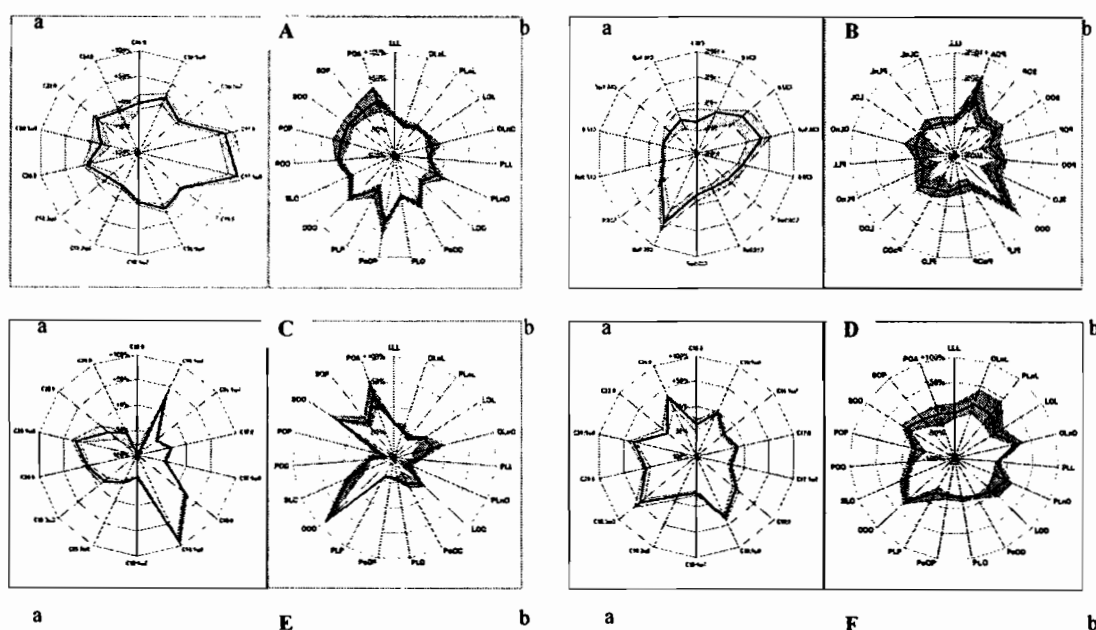
descripteur très spécifique en premier. C'est le cas pour le descripteur "prune" sur l'AOC Nîmes. La présence d'un descripteur spécifique en deuxième position comme le descripteur "banane verte" sur l'AOC "Haute-Provence", est aussi très informatif. Par contre, le descripteur "fleur de genêt" de l'AOC Nice, bien que très spécifique, est trop souvent minoritaire ou non perçu pour être utilisé dans une grille de contrôle de profil.

### 3.2. Analyses chimiques

Tous les échantillons étudiés possèdent les mêmes 14 acides gras et les mêmes 19 triglycérides mais à des taux différents suivant les AOC (Ollivier et al., 2006 ; Ollivier et al., 2007). Les compositions en acides gras et en triglycérides des six AOC sont présentées sous forme de morphogrammes basés sur la banque de données (FATG-BD02). Les AOC Haute-Provence, Nice, Nîmes et Nyons qui sont constitués d'une seule variété principale, ont des morphogrammes différents et caractéristiques pour les acides gras (a) et les triglycérides (b) (Figure 1). L'AOC Haute-Provence (cv *Aglandau*) (Figure 1A) est caractérisée par des valeurs voisines de 100% pour les acides margarique (17:0) et margaroléique (17:1n-8). Les autres acides sont proches de la moyenne (0%) à l'exception des acides linoléique (18:2n-6) et linoléique (18:3n-3) qui y sont légèrement inférieurs. Les triglycérides sont voisins de la moyenne sauf PoOP qui avoisine le niveau de 50% tandis que LLL, OLnL, PLnL, LOL et PLL sont inférieurs à 0%.

L'AOC Nice (cv *Cailletier*) (Figure 1B) possède des valeurs comprises entre 0 et 50% pour les acides oléique (18:1n-9), gondoïque (20:1n-9) et béhénique (22:0). Les autres acides se situent à des valeurs proches ou inférieures à 0% notamment les acides palmitique (16:0), palmitoléique (16:1n-7), margarique (17:0) et margaroléique (17:1n-8). L'acide stéarique (18:0) dont le taux est le plus bas de toutes les AOC caractérise l'AOC Nice. Les triglycérides sont en accord avec les acides gras avec OOO proche de 50% et des valeurs proches de 0% ou inférieures pour les autres triglycérides.

L'AOC Nyons (cv *Tanche*) (Figure 1C) est caractérisée par des valeurs voisines de 50% pour les acides hypogéique (16:1n-9) et stéarique (18:0) et une valeur supérieure à 50% pour l'acide oléique (18:1n-9). En revanche, l'acide palmitique (16:0) est proche de 100% et les acides palmitoléique (16:1n-7), margarique (17:0), margaroléique (17:1n-8) et Z-vaccénique (18:1n-7) ont des pourcentages de l'ordre de 50%. Cette AOC est la plus riche en acide oléique (18:1n-9) et trioléine (OOO) des six AOC. L'AOC Nîmes (cv *Picholine*, *Négrette*) (Figure 1D) présente des morphogrammes spécifiques. Nîmes est particulièrement riche en acides linoléique (18:2n-6) (~50%) et gondoïque (20:1n-9) (~50%) et a des valeurs de l'ordre de 50% pour les acides palmitique (16:0), palmitoléique (16:1n-7), margaroléique (17:1n-8) et Z-vaccénique (18:1n-7). Les triglycérides OLnL et OLnO sont voisins de 50% tandis que PoOO, PoOP et POP et SOP sont proches de 50%.



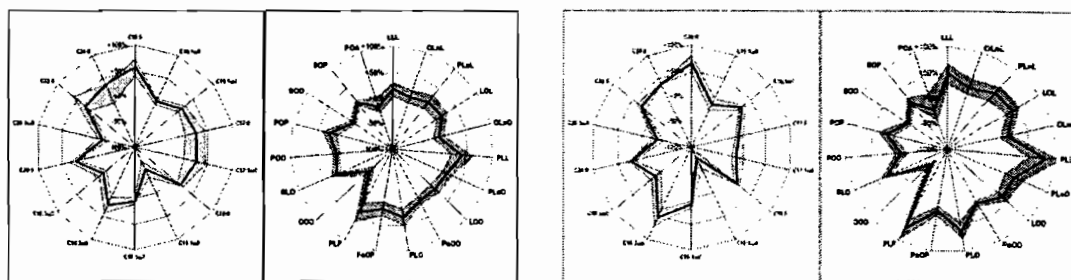


**Figure 1:** morphogrammes des acides gras (a) et des triglycérides (b) des AOC Haute-Provence (A), Nice (B), Nyons(C), Nîmes(D), Aix-en-Provence (E) et Vallée des Baux de Provence (F).

Les Figures 1E et 1F montrent les acides gras et les triglycérides des AOC Aix-en-Provence (cv : *Aglandau*, *Salonenque*, *Cayanne*) et Vallée des Baux de Provence (cv : *Salonenque*, *Aglandau*, *Grossane*, *Verdale des Bouches du Rhône*) qui résultent des variétés qui les constituent. Les formes des représentations des deux AOC présentent de fortes similitudes. Ce résultat était prévisible puisque les deux AOC ont en commun deux variétés principales *Aglandau* et *Salonenque*. Une analyse fine du morphogramme des acides gras de l'AOC Aix-en-Provence montre que cette AOC possède le cultivar *Aglandau* comme variété majoritaire en raison des taux élevés en acides margariques (17:0) et margaroléiques (17:1n-8). Une analyse similaire pour l'AOC Baux de Provence permet de conclure que c'est le cultivar *Salonenque* qui est majoritaire en raison des taux élevés des acides palmitique (16:0) et linoléique (18:2n-6) présents dans cette huile. Ces résultats sont en accord avec les populations des ces cultivars (*Aglandau* et *Salonenque*) sur les aires de production de ces AOC.

### 3.3. Analyse spectroscopique

Les spectres moyens infrarouges sont organisés en matrice et les différentes AOC sont repérées par un codage binaire. Pour une origine considérée, un échantillon est codé 1 lorsqu'il appartient à cette origine et 0 lorsqu'il n'en fait pas parti. Une analyse discriminante est réalisée en utilisant la méthode PLS-DA (Galtier et al., 2008). Une étape préliminaire est réalisée afin de déterminer si les huiles proviennent d'une AOC française ou d'une huile étrangère. Dans ce cas, un modèle d'étalonnage est réalisé avec un total de 100 HOV dont 50 proviennent de France et 50 proviennent des principaux producteurs d'huile d'olive (Espagne, Grèce, Italie, Turquie). Le but étant de savoir si l'HOV est française, un seul modèle PLS-DA est réalisé considérant l'origine "France" par rapport à toutes les autres. On considère, dans ce cas, les quatre origines étrangères comme une seule pour la construction du modèle. Le modèle est ensuite validé sur 100 HOV françaises et sur 45 HOV étrangères n'ayant pas servi au calcul du modèle. Si pour un échantillon d'HOV, l'origine prédite est "France", les modèles correspondant aux six AOC françaises lui sont alors appliqués. Les HOV françaises sont toutes prédites avec une valeur supérieure 0,75, celles des autres origines sont prédites avec une valeur inférieure à 0,25. Tous les échantillons sont donc bien prédits, et aucun ne se trouve proche de la valeur seuil à 0,5. La distinction de l'origine géographique "France" ou "étrangère" ne pose donc aucun problème.

Pour la reconnaissance des différentes AOC françaises, l'étude est menée sur 342 échantillons (250 échantillons sont utilisés pour la calibration et 92 pour la prédiction). Les résultats en prédiction sont rassemblés dans le Tableau 2.

**Tableau 2:** Prédiction de l'appartenance d'huiles d'olive vierges à une AOC française.

|                                      | Aix-en-Provence | Haute-Provence | Nice      | Nîmes   | Nyons     | Baux      |
|--------------------------------------|-----------------|----------------|-----------|---------|-----------|-----------|
| Nombre d'échantillons prédits        | 21              | 13             | 15        | 9       | 13        | 21        |
| Nombre de coefficients de régression | 6               | 6              | 6         | 5       | 4         | 6         |
| Aix-en-Provence                      | 20 (95%)        | 0              | 0         | 0       | 0         | 1         |
| Haute-Provence                       | 0               | 13 (100%)      | 0         | 0       | 0         | 0         |
| Nice                                 | 0               | 0              | 15 (100%) | 0       | 0         | 0         |
| Nîmes                                | 0               | 0              | 0         | 8 (89%) | 0         | 0         |
| Nyons                                | 0               | 0              | 0         | 0       | 13 (100%) | 0         |
| Vallée des Baux de Provence          | 1               | 0              | 0         | 0       | 0         | 21 (100%) |

Régression PLS-DA obtenue après traitement SNV des spectres dans la gamme 4000-650 cm<sup>-1</sup>.

Cette bonne classification des échantillons en fonction de leur AOC peut s'expliquer par l'interprétation des vecteurs de régression, et en particulier du premier de chaque modèle. Il est connu que le premier coefficient de régression est une bonne approximation du composé pur discriminant (Haaland & Thomas, 1988) dans le cas de régression PLS. Donc, les coefficients de régression obtenus pour chaque modèle AOC sont des approximations du composé discriminant des AOC françaises. L'interprétation de ces coefficients est très utile pour permettre la compréhension des modèles AOC en fonction de leur composition chimique.

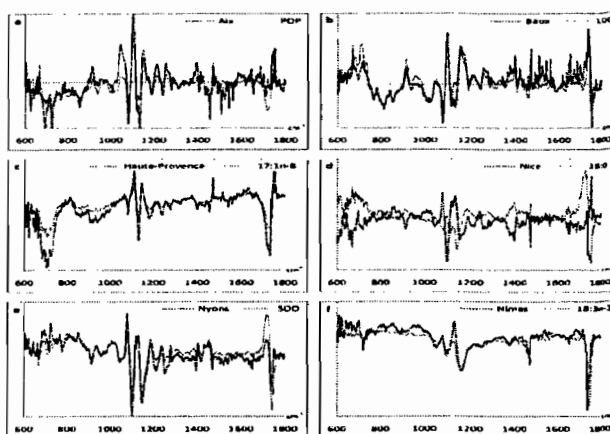


**Figure 2:** Comparaison des coefficients de régression des modèles AOC et des modèles acides gras et triglycérides obtenus à partir des spectres MIR.

Les coefficients de régression obtenus pour les modèles de prédiction des AOC ont été comparés à ceux obtenus pour l'analyse quantitative des acides gras et des triglycérides des HOV (Galtier et al., 2008). Chaque coefficient de régression de chaque modèle AOC présente une grande similitude avec un coefficient de régression des modèles acides gras et triglycérides (**figure 2**) : l'AOC "Aix-en-Provence" et le triglycéride POP ; l'AOC "Haute-Provence" et l'acide gras 17:1n-8 ; l'AOC "Nice" et l'inverse de l'acide gras 18:0 ; l'AOC "Nîmes" et l'acide gras 18:3n-3 ; l'AOC "Nyons" et le triglycéride SOO ; l'AOC "Vallée des Baux" et le triglycéride LOO.

Le Tableau 3 présente le taux de ces six composés dans les six AOC françaises déterminés par chromatographie. Ce tableau montre que ces acides gras ou ces triglycérides sont effectivement caractéristiques pour chacune des AOC. En ce qui concerne les AOC "Aix-en-Provence", "Haute-Provence", "Nyons" et "Vallée des Baux de Provence", le pourcentage des différents composés associés (POP, LOO, SOO et 17:1n-8) est discriminatoire car il est le plus élevé dans chaque AOC associée. Au contraire, pour l'AOC "Nice", le pourcentage de 18:0 est le plus faible par rapport aux autres AOC ; cela explique que les coefficients de régression soient inversés.

**Tableau 3:** Proportions des six composés discriminatoires pour chaque AOC française (résultats chromatographiques).

| AOC                         | 18 :0 (%) | 17 :1n8 (%) | 18 :3n3 (%) | POP (%) | SOO (%) | LOO (%) |
|-----------------------------|-----------|-------------|-------------|---------|---------|---------|
| Aix-en-Provence             | 2,45      | 0,20        | 0,59        | 3,77    | 3,35    | 14,71   |
| Haute-Provence              | 2,32      | 0,31        | 0,54        | 3,38    | 3,66    | 12,04   |
| Vallée des Baux de Provence | 2,36      | 0,13        | 0,59        | 3,68    | 3,10    | 15,81   |
| Nice                        | 2,04      | 0,09        | 0,57        | 2,78    | 3,30    | 12,64   |
| Nîmes                       | 2,34      | 0,14        | 0,85        | 3,10    | 3,62    | 13,62   |
| Nyons                       | 2,58      | 0,08        | 0,56        | 2,02    | 4,47    | 12,29   |

#### 4. Conclusion

L'analyse sensorielle et les compositions en acides gras et en triglycérides permettent de caractériser les huiles d'olive vierges des six AOC françaises étudiées. La corrélation existant entre la composition chimique d'une HOV et son origine variétale ou géographique a rendu possible à partir des spectres MIR, d'une part la distinction d'huiles d'AOC françaises d'huiles étrangères, et d'autre part l'identification de l'appartenance à une AOC des HOV françaises.

La comparaison des coefficients de régression des modèles de prédiction des AOC déterminés par spectroscopie infrarouge à ceux déterminés à partir des analyses chromatographiques montre des similitudes importantes. Chaque AOC possède un composé susceptible de la différencier des cinq autres.

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## Authentification de l'huile d'olive dans l'alimentation animale par la technique de RT-PCR

George Perrakis, Emmanuel Politis & George Siragakis

### Résumé

Pendant les deux ans derniers, plusieurs fermiers utilisent de l'huile d'olive dans le feedstuffs pour avoir la meilleure qualité de viande. Le but de cette étude était d'établir un essai analytique à base d'ADN sensible pour la détection d'huile d'olive dans la nourriture d'animal. Pour l'isolement d'ADN 3 échantillons de fourrage ont été utilisés leur contenu de pétrole après soxhlet l'extraction. En plus, l'huile d'olive pure dans de différentes quantités (conforme à 100 %, 20 %, 10 %, 5 % et 1 % d'huile d'olive dans la nourriture d'animal) a été appliquée pour déterminer la limite de cette technique de détection. L'essai a été davantage évalué sur la nourriture d'animal après l'enrichissement avec 20 % et 10 % d'huile d'olive. Une étude comparative a été faite de deux différents kits commerciaux (Quiagen et r-Biopharm) pour la récupération d'ADN d'arbre vert olive de haute qualité. L'effet boule de neige polymérase ultérieure (PCR) a été conçu en utilisant des abécédaires d'ADN universels amplifiant le chloroplaste trnL (UAA) intron. La traçabilité de l'huile d'olive pourrait être fondée sur le polymorphisme de cet intron. Pour *Olea europaea* cela a une grandeur de 548 bp. La limite de détection a été déterminée comme 20 %.

**Mots clés :** Huile d'olive, détection dans les aliments, polymorphisme, DNA, RT-PCR.

### Olive oil identification in feed by RT-PCR

#### Abstract

During the last two years, several farmers use olive oil in the feedstuffs in order to have better quality of meat. The aim of this study was to establish a sensitive DNA-based analytical assay for the detection of olive oil in animal feed. For the isolation of DNA 3 fodder samples were used their oil content after soxhlet extraction. In addition, pure olive oil in different quantities (corresponding to 100%, 20%, 10%, 5% and 1% of olive oil in animal feed) was applied in order to determine this technique's limit of detection. The assay was further tested on animal feed after enrichment with 20% and 10% of olive oil. A comparative study was made of two different commercial kits (Quiagen and r-Biopharm) for the recovery of high quality olive tree DNA. Subsequent polymerase chain reaction (PCR) was designed by using universal DNA primers amplifying the chloroplast trnL (UAA) intron. Olive oil traceability could be based on this intron's polymorphism. For *Olea europaea* it has a size of 548 bp. The limit of detection was determined as 20%.

**Key words:** olive oil, identification, feed, DNA, RT-PCR.

#### 1. Introduction

Over the past few years olive oil nutritional value has been highly appreciated by consumers from all over the world regardless their own national diet habits. Olive oil is considered to be a bioactive food not only in the Mediterranean basin where it is mainly produced and consumed but throughout the globe, even in distant countries from olive tree's natural habitat such as China and Japan. This was also noticed during the recent commercial exhibition "Elaiotechnia" (olive oil art) where participating exhibitors were even from Asia and visitors coming to learn about olive oil and promote it to their countries even from far-away Malaysia.

## 2. Material and Methods

### 2.1. New products with olive oil

Meat and products originating from it are considered to be responsible for causing a series of contemporary health syndromes such as cardiovascular disease, obesity etc. The new trend in food industry is to use the positive mind-set of the consumers regarding olive oil in a way that other products, considered up to now as less nutritional and healthy, to be promoted. It is worth mentioning some examples: the biggest Fast Food brand in Greece decided to use olive oil for frying, the two major greek sausage and cold meat product companies use olive paste and olive oil in their products, one of the biggest dairy industry launches Mediterranean yogurt with olive oil and much more similar cases.

Except from the above mentioned, several products can be found in the market based on olives such as olive sweets prepared the same way as greek traditional syrup fruit sweets, olive liquor e.t.c. that are currently promoted excessively because of olive's oil positive and dynamic impact on consumers. Olive oil is even used in animal feed in order to improve meat quality. At least three poultry production units in Greece are following this approach.

Food Allergens Lab with a specialized scientific group in all its three laboratories (Crete-Athens-Cyprus) counts many years of experience in olive oil research. On September 2009, a proposal was submitted from our laboratory to the Institution of Research Promotion of the Cyprus Republic and it is expected to be approved in the "New Products and Services" framework. This is a proposal about a research program concerning the production of ice-cream with cyprus olive oil.

This kind of actions assist to further develop and expand olive tree cultivation and olive related culture globally thus giving hope to olive tree cultivators who watch their products getting constantly a lower price.

### 2.2. New identification method

Is it possible to confirm a manufacturer's claim that its product contains olive oil? The main difficulty is that olive oil is present in foodstuffs in a mixture of other oils and fat ingredients. This means that it can not be detected by classical analytical methods such as fatty acid or sterol profiling by the use of gas chromatography because olive oil's lipid ingredients exist in other food materials as well and no distinguishing of their source is possible. Hence a more accurate, sensitive and selective way is necessary to be found.

Food Allergens Lab has developed a method based on molecular biology techniques in order to detect olive oils' presence in complex food matrices so that its addition in different products can be validated. The innovation in this new analytical method is the use of Polymerase Chain Reaction (PCR) which identifies olive oil by means of olive tree DNA presence and is characterized by high sensitivity and selectivity.

An overview of the experimental procedure can be seen in Figure 1.

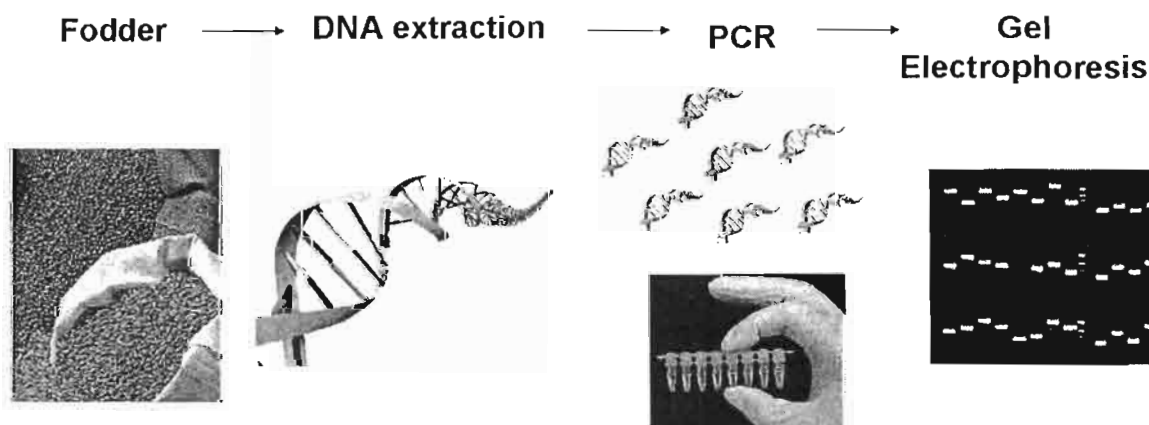


**Figure 1:** Overview of the experimental set up.

### Samples:

Firstly it was necessary to test different samples from where olive DNA could be extracted. Fodder with olive oil enrichment claim and animal feed with olive oil added by us were used as starting materials. Fodder samples were mixtures of wheat soy, corn, plant oils and other ingredients of plant origin. In addition, pure olive oil in different quantities (corresponding to 100%, 20%, 10%, 5% and 1% of olive oil in animal feed) was applied in order to determine this technique's limit of detection. Except from the above mentioned, DNA from *Olea europaea* leaves was also isolated so that it could be used as a positive control. Leaves were chosen as a DNA source because of their higher DNA extraction yields and easier DNA isolation.

### DNA extraction:

For the recovery of high quality olive tree DNA, extraction took place by using two different commercial extraction kits (Quiagen and r-Biopharm) according to their manufacturers' protocols and a comparative study was made. In order to achieve better extraction performance, samples were manipulated not only per se, but also after soxhlet extraction for a more concentrated and clean oil content. In this case (fat content after soxhlet extraction) or when pure olive oil was used for the isolation of DNA, successive centrifugation steps took place in order to acquire an adequate amount of pellet (plant cells and other debris of plant origin) that then went through the DNA extraction process.

### Polymerase Chain Reaction:

Subsequent PCR was designed by using universal DNA primers amplifying a noncoding region, the chloroplast trnL (UAA) intron which varies in size depending on the plant. Its sequences have been widely used for reconstructing phylogenies between closely related species or for identifying plant species<sup>[1,2]</sup>. Olive oil traceability could be based on this intron's polymorphism. For *Olea europaea* it has a size of 548 bp. Other plant species result in PCR products of different DNA size. For instance, soy has an intron size of 585 bp. The primers' sequences (Spaniolas et al. 2008) are:

**A1:CGAAATCGGTAGACGCTACG**

**A2: GGGGATAGAGGGACTTGAAC**

In every PCR reaction the following reagents and the applied PCR program are presented in the Table 1.

**Table 1:** PCR experimental conditions used and The PCR program applied was:

| REAGENT           | VOLUME (μl)                                                 |                  |           |
|-------------------|-------------------------------------------------------------|------------------|-----------|
| Buffer            | 5                                                           | 95 °C for 10 min |           |
| MgCl <sub>2</sub> | 5                                                           | 95 °C for 30 sec |           |
| dNTPs             | 1                                                           | 50 °C for 30 sec | 40 cycles |
| Primer Plant A1   | 1,5                                                         | 72 °C for 1 min  |           |
| Primer Plant A2   | 1,5                                                         | 72 °C for 10 min |           |
| DNA Polymerase    | 0,25 (AmpliTa <sub>q</sub> ) , [or 0,50 (Ta <sub>q</sub> )] | 4 °C constantly  |           |
| DNA               | 5                                                           |                  |           |
| H <sub>2</sub> O  | 30.75                                                       |                  |           |

Apart from the samples, some controls were also applied in order to ensure that PCR has worked properly. Except from the positive control (mentioned previously) an extraction blank and amplification blank were necessary in order to ensure that at any stage of the process no contamination took place (Taberlet et al. 2007) . If the extraction blank or the amplification blank give birth to a DNA band at the end, then during DNA extraction or during the preparation of the PCR reactions something went wrong and plant DNA from the environment or from other samples was present. In addition, controls were added that check for inhibition of the PCR by substances present in the DNA extract, by spiking samples with olive tree DNA.



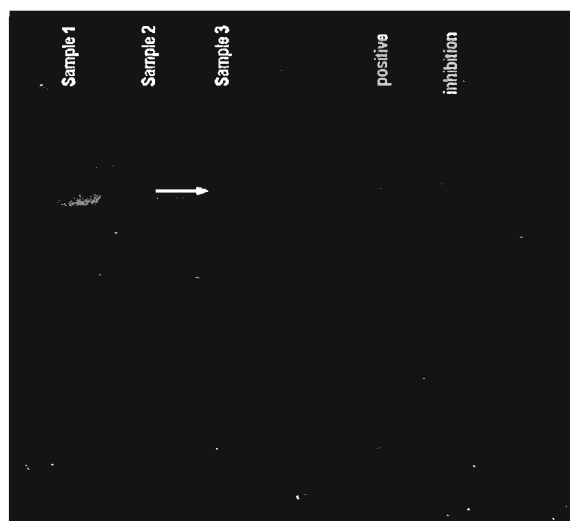
### Gel Electrophoresis:

Visualization of the results was performed by gel electrophoresis of the PCR products. Each plant species' was separated on a DNA size basis and olive tree DNA could be identified after comparison of the amplicon size with a suitable ladder. For best separation results, agarose gel was made very dense and long. Electrophoresis was applied at 120 Volt for approximately 100 min and DNA bands were visible when exposed to UV light.

### 3. Results and Discussion

DNA extraction was complicated and sometimes problematical due to the oily texture of the samples. The 2 commercial DNA extraction kits did not differentiate regarding DNA yield and DNA purity. The amount of isolated DNA was increased after consecutive centrifugations of the olive oil. This can be explained by the fact that plant debris and cell residues were condensing into pellets that were then all resuspended in the same extraction buffer and treated as one sample.

**Figure 2:** DNA bands after PCR and agarose gel electrophoresis. From the right to the left: Inhibition control, Positive control (*Olea europaea* DNA obtained from olive tree leaves), DNA ladder, and 3 fodder samples. White arrows indicate DNA from olive oil and red arrows DNA from other plants.



PCR did not always give the expected results because of impurities inhibiting the reaction. Hence many repetitions were made in order to achieve having no contamination, no inhibition and the production of amplicons corresponding to olive tree. DNA gel electrophoresis revealed that, except from *Olea europaea*, other plant species such as soy (amplicon size 585 bp) gave DNA bands (see Figure 2, bands indicated by red arrows). Occurrence of multiple bands was something normal since several plant materials took part for the production of animal feed. Olive oil was detected at a percentage as low as 20% (v/w). Smaller amounts could not be recovered and amplified by PCR. Therefore the limit of detection was determined as 20% (Figure 3). Variations and modifications on DNA extraction and PCR protocols did not result in higher sensitivity.

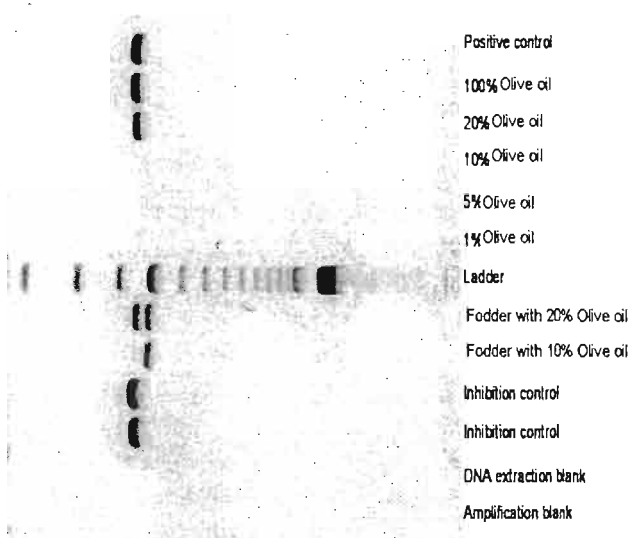


**Figure 3:** Visualization of results after gel electrophoresis. Above the ladder: 100%, 20%, 10%, 5% and 1% of olive oil in animal feed (for the determination of this technique's limit of detection). Olive oil was not detected when it was less than 20% (v/w). Below the ladder: samples compared to PCR controls.

A limit of detection equal to or lower than 0.5% (v/w) is necessary in order for this method to be used for quality control applications. Consequently improvements, optimization and standardization of this method need to be done. Nonetheless, this study indicates that it is a first step towards establishing a sensitive DNA-based analytical assay for the detection of olive oil in food.

#### 4. Conclusion

There is an international trend towards the enrichment of novel and functional foods with olives and olive oil. The identification of olive oil presence in complex foodstuff could be accomplished by the use of molecular techniques. The detection limit of the method developed by Food Allergens Lab has been determined at 20% (v/w). This new technique is currently used more extensively in many food matrices and the results are indicative that it could be exploited for quality control applications provided that it can be further optimized. It has been already presented excessively in two recent international conferences: at the 3<sup>rd</sup> Greek Lipid Forum on June in Athens and at the 10<sup>th</sup> Chemistry Conference of Greece and Cyprus on July in Heraclion.

#### Acknowledgment

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## **L'huile d'olive aromatisée : Elaboration et évolution des caractéristiques chimiques en fonction du stockage**

Grati Kamoun Naziha, Amani Rebai, M.T Hamdi et M.N Arous

### **Résumé**

Devant le manque de diversification de la gamme commerciale de l'huile d'olive tunisienne mise sur le marché, nous nous sommes proposé d'élaborer une nouvelle gamme d'huile d'olive de qualité, ayant des vertus nutritionnelles et thérapeutiques, « huiles d'olive aromatisées ». Ces huiles sont obtenues par l'addition de plantes aromatiques et des arômes naturels. Elles sont gardées dans un endroit frais à une température moyenne de 20 °C et leur stabilité oxydative en fonction du temps de stockage a été suivie.

Les résultats obtenus montrent que l'addition des plantes aromatiques entraîne une augmentation des pigments (chlorophylles et carotènes) et des antioxydants (polyphénols et tocophérols). L'huile aromatisée au romarin est la plus riche en ces pigments et en tocophérols. Pour les teneurs en polyphénols, les huiles aromatisées à la lavande et au basilic possèdent les teneurs les plus élevées. Par contre, l'aromatisation n'a pas influencé significativement la composition acide étant donné que ce paramètre dépend essentiellement du génotype.

Des tests de préférence réalisés auprès de 300 consommateurs représentant différentes catégories d'âge et de niveau social ont mis en évidence que l'huile aromatisée au citron est la plus appréciée au niveau du goût et de l'odeur alors que l'huile aromatisée au romarin et au thym sont les plus appréciées au niveau de la couleur. Par ailleurs, l'étude de la stabilité oxydative des huiles d'olive aromatisées montre que les critères de qualité (acidité, K270 et indice de peroxyde) augmentent au cours du stockage alors que la teneur des composés mineurs (polyphénols, tocophérols, chlorophylles et carotènes) diminuent. Ce sont les huiles aromatisées au romarin qui présentent une stabilité oxydative plus élevée par rapport aux huiles témoins et les huiles additionnées par d'autres arômes.

**Mots clés:** Huile d'olive, aromatisation, stabilité oxydative, stockage.

## **Flavoured Tunisian Olive Oils: Production and Study of the physicochemical characteristics evolution during the storage**

### **Abstract**

The aims of the present work were to produce several flavoured olive oils and to evaluate the physico-chemical characteristics and oxidative stability change of these oils during the storage

The aromatized olive oils that present the dietetic and therapeutic virtues were prepared by maceration for 10 days of fresh selected Mediterranean aromatic plant (3% w/w of rosemary, lavender, lemon, basil and thyme) and addition of certain natural extract aromas plant. Oils samples were kept in glass bottles and stored at fresh place at 20°C.

The resistance to oxidation of these selected flavoured oils was compared to a control samples by measuring PV, K232 and K270 values and change in chlorophyll, carotenes and polyphenols contents during storage. Obtained results confirmed the previous ones and demonstrated that addition of aromatic plants causes a slight increase in free acidity and viscosity, no significant change in the acid composition but a significant enhances pigment and antioxidants content.

Moreover, the results show that rosemary olive oil is the richest aromatized olive oil in the pigment and tocopherols. Concerning the polyphenols, the flavoured olive oils prepared with the addition of lavender and basil have the highest content.

A flavoured olive oil should not only satisfy the sensory requirements of consumers but also should present some other qualities appreciated in the food market, e.g. improved keeping quality compared to that of the plain oil. Furthermore, the investigation beside 300 consumers demonstrated that aromatized olive oil with the citron was the most appreciated.

The following of the change of oxidative stability and quality criteria's (acidity, PV, K232 and K270) of flavored olive oils show the increase of these parameters during the storage. However, we observed a significant reduce of pigment and antioxidants content. A rosemary flavoured olive oil was most stable against oxidation during the storage.

**Key words:** olive oil, aromatization, oxidative stability, storage

## **Thème 6**

**Nouveaux usages et alternatives de valorisation des produits de l'olivier (nutrition, santé, cosmétique, énergie, environnement)**

## **Présentation du projet RESOLIVE pour la production d'énergie renouvelable par digestion anaérobie des margines et des margions de l'industrie oléicole en Méditerranée**

Bárbara De Mena Pardo, Dr. Gerhard Schories

### **Résumé**

L'UE comprend les pays principaux des producteurs d'huile d'olive, un secteur qui a un potentiel énorme pour l'implémentation des énergies renouvelables. Les activités prévues pour le RESOLIVE projet, subventionné sous FP7 dans le cadre du thème <<Recherche au profit de SMEs>> s'adressent directement à l'adaptation de solution d'énergies renouvelables pour l'industrie d'huile d'olive. Un objectif principal de ce projet sera la définition des conditions spécifiques pour l'implémentation des solutions des énergies renouvelables dans l'industrie d'huile d'olives. Dans RESOLIVE, des tests laboratoires sur la digestion anaérobique seront exécutés pour optimiser les techniques actuelles pour la production de biogaz. Ce papier présentera le progrès obtenu, y compris une analyse des résultats.

### **Anaerobic digestion of olive mill wastewater and 2-phase olive mill residues in the framework of the FP7 project RESOLIVE (Adaptation of renewable energy solutions for the olive oil industry)**

### **Abstract**

The EU comprises leading producer countries of olive oil, a sector that has a huge potential for the implementation of renewable energies. The activities carried out in the project RESOLIVE, funded under FP7 in the "Research for the benefit of SMEs" scheme, directly address the adaptation of renewable energy solutions for the olive oil industry. The one of the main objectives of this project is defining specific conditions for the implementation of renewable energy solutions in the olive oil industry. Within RESOLIVE a full program of laboratory scale tests on anaerobic digestion is being carried out in order to optimize the existing techniques for biogas production. This paper will present the progress obtained, including an analysis of the results.

## Medicinal properties of olive leaves phenolic compounds in cholesterol fed rats

Jemai Hedya, Ines Feki, Mohamed Bouaziz, Abdelfatteh El Feki, Sami Sayadi

### Abstract

The positive correlation existing between phenolic compounds consumption and cardiovascular diseases decreased prevalence is well established. This fact is related to the polyphenols capacity in reducing some cardiovascular risk indicators mainly hypercholesterolemia and LDL- C oxidation. Indeed, the medicinal properties of phenolic compounds are due to their antioxidant effects. The present study was designed to test the lipid- lowering and antioxidant activities of olive leaves polyphenols in hypercholesterolemic diet fed rats. Our results show that olive leaves polyphenols have significantly restored the lipid metabolism, the hepatic antioxidant enzymes activities and the histological organisation of the heart, the liver and the aorta. These findings highlight the olive leaves and confirm that olive by- products polyphenols beneficially affect the risk factor of cardiovascular diseases.

**Key words:** Antioxidant, Cardiovascular diseases, Hypercholesterolemia, Olive leaves, Phenolic compounds

### Propriétés médicinales des composants phénoliques contre le cholestérol pour l'alimentation des rats nourris avec des feuilles d'olivier

### Résumé

La corrélation positive qui existe entre la consommation des composés phénoliques et la diminution de la prévalence des maladies cardiovasculaires est bien établie. Cet effet est lié principalement au pouvoir des polyphénols à modérer certains indicateurs de risque cardiovasculaire, principalement l'hypercholestérolémie et l'oxydation des LDL-C. En effet, les propriétés médicinales des composés phénoliques sont dues à leurs effets antioxydants. La présente étude a été conçue pour tester les activités antioxydantes et hypo- lipidique des polyphénols issus des feuilles de l'olivier chez des rats hypocholestérolémiques. Nos résultats montrent que ces polyphénols ont significativement rétablie le métabolisme lipidique et les activités des enzymes antioxydantes hépatiques. Ces résultats mettent en valeur les feuilles de l'olivier et confirment leur implication dans la diminution de l'incidence des facteurs de risque des maladies cardio-vasculaires.

**Mots-clés:** Antioxydant, Maladies cardiovasculaires, Hypercholestérolémie, Feuilles de l'olivier, Composés phénoliques

### 1. Introduction

Atherosclerosis, the principal contributor to the pathogenesis of myocardial and cerebral infarctions, is known to be one of the leading causes of morbidity and mortality worldwide and it is essentially caused by hyperlipidemia resulting from lipid metabolic changes (Aygustin (1999)). Phenolic compounds have been reported to prevent LDL oxidation *in vitro* and show marked hypolipidemic activity *in vivo*, suggesting the effectiveness of polyphenols for the prevention of atherosclerosis (Dufull et al (2003)). Olive leaves are considered as a cheap raw material which can be used as a useful source of high-added value products, especially phenolic compounds (Briante et al (2002)). The main phenolic compounds in olive leaves are the glycosylated forms of oleuropein and ligstroside (Amro et al (2002)). Numerous *in vitro* studies have shown that oleuropein and its derivatives have various biochemical roles (Mercier (1997), Amro et al. (2002)). Moreover, it was reported that these polyphenols are able to prevent low- density lipoprotein oxidation Amro et al (2002). Hydroxytyrosol is the principal oleuropein derivative; it is used to prevent diseases because it is endowed with an

important antioxidant property (Capasso (1999)). Moreover, oleuropein aglycone is obtained by enzymatic hydrolysis of oleuropein by  $\beta$ -glucosidase (Konno (1999)). Oleuropein aglycone has many biological activities and it has been shown that it is more efficient than hydroxytyrosol and oleuropein as anti-proliferative and pro-apoptotic agent in the prevention of some cancer Menendez (2007). Therefore, the aim of this study was to investigate the effects of these olive leaf extracts on serum lipid levels and antioxidant enzymes activities in rats fed a cholesterol-rich diet.

## 2. Materials and methods

### 2.1. Preparation of the phenolic- rich extracts

Samples of fresh green leaves were dried and powdered (50 g), and extracted with a mixture of methanol and water (200ml, 4:1 v/v). Then, the extract was concentrated by evaporation to dryness. The acid hydrolysis was carried out as following: one gram of the ethyl acetate extract was dissolved in 10 ml of a MeOH/H<sub>2</sub>O (4:1) mixture in sealed vial. The solution was hydrolysed at 100 °C for 1 h using 5ml of a 2 M HCl solution (Prolabo, France). After hydrolysis, the reaction was cooled and diluted with water (10 ml) and the hydrophobic fraction was extracted by a separatory funnel, three times with 25 ml of ethyl acetate, which was subsequently removed by evaporation. Finally, the enzymatic hydrolysis was carried out using  $\beta$ - glucosidase from almond (Sigma) as previously described (Bouaziz et Sayadi, 2005). A reverse-phase high-performance liquid chromatography technique was developed to identify and quantify the major phenolic compounds.

### 2.2. Experimental design

Fifty male Wistar rats weighing between 160 $\pm$  10g were used. The animals were maintained in stainless steel cages under controlled conditions (23 $\pm$ 1°C, 12h light–dark cycle, and had access to a standard diet (SICO, Sfax, Tunisia) and drinking water. The animals were housed according to the EEC 609/86 Directives. They were divided into 5 groups (n=10). **Group 1** was fed a standard laboratory diet (CD). **Group 2** was fed a cholesterol- rich diet (HCD) (normal diet + 1% cholesterol and 0.25% bile salts). **Groups 3, 4 and 5** received HCD with, respectively; olive leaf, acid hydrolysate and enzymatic hydrolysate extracts (3mg/kg b.w oleuropein, hydroxytyrosol and oleuropein aglycone, respectively) which were dissolved in drinking water. The experiment was conducted over a period of 16 weeks and at the end, rats were killed by decapitation and blood samples were collected and stored at -80 °C.

### 2.3. Serum lipids

Concentrations of total cholesterol (TC), total triglycerides (TG), low-density lipoprotein (LDL-C) and high density lipoprotein HDL-C in serum were determined by enzymatic colorimetric methods using commercial kits (Kyokuto Pharmaceutical, Japan).

### 2.4. Antioxidant enzyme activities

CAT and SOD activities were evaluated in liver tissue homogenate. CAT activity was measured using the method of Regoli and Principato (Regoli et Principato, 1995) based on the spectrophotometric measure of the decomposition rate of H<sub>2</sub>O<sub>2</sub> at 240 nm for 1 min. One unit of CAT was defined as the 1 mole H<sub>2</sub>O<sub>2</sub> decrease/ ((mg protein) min). SOD activity was measured according to the method of Marklund and Marklund based on pyrogallol oxidation by superoxide anion (O<sub>2</sub><sup>-</sup>) and its dismutation by SOD (Marklund et Marklund, 1974). One unit was determined as the amount of enzyme inhibiting the oxidation of pyrogallol by 50%. The activity was measured at 440 nm expressed as units/mg protein.

### 2.5. TBARS assay

TBARS (thiobarbituric acid-reactive substances) concentrations were measured in the liver homogenates using the method of Park et al. (Park et al. (2002)). The homogenate solution was mixed with 600 $\mu$ l of distilled H<sub>2</sub>O and 200 $\mu$ l of 8.1% (w/v) SDS. The reaction mixture was heated at 95 °C for 1 h after the addition of 1.5ml of 20% acetic acid (pH 3.5) and 1.5ml of 0.8% (w/v) TBA. After cooling the reaction, 5.0 ml of butanol: pyridine (15:1) were added. After centrifugation, the resulting coloured layer was measured at 532 nm using malondialdehyde (MDA) as standard.



## 2.6. Statistical analysis

Data are given as means  $\pm$  S.E. Statistical differences were calculated using a one-way analysis of variance (ANOVA), followed by Student's t-test. Differences were considered significant at  $P < 0.05$ .

## 3. Results and discussion

### 3.1. Olive leaves and hydrolysate extracts characterization

Fig.1 shows a high concentration of oleuropein (4.32 g/100g dry weight). The identification of oleuropein was based on of the chromatographic retention time. Various extraction methods were used to extract this compound and its amount varied with the olive cultivar (Bouaziz et Sayadi, 2005). Acid treatment of the leaf extracts induced hydrolysis of oleuropein. The most notable effect seen was the increase in hydroxytyrosol concentration. It is well known that acid hydrolysis of olive leaf extract leads to a high quantity of hydroxytyrosol (Bouaziz et al. (2006)). HPLC profile of acid hydrolysate extract showed that hydroxytyrosol was the major compound which concentration reached 1.4g/100 g dry weight.

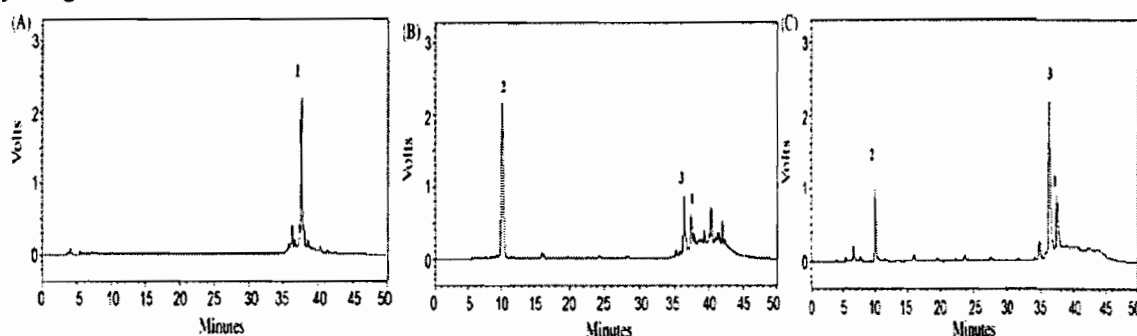


**Figure 1:** HPLC chromatograms at 280 nm of olive leaf extracts (A) olive leaf extract after acid hydrolysis (B) and olive leaf extract after enzymatic hydrolysis (C). (1): Oleuropein; (2) hydroxytyrosol; (3) oleuropein aglycone.

Moreover, the biotransformation of olive leaf extract by  $\beta$ - glucosidase carried out at 37 °C and pH 7 showed a small amount of hydroxytyrosol and high concentration of oleuropein aglycone after 2 h of incubation time. The concentration of the latter reached 3.82 g/100 g dry weight. The aglycone is well known as pharmacologically active molecule. In fact it is described for its potential application as an antioxidant and antimicrobial agent in some fairly common diseases of olive trees (Bouaziz et al. (2006)).

### 3.2. Serum lipids

After the treatment, the TC, TG and LDL-C concentrations of rats fed a cholesterol-rich diet (HCD) showed a significant increase compared with control group (CD) (Fig. 2). However, a decrease of HDL-C concentration of rats in the HCD group was observed ( $P < 0.05$ ). Rats orally administrated with olive leaf, acid hydrolysate and enzymatic hydrolysate extracts had lower concentrations of TC, TG and LDL-C than rats receiving an HCD. Olive leaves extracts significantly reduced the TC, TG and LDL-C levels. Moreover, the treatment of HCD fed groups with phenolic extracts significantly re-established their HDL-C level ( $P < 0.05$ ). Lowering levels of TC and LDL-C and improving level of HDL-C has been linked to a lower risk of CHD (Libby et al, 2000). In fact, it was reported that the decrease in LDL-C concentration and the increase of HDL-C level could fasten the removal of cholesterol from peripheral tissues to liver for catabolism and excretion (Young et al, 2004). In addition, the increase in HDL-C concentration could protect the LDL against oxidation *in vivo* because lipids in HDL are preferentially oxidized before those in LDL (Young et al. (2004)). Therefore, the results of the present study indicate that olive leaf phenolics may reduce the incidence of CHD.

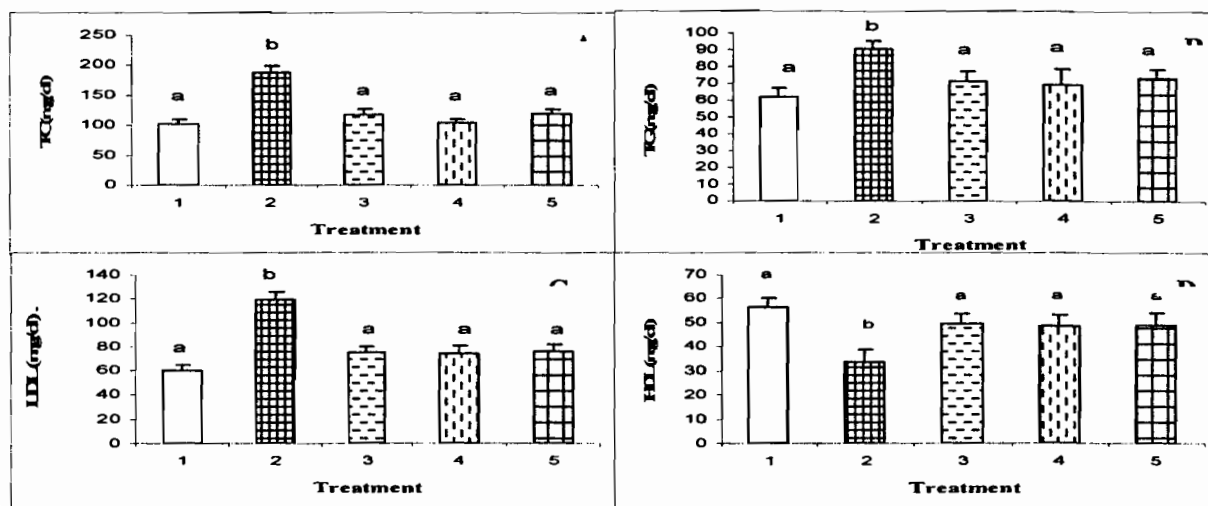


**Figure 2:** Effects of olive leaf extracts on rat total cholesterol (TC) (A), triglycerides (TG) (B), low-density lipoprotein cholesterol (LDL-C) (C), and high-density lipoprotein cholesterol (HDL-C) (D) levels. Group: 1, CD (standard diet); 2, HCD; 3, HCD+ olive leaf extract; 4, HCD+ acid hydrolysate extract; 5, HCD+ enzymatic hydrolysate extract. Each bar represents mean  $\pm$  S.E. from 10 rats. Bars with different letters differ significantly;  $P < 0.05$ .

### 3.3. Hepatic antioxidant enzyme activities

The CAT and SOD activities significantly decreased in livers of rats fed a cholesterol-rich diet compared to those fed a control diet. The decrease was significantly restored ( $P < 0.05$ ) in the presence of the olive leaves and the hydrolysate extracts (Fig. 3). By the way, it has been reported that a high fat diet lowered the activities of antioxidant enzymes, which is possibly due to their increased implication in fighting excessive oxidative stress in hypercholesterolemic rats (Wang et al. (2008)). The increase of hepatic antioxidant enzyme activities and the serum antioxidant potential observed in olive leaf phenolic-supplemented rats may be due to the removal toxic reactive species resulting from the high cholesterol feeding.

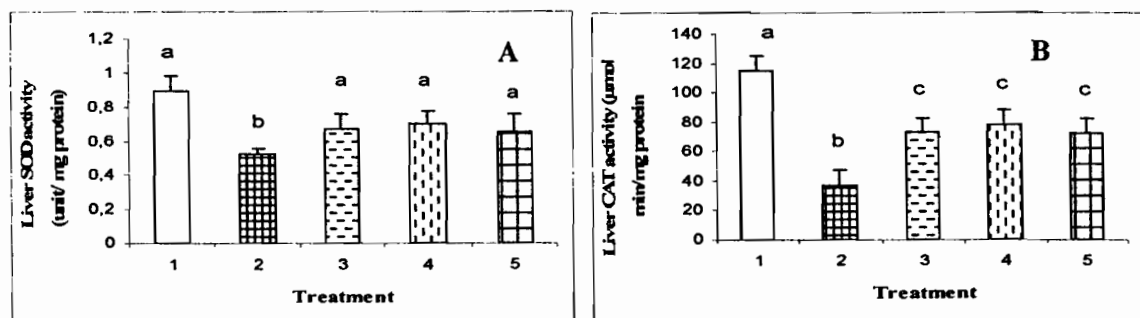


**Figure 3:** Effects of olive leaf extracts on SOD (B) and CAT (A) activities in liver. Group: 1, CD (standard diet); 2, HCD; 3, HCD+ olive leaf extract; 4, HCD+ acid hydrolysate Extract; 5, HCD+ acid hydrolysate extract. Each bar represents mean  $\pm$  S.E. from 10 rats. Bars with different letters differ significantly;  $P < 0.05$ .

### 3.4. TBARS levels

The olive leaf-deriving preparations were revealed to slow down the lipid peroxidation (Fig. 7). The TBARS levels were significantly increased ( $P < 0.05$ ) in liver, heart, kidneys and aorta within animals fed high cholesterol diet compared to the control diet group. This increase was significantly reduced after treatment with phenolic extracts from olive leaves. TBARS are the major oxidation products of peroxidized polyunsaturated fatty acids; thus, increased TBARS content is an important indicator of lipid peroxidation (Prasad (1999)).

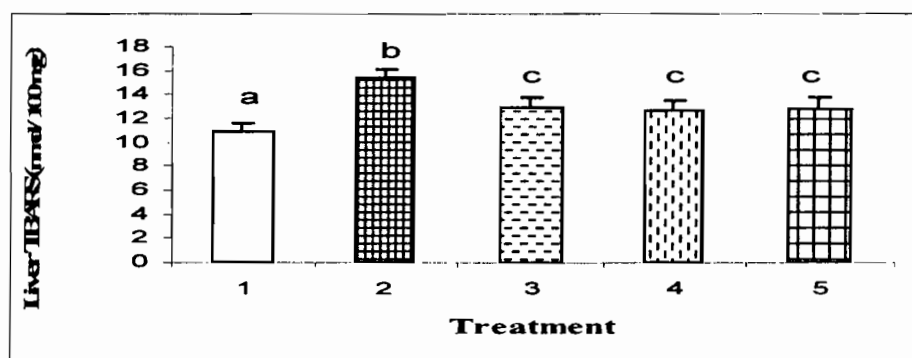


**Figure 4:** Effects of olive leaf extract (3) and hydrolysate extracts (4 and 5) on rat liver TBARS levels. Each bar represents mean  $\pm$  S.E. from 10 rats. Bars with different letters differ significantly;  $P < 0.05$ .

The reduced lipid peroxidation observed in the olive leaf extract-treated animals may be attributed to the important role of oleuropein, oleuropein aglycone and hydroxytyrosol as antioxidants. This power may be attributed to their ability to decompose free radicals by quenching reactive oxygen species and by trapping radicals before reaching their cellular targets (Srinivasan et al. (2007)).

#### 4. Conclusion

By the presented results, we demonstrate that polyphenols recovered from olive leaf extracts, oleuropein, hydroxytyrosol as well as oleuropein aglycone, exhibited a pronounced hypolipidemic and antioxidant effects. Subsequently, olive tree by-products are a source of antioxidants able to reduce the frequency of cardiovascular diseases.

#### Acknowledgements

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## Olive orchard amended with olive mill wastewater: Effects on olive fruit and olive oil quality

Mechri Baligh, Manel Issaoui

### Abstract

The aim of this work was to study the effects of agronomic application of Olive mill wastewater (OMW) in a field of olive trees on olive fruit and olive oil quality. Agronomic application of OMW increased significantly the fungal: bacteria ratio, whereas the root colonization and the photosynthetic rates decreased significantly. Consequently, the oil content expressed as a percentage of dry weight, decreased significantly after agronomic application of OMW. Land spreading of OMW altered the relative proportion of individual olive fruit sugar and decreased significantly the nitrogen (N) and phosphorus (P) of the fruit. A significant increase was observed in total phenol content of oil after agronomic application of OMW.  $\alpha$ -Tocopherol content, on the contrary, decreased with OMW application. The fatty acid composition of the oil was not affected by the treatments. To our knowledge, this is the first report of change in the olive fruit and olive oil quality following agronomic application of OMW.

**Mots clés:** Olive mill wastewater, olive oil,  $\alpha$ -Tocopherol, fungal: bacteria ratio.

## Contrôle des pathogènes d'olivier en utilisant les eaux usées du moulin et quelques uns de ses dérivés chimiques et biologiques

A. Rhouma, M.A. Triki, K. Gargouri, T. Yangui, A. Dhouib and S. Sayadi

### Résumé

La margine et certains de ses dérivés chimiques et biologiques ont été testé *in vitro* et *in vivo* contre quelques agents phytopathogènes de l'olivier (*Rhizoctonia bataticola*, *Fusarium solani*, *Verticillium dahliae* et *Pseudomonas savastanoi* pv. *savastanoi*).

L'incorporation de la margine dans le milieu de culture a permis d'exercer un effet fongicide contre les champignons testés. Les polyphenols (hydroxytyrosol) possèdent des activités antifongiques et bactéricides.

Les bactéries isolées à partir de la margine se sont montrées efficaces contre les champignons et les bactéries phytopathogènes *in vitro* et *in vivo*. L'identification moléculaire basée sur le séquençage l'ARNr 16S a permis d'identifier plusieurs souches de *Bacillus* résistantes aux polyphenols.

Les essais menés *in vivo* ont mis en exergue l'effet de la margine dans la réduction des pourcentages d'attaque causés par *Rhizoctonia bataticola* et *Fusarium solani*. Les polyphenols se sont révélés très efficaces en inhibant complètement la formation de tumeurs causées par *Pseudomonas savastanoi* pv. *savastanoi*.

**Mots clés :** *Olea europea*, margine, Polyphenols, hydroxytyrosol, agents phytopathogènes.

## Control of olive tree pathogens using olive mill waste water and some of its chemicals and biological derivatives

### Abstract

The olive mill waste water (OMW) and some chemical and microbial derivatives have been tested *in vitro* and *in planta* experiments against some plant pathogens of olive tree (*Rhizoctonia bataticola*, *Fusarium solani*, *Verticillium dahliae* and *Pseudomonas savastanoi* pv. *savastanoi*). The incorporation of the OMW in the culture medium resulted in a fungicidal effect against the tested plant pathogenic fungi. Polyphenols (hydroxytyrosol) have shown a fungicide and fungistatic activity. On the other hand, the application of the volatile fraction resulted in an inhibition of the mycelia growth of the tested fungi as well as bactericide effect against *Pseudomonas savastanoi*. Indigenous bacterial strains isolated from Olive Mill Waste Water were proved efficient in the control of olive tree pathogens *in vitro* and *in planta*. Molecular identification using 16S sequencing has proved the identification of several strains of *Bacillus* resistant to polyphenols. The *in vivo* experiments have shown a very substantial effect of the OMW basing on the significant reduction of plants attacked by *Rhizoctonia bataticola* and *Fusarium solani*. Polyphenols were proved very efficient in preventing tumour formation caused by *Pseudomonas savastanoi* pv. *savastanoi*.

**Keywords:** *Olea europea*, Olive mill waste water, Polyphenols, Flavonoides, Plant pathogens.

## Ethanol Production from Olive Cake Biomass Substrate

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### Abstract

The inexpensive production of sugars from lignocellulose is an essential step for the use of biomass to produce fuel ethanol. Olive cake is an abundant by-product of the olive oil industry and represents a potentially significant lignocellulosic substrate for bioethanol production in the Mediterranean basin. Converting olive cake to ethanol could add further value to olive production. In the present study, olive cake was evaluated as a feedstock for ethanol production. To this end, the lignocellulosic component of the olive cake was dilute-acid pretreated at 13.5% olive cake loading with 1.75% (w/v) sulfuric acid and heating at 160°C for 10 min., followed by chemical elimination of fermentation inhibitors. Soluble sugars resulting from pretreatment were fermented using *E. coli* FBR5, a strain engineered to selectively produce ethanol. 8.1 g ethanol/L was obtained from hydrolysate containing 18.1 g soluble sugars. Increasing the pretreatment temperature to 180°C resulted in failed fermentations, presumably due to inhibitory by-products released during pretreatment.

**Mots clés:** Ethanol, lignocellulose, fermentation, olive cake.

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## **Agrochemical characteristics of the compost made from “Alperujo” mixed with other plant and animal wastes**

Juan Cegarra, J.A. Alburquerque, G. Tortosa, G. Ait-Baddi

### **Abstract**

Around four millions tons/year of the wet solid lignocellulosic olive-mill waste called “alperujo” (AL) is generated by the olive oil extraction industry, due to the generalised use of the two-phase centrifugation method in Spain. Its high non-stabilised organic load includes phytotoxic compounds such as fats, organic acids and a short but active content of water soluble phenols, which make AL unsuitable for direct soil application.

Co-composting AL with other solid and liquid wastes, either from plant or animal origin, has been successfully performed, thus the procedure is nowadays considered a suitable alternative for AL disposal and leads to obtain non-phytotoxic soil organic amendments and fertilizers rich in partially humified lignocellulosic matter, as revealed by the maturity and humification indices. They usually contain abundant potassium, but also changeable amounts of other nutrients depending on the materials added to the AL.

**Key words:** Agrochemical characteristics, two-phase olive mill waste « alperujo », co-composting

## **Caractéristiques agrochimiques du compost fabriqué à partir du “Alperujo” mélangé avec autres déchets d’origine végétale et animale**

### **Résumé**

En Espagne, la production des déchets lignocellulosiques appelés communément “alperujo” (AL) générés par les huileries d’olives est très importante (à peu près 4 millions de tonnes/an). Ces déchets, résultent de l’extraction d’huile d’olive par des systèmes généralisés de centrifugation à deux phases. Leur richesse en matière organique non stabilisée leur confère un caractère phytotoxique, ce qui empêche leur application directe au sol. Le co-compostage de ces déchets en mélange avec d’autres déchets organiques d’origine animale ou végétale présente une meilleure solution pour limiter le pouvoir toxique de ces déchets et pour produire un amendement et un fertilisant organique riche en matière organique partiellement humifiée. A ces caractéristiques, s’ajoute aussi l’enrichissement en potassium et en d’autres nutriments, dépendamment de l’origine du matériel composté avec AL.

### **1. Introduction**

The main by-product generated by the Spanish olive oil industry is “alperujo” (AL), which is annually produced in huge amounts (approximately four million tons/year) because the two-phase centrifugation method is used by more than 90% of olive mills. Apart from the treatments to recover its residual oil and further use for thermal and electrical energy generation, AL composting is increasingly considered a good way of improving profits of the olive oil production process, leading to obtain organic amendments and fertilizers containing “humic-like” compounds, which are known to be the most active fraction in compost and positively affect soil ecology, structure, fertility and productivity.

AL presents high contents of lignin, cellulose and hemicellulose, a rather scarce nutrient content, unbalanced C/N ratio, potentially phytotoxic compounds such as polyphenols, and slightly acidic pH values (Alburquerque et al., 2004). In addition, it is a scarcely porous material very susceptible to compaction, which is a result of the conjunction of its high moisture content, small particle size and high lipid content; all these properties being unfavourable for a correct distribution of air, water and dissolved substances during composting. Therefore, bulking agent addition is necessary to improve the AL physical structure and to amend its nutrient content, in order to ensure a proper composting performance.



In this report, the most relevant results gained in successive co-composting experiments of AL mixed with several organic wastes as bulking agents, and the main characteristics of the composts obtained are summarized.

## 2. Materials and methods

### 2.1. Characteristics of raw materials

Three agricultural wastes and four animal manures were employed as bulking agents of the AL in several composting experiments (Table 1), the former had the highest C/N and the lowest pH and EC values whereas the latter generally showed the lowest lignin and the highest nutrient contents. These materials clearly showed lower moisture and lignin contents than AL (Table 3), but generally higher pH values.

**Table 1:** Main characteristics of bulking agents (g/kg, dry weight basis): CW, cotton waste ; GS, grape stalk ; OL, olive leaf ; FCB, fresh cow bedding ; HM, horse manure ; PM, poultry manure and SM, sheep manure.

| Parameters                            | Agricultural wastes |       |       | Manures |       |       |       |
|---------------------------------------|---------------------|-------|-------|---------|-------|-------|-------|
|                                       | CW                  | GS    | OL    | FCB     | HM    | PM    | SM    |
| Moisture (% f.w.)                     | 11.5                | 5.9   | 7.3   | 46.1    | 19.5  | 20.1  | 38.5  |
| pH <sup>1</sup>                       | 6.8                 | 4.4   | 5.6   | 7.5     | 7.5   | 7.5   | 8.5   |
| EC <sup>1</sup> (dS m <sup>-1</sup> ) | 4.1                 | 4.2   | 1.7   | 7.5     | 9.3   | 8.5   | 11.3  |
| Organic matter                        | 933.0               | 934.1 | 906.0 | 664.0   | 578.0 | 805.5 | 456.5 |
| Lignin                                | 232.0               | 262.3 | 319.1 | 185.0   | 201.1 | 129.7 | 211.4 |
| Total-N                               | 21.3                | 8.0   | 13.3  | 19.4    | 15.3  | 32.3  | 17.7  |
| C/N ratio                             | 22.4                | 60.1  | 39.4  | 19.0    | 20.4  | 12.3  | 14.3  |
| P                                     | 1.8                 | 0.6   | 0.8   | 2.5     | 2.3   | 2.2   | 2.2   |
| K                                     | 17.4                | 20.0  | 5.7   | 35.8    | 21.2  | 13.5  | 16.5  |
| Ca                                    | 23.0                | 6.3   | 26.0  | 63.7    | 58.6  | 47.5  | 100.9 |
| Mg                                    | 4.2                 | 1.5   | 2.4   | 8.8     | 14.9  | 5.5   | 18.7  |

<sup>1</sup> water extract 1:10 and EC: electrical conductivity.

### 2.2. Composting performance

Seven mixtures were made by adding the above bulking agents to the five selected AL samples according to the proportion shown in Table 2, later placed in eight trapezoidal piles. Aeration was first supplied by forced ventilation in the pilot-plant experiments (2-4 tons), which was only revealed to be effective when performed in conjunction with mechanical turning. As a result, only mechanical turning was used in the large-scale experiments (piles 6-8, 10-20 tons) in order to reduce operating costs.

**Table 2:** Composition and management of composting substrates.

| Pile    | Composition (%)               |           | Turnings | Forced ventilation | Composting time (weeks) |
|---------|-------------------------------|-----------|----------|--------------------|-------------------------|
|         | Fresh basis                   | Dry basis |          |                    |                         |
| 1       | 92.6 AL1 + 7.4 CW             | (80/20)   | 1        | yes                | 42                      |
| 2       | 94.6 AL2 + 5 GS + 0.4 urea    | (87/12/1) | 3        | yes                | 49                      |
| 3       | 94.6 AL2 + 5 OL + 0.4 urea    | (87/12/1) | 3        | yes                | 49                      |
| 4 and 5 | 90 AL3 + 9 FCB + 1 AL compost | (87/11/2) | 14       | 4 (no)<br>5 (yes)  | 36                      |
| 6       | 91 AL4 + 9 HM                 | (85/15)   | 18       | no                 | 37                      |
| 7       | 52 AL5 + 48 PM                | (37/63)   | 7        | no                 | 38                      |
| 8       | 65 AL5 + 35 SM                | (57/43)   | 7        | no                 | 38                      |

The active phase of composting was considered as finished when the temperature of the piles was close to ambient and re-heating did not occur, at which time air blowing and/or mechanical turning were stopped and the materials left to mature. Complete details of the composting performance and the analytical methods can be consulted in Albuquerque et al., (2009a, b).

### 3. Results and discussion

#### 3.1. Composting progress

Both temperature and pH increases were considered clear indicators of the AL composting progress, low increases being related to deficient substrate aeration conditions. High thermophilic temperatures were quickly recorded in piles containing manures in contrast to piles made of agricultural wastes which showed a long lag-phase (data not shown). This fact must be related to the greater nutrient availability of manures and their inoculum effect, but also to the better oxygen distribution favoured by the more frequent turnings which enhanced the composting process by homogenising and re-distributing of microorganisms, moisture and nutrients. Thus, the composting times were considerably shorter in piles 4-8 (Table 2).

The longer time needed for AL composting in comparison to other substrates was due to the paramount predominance of the lignocellulosic fraction in AL substrate, its main component (lignin) is very difficult to be degraded by microorganisms (Whitney and Lynch, 1996). The two other components, cellulose and hemicellulose, were more extensively degraded than lignin (data not shown). The resultant OM-losses (Fig. 1), calculated as a percentage of the initial content, reflected clear differences between piles: pile 5 showed the highest loss (61%) while pile 3 the lowest (34%). The degradation process reduced the amount of non-humified compounds and also resulted in the release of compounds which probably were incorporated into the humification pathways, leading gradually to more humified and polymerized compounds.

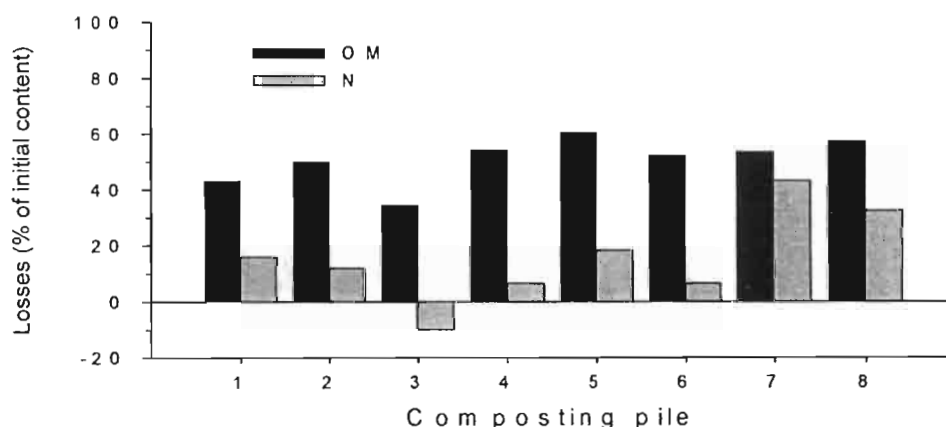


**Figure 1:** Total organic matter (OM) and nitrogen (N) losses at the end of the composting processes.

In all piles, a continuous nutrient concentration effect was recorded as composting progressed due to the mineralization of organic compounds and the subsequent OM-losses. This effect was equally evident for N, even if a clear ammonification process followed by intense ammonia formation and loss by volatilization was generally detected coinciding with the thermophilic period and high pH values (Jeong and Kim, 2001; Liang et al., 2000). As a result, in none of the experiments the  $\text{NH}_4\text{-N}$  decrease was accompanied by a clear increase in  $\text{NO}_3\text{-N}$ , which was only detected at low concentrations at the end of composting (Table 3). The highest total N-loss was detected in pile 7 (Fig. 1) due to the great proportion of poultry manure (rich in  $\text{NH}_4\text{-N}$ ) added to the AL while a final N gain of 10% was registered in pile 3, where the mentioned lowest OM decomposition would have contributed to nitrogen immobilization and subsequent lower ammonia formation and volatilization.

#### 3.2. AL compost characteristics

AL compost showed a pleasantly earthy smell and had alkaline pH, moderate salinity and high OM content, the half being lignin responsible of the relatively high C/N ratio. The end-values obtained for the DH (> 71%) and PAH (> 63%) indices suggest a substantial OM humification and polymerization,

respectively, whereas those of GI indicate a clear detoxification of the initial substrates. The compost also had an acceptable nutrient content, K, Ca and N (mainly organic) showing the most relevant values (Table 3).

In comparison to the raw AL, the compost obtained clearly shows higher nutrient content due to the AL amendment with the bulking agents and as a result of the above mentioned concentration effect. By the contrary, the C/N ratio and contents of fats and water-soluble phenols were much lower in AL compost, accordingly to the mentioned detoxification of the raw materials. When compared with manures of different origin (cow, pig, poultry, sheep and rabbit), organic and inorganic-N, P, Ca and Mg contents were considerably lower in AL compost but that of K was higher. Also, the C/N ratio was higher in compost than in manures in agreement to the higher lignin content in the former.

**Table 3:** Main characteristics of the raw AL, AL compost and manures of different origin (mean values, dry weight basis).

| Parameters                                             | AL (n=5) | AL compost (n=8) | Manures (n=10) |
|--------------------------------------------------------|----------|------------------|----------------|
| pH <sup>1</sup>                                        | 5.1      | 8.8              | 7.8            |
| EC <sup>1</sup> (dS m <sup>-1</sup> )                  | 4.4      | 3.1              | 4.6            |
| Organic matter (g kg <sup>-1</sup> )                   | 929.4    | 804.2            | 756.0          |
| Lignin (g kg <sup>-1</sup> )                           | 364.8    | 402.5            | 167.4          |
| Fats (g kg <sup>-1</sup> )                             | 95.6     | 5.0              | -              |
| Water-soluble phenols (g kg <sup>-1</sup> )            | 11.2     | 3.1              | -              |
| Total-N (g kg <sup>-1</sup> )                          | 12.7     | 22.9             | 31.7           |
| NH <sub>4</sub> <sup>+</sup> -N (mg kg <sup>-1</sup> ) | -        | 130              | 5243           |
| NO <sub>3</sub> <sup>-</sup> -N (mg kg <sup>-1</sup> ) | -        | 77               | 232            |
| P (g kg <sup>-1</sup> )                                | 1.0      | 1.9              | 3.2            |
| K (g kg <sup>-1</sup> )                                | 19.0     | 33.5             | 20.9           |
| Ca (g kg <sup>-1</sup> )                               | 5.9      | 26.7             | 56.4           |
| Mg (g kg <sup>-1</sup> )                               | 2.4      | 5.4              | 9.8            |
| C/N ratio                                              | 42.0     | 18.2             | 12.5           |
| GI (%)                                                 | 0        | 81               | -              |
| DH (%)                                                 | -        | 75.5             | -              |
| PHA (%)                                                | -        | 70.6             | -              |

<sup>1</sup>water extract 1:10, EC: electrical conductivity, GI: germination index, DH: [(HAC+FAC)/TEC]×100, PHA: (HAC/TEC)×100, TEC: total organic carbon extracted in 0.1M NaOH, HAC: humic-like acid carbon and FAC: fulvic-like acid carbon. -: not determined.

The above characteristics make AL compost suitable for agricultural requirements and it has been shown (Albuquerque et al., 2006) that it can be better used than manures as an effective soil amendment due to the strong resistance of its lignocellulosic matrix to edaphic degradation. For plant growth, however, the low nitrogen mineralization rate of AL compost in the short term may require the addition of supplementary nitrogen fertilizers (Albuquerque et al., 2007). In addition, these composts can be classified within the highest quality class according to the limits established for heavy metal concentrations of compost and fertilizer products made from wastes by the Spanish legislation (BOE, 2005), fulfilling the requirements of European Ecolabel criteria for soil improvers and growing media (European Commission, 2001).

#### 4. Conclusions

Both selection of an appropriate bulking agent and mechanical turning were key factors for the proper AL composting performance. The best results were found by combining the use of manure as bulking agent with frequent turnings, which resulted in a clear reduction in the composting time. However, the lignocellulosic nature of AL led to long composting times. The resulting AL compost, free of toxicity, was rich in organic matter substantially humified and polymerized and had an acceptable nutrient content, but rather low in comparison to manures. In addition, AL compost can be considered as an effective product for soil amendment due to the great resistance of its lignocellulosic fraction to the edaphic degradation, but supplementary fertilization with mineral N is required, if used as fertilizer.

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## Valorisation biotechnologique des sous produits de l'olivier par Fermentation en Milieu Solide

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### Résumé

L'oléiculture et l'oléologie ont profondément évolué ces dernières décennies. Cette évolution a notamment entraîné des modifications importantes à tous les niveaux de la filière oléicole. Ainsi, au niveau agronomique, la culture de l'olivier s'oriente vers des productions biologiques avec un minimum d'entrants (engrais, pesticides...); au niveau technologique, les récoltes se sont mécanisées et automatisées permettant de réduire considérablement les délais entre la récolte et la trituration. La généralisation des systèmes de trituration continus a permis de traiter les olives sans délai d'attente. La conjugaison de toutes ces évolutions a conduit d'une part à des huiles d'olive vierges de grande qualité organoleptique et d'autre part à l'obtention de co-produits (margines, grignons, feuilles, bois...) de bonne qualité, ce qui permet d'orienter les recherches vers la valorisation biotechnologique de ces matières premières. Ces produits nouveaux constituent un SOS (Solid Olive Substrate) pour le traitement des margines utilisées comme substrat en biotechnologie pour la culture de champignons filamenteux en Fermentation en Milieu Solide (FMS). Quelques exemples d'applications (production de biopesticides, d'enzymes et des champignons comestibles médicinaux) seront détaillés pour démontrer le potentiel de ces matières premières pour un développement durable du secteur oléicole dans les pays Méditerranéens.

**Mots clés :** Grignons d'olive, margine, FMS, champignons, enzymes.

### Abstract

Oleiculture and oleology have deeply progressed for these last decades. This progress has notably induced important changes in all olive chain levels. Also, in the agronomic level, olive tree culturing is directed towards biologic productions with a minimum of volumes (fertilizers and pesticides); in the technological level, harvests are mechanized and automatized enabling to reduce considerably the time between the harvest and tituration. The generalisation of continuous trituration systems has enabled to treat olives without time out. The conjugation of all these evolutions has led to virgin olive oils of a high organoleptic quality, on the one hand, and to the obtaining of co-products, in the other hand (margins, pomace, leaves, wood...) of good quality, enables to direct researches towards biotechnological valorisation of these raw materials. These new products constitute an SOS (Solid Olive Substrate) for the treatment of the margins used as substrate in biotechnology for the culturing of filamentarous fungi in fermentation in solid areas (FMS). Some application examples (biopesticides production, edible medicinal enzymes and fungi) will be detailed to demonstrate the potential of these raw materials for a sustainable development of the olive sector in the Mediterranean countries.

**Key words:** olive pomace, margins, FMS, fungi, enzymes.

### 1. Introduction: La culture de l'olivier dans les pays Méditerranéens

En l'absence de tout apport extérieur, l'exploitation d'une oliveraie se traduit par un appauvrissement progressif du sol en éléments (C, N, P, sels minéraux) nécessaires à la production des olives et à la biomasse des arbres (Therios, 2006). Une conduite intensive mais écologique et durable des oliveraies ne peut donc s'envisager qu'à la condition de rapporter au sol ces éléments sous la forme des sous-produits de l'exploitation, c'est-à-dire les déchets verts résultants de la taille et les résidus des moulins à huile qui peuvent être pâteux (margions) ou liquides/solides (margines/grignons) en fonction de la technologie utilisée pour extraire l'huile (biphasique ou triphasique).

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Les résidus bruts sortant des chaînes de trituration d'olives ne peuvent cependant être retournés aux champs directement que dans des conditions précises et limitées car ils contiennent des substances phytotoxiques et antimicrobiennes (phénols, acides gras et acides organiques). Leur fort contenu en matières organiques non stabilisées et en matières minérales (N, P, K) représente de toute façon un risque de pollution pour l'environnement (Kapellakis et al., 2008). La nouvelle loi cadre française sur l'eau et les milieux aquatiques parue en 2006 afin de répondre à la directive européenne 2000/60/CE sur l'eau impose d'ailleurs à l'industrie oléicole française de développer de nouvelles voies d'élimination de ces déchets.

Dans cette présentation, les différents procédés d'extraction d'huile d'olive à une ou à deux phases seront évoqués; la nature et la quantité des produits et sous produits obtenus seront décrites; les différents procédés de conservation et de biotransformation des sous produits seront brièvement présentés. Le but de cette tâche est donc de définir dans quelles conditions opératoires des techniques telles que l'ensilage, les fermentations en milieu solide, le compostage et le lombricompostage permettent de stabiliser et détoxifier les résidus de trituration en vue de leur réincorporation sans risque dans les oliveraies. Le degré de maturation des composts et lombricompost sera déterminé avec précision afin de proposer des produits de qualité répondant à la norme et ne nécessitant ainsi aucune contrainte d'épandage.

## 2. La production d'olives, d'huiles d'olive et des sous produits dans le monde

La culture de l'olivier, à forte valeur culturelle et patrimoniale en région méditerranéenne, et la production d'huile d'olive, représentent environ 97% de la production mondiale (Fig. 1). Cette production s'accompagne de l'apparition de sous produits (restes de taille des oliviers, grignons et margines) peu ou pas valorisés à l'heure actuelle.

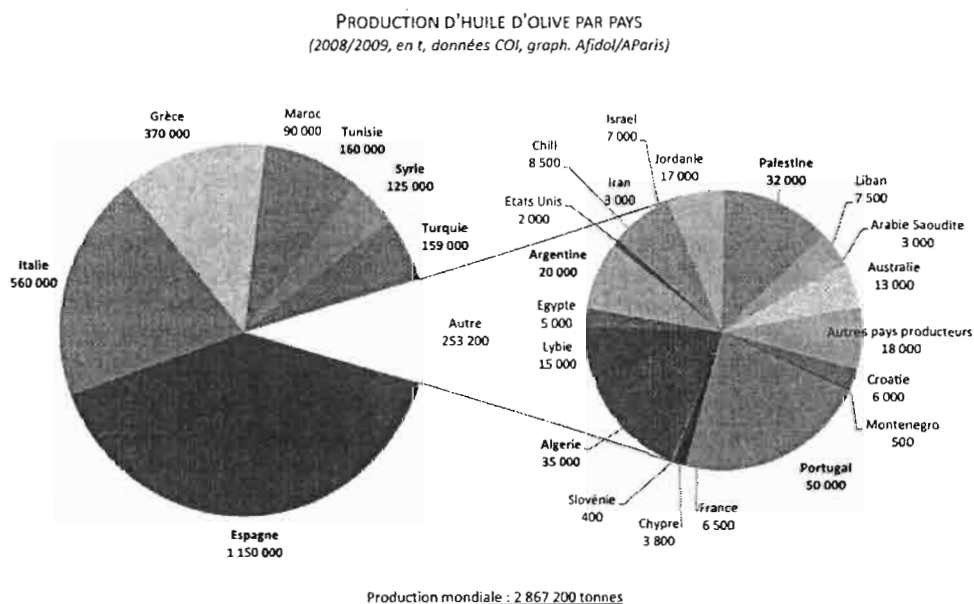


Figure 1: Production mondiale d'huile d'olive 2008/2009 (Source : AFIDOL).

## 3. Valorisation biotechnologique des sous produits de l'olivier pour une agriculture raisonnée

Les olives ne sont pas des fruits très riches en huile, comparées aux graines des oléagineux. Ainsi, la composition moyenne des olives est: eau 40 à 50% (eau de végétation ou margines); matières solides 25-35% (grignons); huile 20-25%. Les huiles d'olive vierges (HOV), obtenues uniquement par des moyens mécaniques, sont des systèmes chimiques complexes constitués de plus de 250 composés. Ils peuvent être classés en deux grands groupes: les substances saponifiables (de 96 à 98% de l'huile) et les substances insaponifiables (de 2 à 4% de l'huile).

Les composés phénoliques sont caractéristiques des huiles d'olives vierges et leur confèrent des propriétés particulières (stabilité oxydative, saveur...). La production d'huiles d'olive vierges nécessite un broyage des olives, suivi d'un malaxage de la pâte obtenue afin d'optimiser le rendement

d'extraction. La séparation des phases liquides (huile et margines) de la phase solide (grignons) est réalisée à l'aide de différents équipements au sein desquels la pâte d'olive est soumise à l'action de forces diverses qui, en fonction du système employé, peuvent être : la pression (système discontinu ou traditionnel) ou la force centrifuge (système continu).

Le système discontinu sépare les deux phases liquides de la phase solide. Les deux phases liquides sont ensuite séparées par décantation ou centrifugation. Le système continu se subdivise en trois modes de fonctionnement possible : (i) le mode trois phases qui permet de séparer en une seule opération les deux phases liquides de la phase solide mais qui nécessite l'ajout d'eau dans la pâte ce qui conduit à environ 120 L de margines/100 kg d'olive, (ii) le mode deux phases qui sépare l'huile des margines plus les grignons et qui généralement ne nécessite pas d'ajout d'eau. Ainsi, il est produit un grignon dit humide (contenant 60-70% d'eau) mais plus de margines, (iii) le mode deux phases et demie nécessite un ajout d'eau de 5 à 15 L d'eau/100 kg d'olives. Ce mode permet de séparer l'huile d'un grignon moins humide que précédemment (contenant 53 à 58% d'eau) et une production réduite de margines allant de 5 à 20 L/100kg d'olives. L'addition d'eau dans les procédés d'extraction centrifuge entraîne une diminution de la teneur en composés phénoliques de l'huile, une augmentation de leur teneur dans la phase aqueuse et un accroissement du volume des margines. En effet, ces composés se répartissent lors du malaxage et de la centrifugation entre les phases huileuse et aqueuse.

L'industrie oléicole mondiale, en plus de sa production principale qui est l'huile (huile d'olive vierge et huile de grignons) qui a été de 2.867.200 tonnes pour année 2008-2009, génère deux résidus : l'un liquide (les margines) et l'autre solide (les grignons). De plus, l'olivier à travers la taille (annuelle, bisannuelle, de rajeunissement, etc.) ou la récolte mécanique engendre des résidus tels que du gros bois et selon les estimations de nombreux pays, 25 kg de feuilles et brindilles (diamètre inférieur à 4 cm) sont produits par an et par arbre. Ceci se traduit par une production annuelle dans le monde d'environ 20 millions de tonnes de feuilles et brindilles fraîches qui pourraient être utilisées en alimentation animale (Nefzaoui, 1988), ou compostées sur place en les mélangeant avec des margines.

En Espagne, premier pays producteur d'huile d'olive, le système biphasique a été introduit vers les années 1970 et actuellement il y a une production de 4.000.000 de tonnes de « alperujo », un sous produit solide, composé essentiellement de lignine (31%), d'hemicellulose (24%), de cellulose (14%) de matières grasses (11%) de protéines (6%) de sucres solubles (6,5%), de phénols solubles (1,5%) et de nombreux sels minéraux étant donné que les cendres représentent 6% de matière sèche (Alburquerque et al. 2004).

Les sous-produits de l'olivier sont donc nombreux, de compositions différentes et d'utilisations très variées suivant les différents pays. Les grignons d'olive sont des sous produits solides essentiellement ligno-cellulosiques contenant la pulpe d'olive et du bois mais aussi des matières grasses, des sucres, des aminoacides, des polyphénols et des sels minéraux. La valorisation des grignons se fait dans diverses applications suivant les pays et le contexte (Tomati et Goli, 2006):

### **3.1. Valorisation des grignons d'olive en alimentation**

Il convient avant toute alimentation de séparer les noyaux éclatés de la pulpe. En 2006, la Société Pieralisi a mis au point de nouvelles machines capables de séparer à partir des grignons d'olive d'une part la pulpe d'olive et d'autre part le bois des noyaux d'olives (Digiovacchino et Prezinso, 2006). Les produits ainsi obtenus peuvent être valorisés séparément, la pulpe pour l'alimentation, les noyaux en biocombustible ou autre usage.

### **3.2. Valorisation biotechnologique des grignons d'olive**

Les grignons d'olive ont été utilisés comme substrat pour la culture de champignons filamenteux thermophiles par fermentation en milieu solide pour la production de lipases thermostables de *Rhizopus oligosporus* (Projet PRAD 02/13). Les matières grasses résiduelles des grignons d'olive favorisent la production importante de biomasse et des enzymes comme les lipases (Ismaili-Alaoui et al. 2002).



### 3.3. Compostage des grignons d'olive

Les résidus solides ou pâteux générés de l'extraction de l'huile d'olive sont riches en matière organique et constituent un aliment de choix pour la croissance de microorganismes.

Le compostage est la méthode la plus utilisée pour la préparation des amendements organiques et pour la fertilisation des sols. Il permet de détoxifier ces résidus solides contenant des substances phytotoxiques et antimicrobiennes à cause de la présence de phénols, des acides gras et des acides organiques. Souvent pour le compostage efficace des grignons on y ajoute des déchets végétaux ou des déchets urbains. Il existe deux sortes de compost obtenus avec un mélange de déchets de l'olivier (Feuilles oliviers + biomasse de taille d'olivier + margines + grignons d'olive) ou d'un mélange de déchets urbains verts + Pailles de céréales + pailles de céréales + déchets de l'olivier). L'AFIDOL (Association Française Interprofessionnelle de l'Olive) a déjà expérimenté en France des compostages à grande échelle avec succès (Tableau 1).

**Tableau 1:** Composition moyenne des mélanges pour le compostage à grande échelle des sous produits liquides et solides de l'industrie oléicole.

| Caractéristiques           | Mélange 1                                                                                                                                     | Mélange 2                                                                                                                                                                | Mélange 3                                                                                                                                                                                                  |
|----------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Composition                | • 9 tonnes (9m <sup>3</sup> ) de grignons humides issus d'un décanteur à 2 phases<br>• 9,5 tonnes (30m <sup>3</sup> ) de déchets verts broyés | • 9,9 tonnes (9,9 m <sup>3</sup> ) de grignons humides issus d'un décanteur à 2 phases<br>10,2 tonnes (28 m <sup>3</sup> ) de déchets verts broyés<br>40 kg d'urée à 46% | 12,8 tonnes (13,5 m <sup>3</sup> ) de grignons humides issus d'un décanteur à 3 phases<br>10,2 tonnes (28m <sup>3</sup> ) de déchets verts broyés<br>6 m <sup>3</sup> de margines issues moulin à 3 phases |
| Densité                    | 0,50                                                                                                                                          | 0,55                                                                                                                                                                     | 0,62                                                                                                                                                                                                       |
| Teneur en eau des mélanges | 49%                                                                                                                                           | 49%                                                                                                                                                                      | 56%                                                                                                                                                                                                        |

Source AFIDOL

La Durée approximative du compostage est compris entre 6 à 8 mois, voire davantage pour un compost plus mûr. Par contre pour la réussite d'un compostage, il est impératif de travailler avec des teneurs en eau faibles pour les différents mélanges, d'où l'intérêt d'ajouter de déchets verts broyés.

### 3.4. Ensilage et lombricompostage des grignons d'olive

Une recherche sur *ISI web of knowledge* montre que plusieurs articles ont été publiés tant sur l'ensilage que le lombri-compostage des résidus de trituration des olives. Il en est de même pour la filtration des margines sur membrane. Ces modes prometteurs de détoxification et de valorisation des sous-produits de l'industrie oléicole sont donc pratiquement vierges et les conditions optimales de leur mise en œuvre ainsi que leur viabilité restent à définir.

Les quelques essais d'ensilage réalisés jusqu'ici n'ont porté que sur les grignons et avaient exclusivement pour but la production d'aliment pour le bétail (Weinberg et al., 2008) sans entrevoir la possibilité d'utiliser les produits ensilés à d'autres fins comme, par exemple, en tant que substrat pour la culture de champignons.

Les résultats disponibles dans la littérature sur le lombri-compostage aboutissent à la conclusion qu'il n'est envisageable que sur le résidu solide généré lors de la récupération au moyen de solvants de l'huile résiduelle présente dans les grignons et les margines préalablement séchés (Moreno et al., 2000). Les autres sous-produits de trituration (grignons, margines, margions) ne permettraient pas par contre pour des raisons de toxicité un développement optimal des vers de terre, du moins dans leur état brut. Rien ne permet cependant de présager d'un échec du lombri-compostage sur ces produits après un prétraitement comme l'ensilage ou après mélange à d'autres résidus agricoles.

Plus récemment, la société Cabries-lombricompostage, a utilisé avec succès, les grignons d'olive pour l'élevage de vers de terre (<http://desbois-lombriculture.info/1.html>). Les essais de co-compostage et de lombri-compostage des sous-produits seront conduits selon la technique classique des andains retournés. Des andains expérimentaux (hauteur 1,50 m x largeur 4 m x longueur 30 m) ont été utilisés pour tester les meilleures proportions entre sous-produits des moulins et pailles de céréales compostés. Dans un second temps, des andains pilotes de plus grande taille (50 m de longueur) seront mis en place.



### 3.5. Conservation par ensilage

L'ensilage des résidus saisonniers de l'industrie oléicole, permet d'envisager une utilisation annuelle pour diverses valorisations tels que la lombriculture, la production de champignons supérieurs ou encore alimentation animale. L'ajout de bactéries lactiques endogènes sélectionnées permettra d'obtenir des ensilages contrôlés (Perraud-Gaime et al. 2009).

### 4. Les margines

Comprennent deux fractions, l'une insoluble (matière organique 64,6%) est essentiellement constituée de pulpes d'olives, l'autre soluble et contient les sucres (12%), les lipides (4,2%), les sels minéraux (7,2%) et les composés phénoliques (2,2%). Des études de toxicité et de biodégradabilité de ces margines ont montré que la toxicité est due à la fraction soluble et que les composés phénoliques de type anthocyanes et monomères aromatiques sont très toxiques mais biodégradables. Par contre, les composés phénoliques responsables de la coloration noire sont peu toxiques et très difficilement biodégradables (Hamdi, 1991). Pour des quantités importantes, le compostage des margines est la manière la plus simple pour les recycler et consiste à les mélanger avec des substrats agricoles solides (Paredes et al. 1999 ; Paredes et al. 2002). Cependant la tendance actuelle vise à récupérer d'abord des molécules antioxydantes comme l'hydroxytyrosol, avant d'orienter les margines vers le compostage ou le traitement d'épuration de l'eau (Fki et al. 2005). Les margines sont utilisées en épandage comme fertilisant, et elles servent surtout à d'autres procédés biotechnologiques (extraction de composés aromatiques et phénoliques). Elles interviennent également dans la fabrication de compost pour la production de champignons (Paredes et al. 2005 ; Olivieri et al. 2006 ; D'Annibale et al. 2004).

### 5. Description du SOS ou COS

Un mélange de coproduits d'olivier (grignons d'olives, bois de la taille, margines) a été mis au point pour fabriquer le COS (Coproduits de l'Olivier Solides) et l'utiliser à la fois comme substrat et support solide, pour la culture des microorganismes par Fermentation en Milieu Solide, en particulier des champignons filamenteux entomopathogènes, nématophages pour la production de biopesticides ou pour la culture du mycélium de champignons comestibles et médicinaux comme le Shiitake (Lakhtar 2009).

#### 5.1. Production de lipases

Des grignons d'olive mélangés avec de la bagasse de canne à sucre ont été utilisés pour la croissance de champignons filamenteux thermophiles pour la production de lipases thermostables(> 80°C). La technique de Fermentation en Milieu Solide s'avère plus adaptée pour la croissance des champignons thermophiles que la culture liquide, la production de lipases est plus élevée (10 à 20 fois) et ces enzymes sont extracellulaires. La présence dans les grignons d'olive d'acide oléique, utilisé comme inducteur, augmente la production de lipases en FMS (8 fois). L'addition à la bagasse de canne à sucre, de grignons d'olive augmente la production de lipases (26 fois). Ces enzymes sont stables pendant la culture (Cordova et al. 1998).

#### 5.2. Production de biopesticides

Les grignons d'olive mélangés à d'autre déchets agroindustriels (margines, feuilles d'olivier, autres substrats lignocellulosiques) constituent un excellent substrat solide pour réaliser des cultures de champignons filamenteux entomopathogènes ou nématophages (Hassouni 2007). Dans la mesure où des conditions optimales de culture sont obtenues pour chaque microorganisme, le produit fermenté obtenu, enrichi en mycélium de ce type de champignon, pourrait s'avérer, après retour au champ, être un excellent agent pour une lutte biologique contre certains des agresseurs de l'olivier. Les procédés les plus prometteurs sont validés à une échelle pré-industrielle.

#### 5.3. Production de champignons comestibles médicinaux

Une étude récente réalisée dans notre laboratoire vise à la détermination d'une formule originale d'un mélange (20/30/50) pour la préparation des différents composants des coproduits de l'industrie oléicole (bois d'olivier, grignons d'olive et des margines d'olives) afin de réaliser des cultures des

champignons comestibles et médicinaux sur un Substrat Oléicole Solide (SOS) par fermentation en milieu solide (Lakhtar 2009). Cette culture permet d'assurer d'une part la production de la biomasse fongique pour l'alimentation humaine et d'autre part la détoxification des coproduits (margines) pour servir à l'alimentation animale. La saisonnalité de substrats d'oliviers (les grignons d'olives et les margines) a été remédiée par le séchage solaire sous serre, une étude qui sera présentée en détaille dans une autre présentation (Lakhtar et al. 2010). Un test de criblage des souches de *Lentinula edodes* (champignons comestible et médicinal, connu sous le nom de Shiitake) a été effectué afin d'obtenir une souche efficace et capable de dégrader les polyphénols dans les margines. La préparation d'inoculum de la souche sélectionnée a été optimisée par l'induction du mycélium à la production des laccases. Les conditions de culture du champignon ont été optimisées et la cinétique de croissance de shiitake sur le mélange ainsi que la transformation de la matière organique ont été étudiées.

## 6. Conclusion de l'existant et du potentiel

On peut dire que l'olivier par son huile d'olive, est une source de produits alimentaires et cosmétiques, par ses grignons une source alimentaire pour le bétail, mais aussi une source de carbone et d'énergie par ses résidus et ses tailles, pouvant servir de substrat pour la croissance de microorganismes dans les procédés biotechnologiques, en particulier pour la production de biopesticides (champignons filamenteux entomopathogènes, nématophages ou des antagonistes de phytopathogènes). Ainsi, ces actions s'inscrivent dans une politique de développement durable et devraient pouvoir être généralisées à divers sous-produits ligno-cellulosiques, Roussos et al. (2006). Les moulins ont tout intérêt à valoriser ces sous-produits de façon d'une part à obtenir des coproduits ayant une valeur marchande et d'autre part ne pas devoir payer la redevance de l'Agence de l'Eau. On rappelle que suivant les rejets, l'Agence de l'Eau peut percevoir une redevance (principe pollueur –payeur) pouvant atteindre environ 300 €/par tonne d'huile d'olive. Parmi les pays producteurs d'huile d'olive, l'Espagne et la Tunisie sont les pays pionniers quand à la valorisation industrielles des sous produits solides et liquides de l'industrie oléicole. L'Italie, est le seul pays européen ayant développé une législation lui permettant d'utiliser l'épandage des margines dans les champs d'oliviers, sous des conditions bien réglementées. En conclusion, les sous produits de l'olivier représentent des tonnages plus importants que l'huile d'olive, leur composition les destine à une multitude d'usages, en particulier comme matière première dans les procédés biotechnologiques et dans l'avenir, ils vont jouer un rôle primordial pour un développement durable du secteur oléicole dans les pays Méditerranéens ; il est donc important de les considérer comme des matières premières au même titre que l'huile.

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## **Extraction par solvant de l'huile de grignon d'olive assistée par micro-ondes. Comparaison avec l'extraction conventionnelle**

Fatiha Amarni , Hocine Kadi \* , Ramdane Moussaoui

### **Résumé**

L'extraction par solvant assistée par micro-ondes (ESAM) a été utilisée pour la récupération de l'huile à partir du grignon d'olives. Comparée à l'extraction par solvant conventionnel (ESC), cette nouvelle technique produit de meilleurs rendements en des temps très courts. Les différentes analyses réalisées (acidité, indice de peroxyde, teneur en polyphénols...) ont également montré que l'huile extraite par ESAM est de meilleure qualité. La composition en acides gras est identique pour les deux méthodes.

**Mots clés:** micro-ondes, extraction de l'huile, solvant, olive cake, caractérisation de l'huile

## **Microwave-assisted solvent extraction of oil from olive cake. Comparison with the conventional extraction**

### **Abstract**

The microwave-assisted solvent extraction (MASE) was used for the recovery of oil from the olive cake. Compared with the conventional solvent extraction (CSE), this new technique provided better yields in very short times. Various analyses carried out (acidity, peroxide index, polyphenol content...) also showed that the oil extracted by ESAM is of better quality. The fatty acids composition was identical for the two methods.

**Key words:** Microwave, oil extraction, olive cake, solvent, oil characterization.

### **1. Introduction**

Le grignon d'olive dont la production mondiale a été estimée par Conseil Oléicole International (COI, 1989) à 3000 000 tonnes/an est un sous-produit oléicole qui peut générer des dommages à l'environnement en raison des composés phénoliques (Martinez-Garcia et al, 2006) qu'il contient. Sa valorisation passe par la récupération par solvant de son huile résiduelle (Méziane et Kadi, 2008) qui peut atteindre jusqu'à 8%. Dans la majorité des travaux relatifs à cette extraction solide-liquide, l'hexane apparaît comme le solvant le plus utilisé (Pérez-Serradilla et al, 2007).

Ces dernières années, l'utilisation de l'extraction par solvant assistée par micro-ondes (ESAM) semble être une alternative à l'extraction conventionnelle par solvant (ECS) de l'huile à partir de matériaux végétaux (Molins et al, 1996 ; Chen, 2007 ; Spigno et De Faveri, 2009). En effet, les micro-ondes ont l'avantage de générer la chaleur au cœur du matériau offrant ainsi de nombreux avantages : rapidité de traitement, rendements élevés, sélectivité, réduction de la consommation de solvant.

Le but de ce travail est d'appliquer cette technique à l'extraction de l'huile à partir d'un grignon d'olive pour étudier l'influence de la puissance de la radiation sur le rendement et sur la qualité de l'huile. Les résultats obtenus sont comparés à ceux donnés par l'extraction conventionnelle.

## 2. Matériel et méthodes

### 2.1. Matériau et réactifs

Le grignon d'olive utilisé a été prélevé de l'huilerie « Ifriolive » située à Béjaïa (Algérie). Son humidité initiale qui était de 45.8% a été ramenée par séchage à l'étuve à 6.2%. Le taux d'huile résiduelle déterminée par extraction épuisante sur soxhlet en utilisant l'hexane est de 7.0 %.

Le grignon a été utilisé à l'état brut c'est-à-dire sans subir aucun broyage. Toutes les extractions sont réalisées avec de l'hexane d'une pureté de 95 %.

### 2.2. Extraction par solvant assistée par micro-onde (ESAM)

Le système ESAM utilisé se compose d'un four micro-onde, d'un réacteur et d'un agitateur en verre (fig.1). Le four micro-onde (COBRA, Model: CB-200-28L-G) a été modifié dans notre laboratoire. Sa puissance maximale fonctionnant à 2450 MHz est de 900 W variable avec un pas de 180 W. Les dimensions de sa cavité interne sont 240 mm x 354 mm x 358 mm.

Les extractions sont réalisées dans un réacteur cylindrique en verre de 600 ml équipé d'un agitateur mécanique. Les solides sont séparés du miscella par filtration sous pression réduite. L'huile est récupérée par distillation du miscella à l'aide d'un évaporateur rotatif. Les traces de solvant sont éliminées par séchage dans une étuve à 103°C.

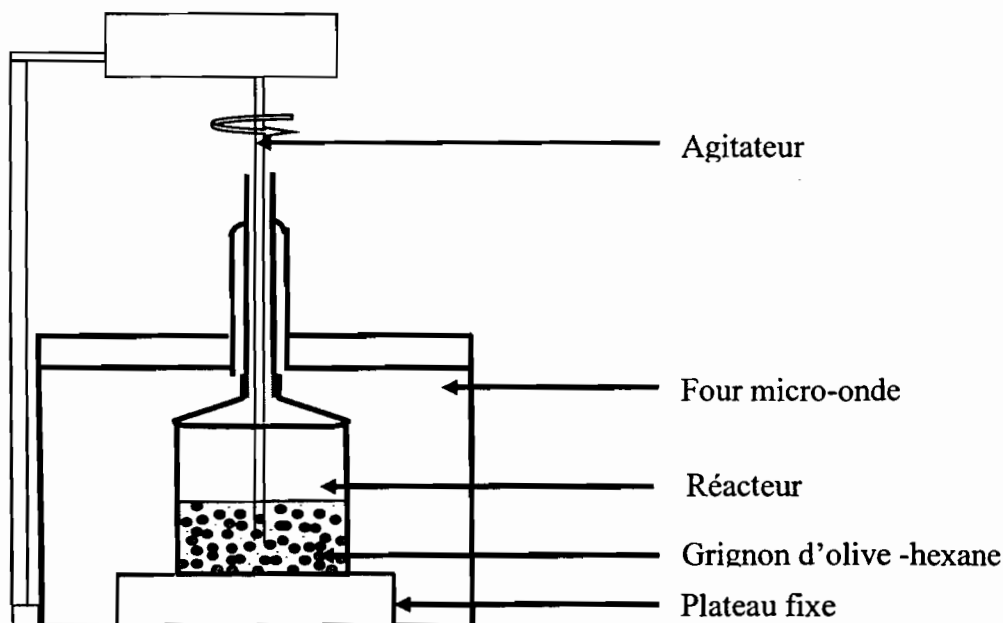


Figure 1: Installation de l'extraction assistée par micro-ondes

Chaque valeur de rendement reportée correspond à une moyenne de trois essais. La masse de grignon utilisée pour chaque essai est de 50 g. Les conditions opératoires sont les suivantes:

- Rapport liquide-solide, (L/S= 3 cm<sup>3</sup>/g)
- Vitesse d'agitation,  $V_a$ = 480 tours/mn
- Temps d'extraction,  $t$ = 1 et 2 mn
- Puissance de la radiation,  $P$ = 180 et 720 W

### 2.3. Extraction par solvant conventionnelle (ESC)

Le système conventionnel ESC est le même que celui utilisé en extraction assistée par micro-onde excepté le fait que la température est contrôlée par immersion du réacteur dans un bain thermostaté. Les conditions expérimentales sont aussi identiques, seule la température est fixée à 25 et 45°C.

Il faut signaler que sous micro-onde, on ne peut pas dépasser un temps d'extraction de 3 minutes surtout pour les puissances de radiation élevées car le solvant entre en ébullition. Pour  $P$ = 720 W, le temps maximum possible est seulement de 2 minutes.

## 2.4. Analyses des huiles extraites

L'huile extraite a été soumise aux analyses suivantes : acidité, absorbance spécifique, indice de peroxyde, indice d'iode, indice de saponification, teneur en insaponifiables et teneur en polyphénols.

### Acidité:

La méthode décrite dans la norme française (NF T60-204, 1968) a été utilisée pour déterminer l'acidité des huiles extraites. Elle est basée sur la mise en solution d'une prise d'essai dans un mélange d'éthanol et d'éther éthylique (1/1, v/v) suivie d'un titrage des acides gras libres à l'aide d'une solution de soude.

### Absorbance spécifique:

L'absorbance à 270 et 232 nm de l'huile extraite dissoute dans l'hexane pur a été déterminée à l'aide d'un spectrophotomètre à double faisceau suivant la norme française (AFNOR, 1978).

### Indice de peroxyde:

La méthode utilisée est celle décrite par la norme française (NFT 60-220, 1968). Le principe consiste à traiter le corps gras en solution dans de l'acide acétique et du chloroforme par une solution d'iodure de potassium. L'iode libéré est titré par une solution de thiosulfate de sodium en présence d'empois d'amidon comme indicateur.

### Indice d'iode:

La méthode utilisée est celle décrite par le règlement N° 2568 / 91 de la CEE dont le principe consiste à mettre en solution une prise d'essai dans un solvant et addition du réactif de Wijs. Au terme d'un laps de temps déterminé, on ajoute une solution d'iodure de potassium et de l'eau avant de procéder au titrage de l'iode libéré par une solution de thiosulfate de sodium.

### Substances insaponifiables:

La teneur en substances insaponifiables a été déterminée par la méthode dite à l'oxyde diéthylique. Cette méthode décrite par française homologuée NF T60 – 205 (1975) consiste d'abord en la saponification du corps gras par traitement à l'ébullition sous reflux avec une solution éthanolique d'hydroxyde de potassium. Les substances insaponifiables sont extraites ensuite de la solution de savon par l'oxyde diéthylique.

### Indice de saponification:

Cet indice est déterminé suivant la méthode donnée par la norme NF 60-206 (déc. 1968). L'échantillon est soumis à une ébullition sous réfrigérant avec reflux avec une solution éthanolique d'hydroxyde de potassium. L'excès d'hydroxyde de potassium est titré avec une solution aqueuse d'acide chlorhydrique.

### Composés phénoliques:

Le dosage des composés phénoliques totaux des extraits obtenus a été effectué conformément à la méthode de Gutfinger (Gutfinger, 1981) dont le principe est le suivant: Cette méthode utilise le réactif de Folin – Ciocalteu qui est constitué par un mélange d'acide phosphotungstique ( $H_3PW_{12}O_{40}$ ) et d'acide phosphomolybdique ( $H_3PM_{12}O_{40}$ ) qui est réduit lors de l'oxydation des phénols en un mélange d'oxydes bleus de tungstène ( $W_8O_{23}$ ) et de molybdène ( $Mo_8O_{23}$ ) (Swain et Goldstein, 1964). La coloration produite estimée par spectrophotométrie visible à 750 nm est proportionnelle au taux de composés phénoliques.

### Acides gras:

Les acides gras ont été transformés en esters de méthyle (NFT 60 – 23, 1977) et analysés par chromatographie en phase gazeuse (AFNOR NF T 60 – 234. 1977). Le chromatographe utilisé est de type Chrompack CP 9002, celui-ci est équipé d'un injecteur Split 1/100, d'un détecteur à ionisation de



flamme et d'une colonne capillaire DB 23 de 30 m de longueur et de 0,32 mm de diamètre intérieur. Les pics ont été identifiés par référence aux temps de rétention des esters de méthyle connus.

Les conditions opératoires de travail sont : Débit du gaz vecteur (azote): 1ml/mn, température de colonne : 190°C, température de l'injecteur : 250°C et température du détecteur : 250°C. La quantité injectée est 0,2 µl.

### 3. Résultats et discussions

#### 3.1. Etude du rendement

Le tableau 1 montre que la température dans l'extraction conventionnelle et la puissance de la radiation dans l'extraction assistée par microonde influent positivement sur le rendement en huile.

En effet, quand la température passe de 25 à 45°C, le rendement augmente de 5.9% pour un temps d'extraction  $t = 1$  mn. Pour  $t = 2$  mn et pour une même variation de la température, le rendement augmente de 6.4%.

S'agissant de la méthode ESAM, quand la puissance de la radiation passe de 180 à 720 W, l'augmentation du rendement est 16.5% pour  $t = 1$  mn. Pour une même variation de la puissance et mais avec  $t = 2$  mn, l'augmentation est de 17.7%.

Comparée à l'extraction traditionnelle, l'extraction par micro-onde donne de meilleurs rendements. On observe sur le tableau 1 que pour un temps d'extraction  $t = 1$  mn, le rendement qui est de 3.71 % à 25°C et 4.37 % à 45°C pour la CSE croît à 4.25% à  $P = 180$  W et à 4.95 à  $P = 720$  W. Pour 2 minutes, il ya toujours une augmentation mais elle est moins importante. Cependant, on observe que pour 2 minutes le rendement est plus élevé avec la ESC à 45°C qu'avec la méthode ESAM à  $P = 180$  W.

**Tableau 1:** Rendement en huile extraite par ESC et ESAM en fonction du temps ( $L/S=3$ ,  $V_a=480$  tours/mn).

| Temps<br>(mn) | ESC      |         | ESAM    |          |
|---------------|----------|---------|---------|----------|
|               | T= 25 °C | T= 45°C | P=180 W | P= 720 W |
| 1             | 3.71     | 4.37    | 4.25    | 4.95     |
| 2             | 4.21     | 4.58    | 4.40    | 5.18     |

#### 3.2. Analyses des huiles

Les huiles soumises aux différentes analyses sont extraites dans les conditions suivantes :  $L/S=3$ ,  $t = 2$  mn,  $V_a=480$  tours/mn. Tous les résultats des analyses sont consignés dans le tableau 2 exceptés ceux relatifs aux acides gras.

##### Acidité :

Les huiles extraites de manière conventionnelle présentent une acidité qui ne semble pas varier avec la température. Dans le cas de l'extraction ESAM, la puissance de la radiation influence légèrement cette acidité qui n'augmente que de 6,3 % quand la puissance passe de 180 à 720W.

Les huiles récupérées par l'extraction ESAM sont moins acides que celles obtenues par la méthode conventionnelle. La diminution de l'acidité varie de 10,7 à 16,8 %

Ces huiles dont les valeurs d'acidité sont supérieures à 3,3 % correspondent à des huiles d'olive lampantes (COI, 2003).



**Tableau 2:** Analyses des huiles extraites (L/S=3, t= 2 mn et Va=480 tours/mn).

|                                                    |        | Extraction conventionnelle |          | Extraction assistée par micro-onde |          |
|----------------------------------------------------|--------|----------------------------|----------|------------------------------------|----------|
|                                                    |        | T= 25 °C                   | T= 45 °C | P= 180 W                           | P= 720 W |
| Acidité (%)                                        |        | 7.4                        | 7.5      | 6.3                                | 6.7      |
| UV                                                 | 232 nm | 3.9998                     | 3.9998   | 1.0462                             | 1.0511   |
|                                                    | 270 nm | 0.6550                     | 0.7357   | 0.1492                             | 0.1611   |
|                                                    | R      | 6.11                       | 5.44     | 7.01                               | 6.69     |
| Indice peroxyde (meq/kg)                           |        | 28.8                       | 30.5     | 22.2                               | 26.1     |
| Indice d'iode<br>(g I <sub>2</sub> /100 g d'huile) |        | 85.6                       | 63.7     | 95.5                               | 88.4     |
| Indice de saponification<br>(mg KOH/g d'huile)     |        | 166.9                      | 167.1    | 189.0                              | 190.4    |
| Teneur en insaponifiables<br>(g/100 g d'huile)     |        | 1.0                        | 1.2      | 1.1                                | 1.3      |
| Teneur en polyphénols                              |        | 562.6                      | 647.9    | 718.8                              | 1158.5   |

**Absorbance spécifique:**

En ESC, l'augmentation de la température n'a pas d'influence sur l'extinction à 230 nm. L'huile présente donc la même oxydation primaire à 25°C comme à 45°C. La croissance de l'extinction à 270 nm indique que l'huile extraite à 45°C contient plus de produits secondaires d'oxydation. La variation du rapport R montre que l'augmentation de la température a une influence négative sur la qualité de l'huile.

S'agissant de l'extraction ESAM, on observe que l'oxydation primaire est accentuée par l'augmentation de la puissance de la radiation qui engendre aussi plus de produits secondaires d'oxydation. La variation du rapport R montre que l'huile est moins oxydée à 180 W.

Les huiles extraites par les deux procédures sont de qualité médiocre. Cependant, l'extraction par micro-onde donne des huiles de moins mauvaise qualité.

**Indice de peroxyde:**

Les huiles extraites par micro-onde présentent des indices de peroxyde inférieurs à celles obtenues que celle obtenues par ESC.

L'indice de peroxyde des huiles extraites augmente avec la température (extraction conventionnelle) et avec la puissance (extraction micro-onde).

Toutes ces huiles dont les valeurs de l'indice de peroxyde sont supérieures à 20 méq d'O<sub>2</sub> /Kg d'huile, sont de mauvaise qualité.

**Indice d'iode:**

On observe que les huiles extraites par microondes ont un indice d'iode supérieur à celles obtenues de manière conventionnelle. On remarque également que la température (ESC) et la puissance (ESAM) provoquent l'altération de l'huile.

**Indice de saponification:**

On observe que la température comme la puissance de radiation ne semblent pas affecter l'indice de saponification. Il faut cependant remarquer que l'huile obtenue par extraction assistée par micro-onde présente un indice plus élevé que dans le cas conventionnel. Cet indice dont la valeur est voisine de 190 mg de KOH/g d'huile se situe dans les limites fixées (184-196) par COI pour les huiles d'olive.

**Teneur en insaponifiables:**

L'extraction par micro-onde permet de récupérer plus de matières insaponifiables. On observe également que la température (ESC) et la puissance (ESAM) favorisent cette récupération.

### Teneur en polyphénols:

L'extraction ESAM conduit à une huile plus riche en polyphénols que celle obtenue par l'extraction ESC. La récupération de ces polyphénols semble favorisée par l'augmentation de la puissance de la radiation et par la température.

### Acides gras:

Les résultats de l'analyse qualitative et quantitative des acides gras des huiles de grignon extraites de façon conventionnelle et micro-ondes sont donnés par le tableau 3.

On remarque que le profil en acides gras des huiles extraites à partir du grignon d'olive semble ne pas être affecté par la nature du procédé d'extraction utilisé, par la puissance de la radiation micro-onde et par la température.

Ce tableau montre également la prédominance de l'acide oléique. Les acides gras essentiels (linoléique et linolénique) sont présents en quantité acceptable. Il faut aussi noter que la teneur en acide gras linoléique est supérieure aux valeurs trouvées dans le cas des huiles d'olive vierges (Talentikite et Ait-Amar, 1988 ; Sánchez Casas et al, 1999). Les autres composés représentés par les acides (palmitoléique, margarique, stéarique et arachidonique) se trouvent en faible quantité.

**Tableau 3:** Analyse des acides gras.

| Acides gras           | ESAM    |         | ESC     |        |
|-----------------------|---------|---------|---------|--------|
|                       | P=180 W | P=720 W | T= 25°C | T=45°C |
| Palmitique C16 : 0    | 12,90   | 12,92   | 13,09   | 12,89  |
| Palmitoléique C16 : 1 | 1,53    | 1,54    | 1,53    | 1,58   |
| Margarique C17 : 0    | 0,08    | 0,08    | 0,08    | 0,08   |
| Stéarique C18 : 0     | 2,45    | 2,45    | 2,49    | 2,43   |
| Oléique C18 : 1       | 68,50   | 68,43   | 68,48   | 68,44  |
| Linoléique C18 : 2    | 13,10   | 13,17   | 12,93   | 13,20  |
| Linolénique C18 : 3   | 1,00    | 1,00    | 0,98    | 0,98   |
| Arachidonique C20: 0  | 0,28    | 0,29    | 0,28    | 0,26   |
| Somme des Saturés     | 15,71   | 15,74   | 15,94   | 15,66  |
| Somme des Insaturés   | 84,13   | 84,14   | 83,92   | 84,20  |

### 4. Conclusion

Les deux méthodes de récupération de l'huile présentent le même profil en acides gras. Comparée à l'extraction conventionnelle, l'extraction assistée par micro-onde présente permet d'obtenir:

- De meilleurs rendements. Ce phénomène peut s'expliquer de la manière suivante : le chauffage au cœur du matériau induit par les radiations provoque l'éclatement des cellules qui libèrent plus d'huile ;
- une huile de meilleure qualité et plus riche en substances insaponifiables et en composés phénoliques.

Les résultats obtenus permettent de penser que l'extraction assistée par micro-onde peut constituer une alternative intéressante à la récupération de l'huile à partir du grignon d'olive.

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## **Conservation des résidus de l'agro-industrie oléicole par ensilage : de l'isolement de bactéries lactiques endogènes à l'étude de faisabilité**

Isabelle Perraud-Gaime, Y. Labrousse, S. Roussos

### **Résumé**

Pour envisager une valorisation annuelle (production de champignons comestibles, lombriculture) des sous produits saisonniers de l'industrie oléicole (grignons, margines, margions), il faut pouvoir les conserver. Cette conférence propose d'exposer les avancées et les résultats sur la faisabilité de la conservation des résidus par ensilage, ensilage naturel ou ensilage contrôlé par l'inoculation par des bactéries lactiques endogènes sélectionnées.

**Mots clés :** résidus oléicoles, conservation, ensilage, inoculation, bactéries lactiques.

## **Presrvation of the residus of olive agro-industry by silaging: from lactic endogenous bacteria isolation to feasibility study**

### **Abstract**

To consider an annual valorization (edible fungi production, lombriculture) of seasonal under-products of olive industry (pomace, margins, margion), we should be able to preserve them. This conference suggests to expose the advances and results on the feasibility of residus preservation by silaging, natural silage or controlled by the inoculation through selected endogenous lactic bacteria.

**Key words:** olive residus, preservation, silaging, inoculation, lactic bacteria.

### **1. Introduction**

La superficie oléicole mondiale est estimée à 8 600 000 hectares, dont 95% se situent dans le bassin méditerranéen. La production moyenne en olive est de 10 millions de tonnes par an dont 92% sont utilisés pour l'extraction d'huile, le reste étant consommé en tant qu'olives de table (Roussos et al., 2006).

L'industrie oléicole, dont le produit final est l'huile d'olive, laisse par le système classique de pressage deux résidus, l'un liquide: les margines (eaux contenus dans l'olive, eaux de lavage, eaux liés au processus de traitement), l'autre solide: les grignons (peaux, résidus de la pulpe, fragments de noyaux). Un nouveau type de résidu apparaît, les margions (terme né de la contraction de margines et de grignons) avec l'essor d'un nouveau système continu utilisant la centrifugation. Ces déchets polluants de l'industrie oléicole sont produits en très grande quantité (Mulinacci et al. (2005), Chimi, 2006).

La transformation des olives et la production de l'huile d'olive sont des activités saisonnières de Novembre à Mars suivant les régions. Le stockage des sous produits, en vu d'une valorisation annuelle, est un problème à résoudre.

Des recherches en cours au sein du laboratoire EMB-IMEP montrent que l'on pourrait utiliser ces résidus pour la production de champignons supérieurs (Lakhtar (2009)), une autre utilisation pourrait être la production de lombricompost. Mais pour cela il faut, avant tout, conserver ces déchets pour avoir une disponibilité annuelle. Une technique de conservation serait l'ensilage. Cette méthode fait intervenir des microorganismes les bactéries lactiques.

Des travaux antérieurs réalisés sur un autre résidu agro-industriel (la pulpe de café) au cours d'un projet européen Biopulca ont démontrés qu'il était possible de conserver ce type de résidus par la technique de l'ensilage (Perraud-Gaime (1995)) et permettre ainsi une possible utilisation annuelle de ce résidu devenu ainsi substrat potentiel à diverses valorisations tels que la lombriculture, la production de champignons supérieurs (Biopulca (2001)).

D'où l'idée d'appliquer cette technologie simple et peu coûteuse aux résidus saisonniers de l'agro-industrie oléicole pour une valorisation annuelle soit par lombriculture, soit par production de champignons supérieurs comestibles et/ou médicinaux, soit pour l'alimentation animale (suivi de la désamérisation), soit pour la production de molécules à haute valeur ajoutée (enzymes et autres molécules).

La finalité de ce travail sera de déterminer la faisabilité de la conservation par ensilage de ces résidus par l'isolement et la caractérisation de la microflore endogène à partir d'ensilages naturels puis la réalisation d'un ensilage contrôlé.

## 2. Matériels et methods

Cette étude porte sur 2 types de sous produits issus de 2 systèmes d'extraction d'huile d'olive du Maroc : le système traditionnel par pressage de Beni Mellal-Maroc qui permet d'obtenir les margines et les grignons séparés et le système moderne par centrifugation de Sidi Bou Othman-Maroc qui permet d'obtenir les margions (margine et grignons mélangés).

Les grignons (35% d'humidité) et les margines issues du système par pressage ont été mélangés pour arriver à une humidité de 63% puis sont mis en microsilos et stockés à l'obscurité (Figure I). Les margions (humidité supérieure à 80 %) ont été directement mis en microsilos (Figure II).

Les micro-silos ont été réalisés entre Décembre 2007 et Février 2008 en fonction des différents sous produits (Tableau I)

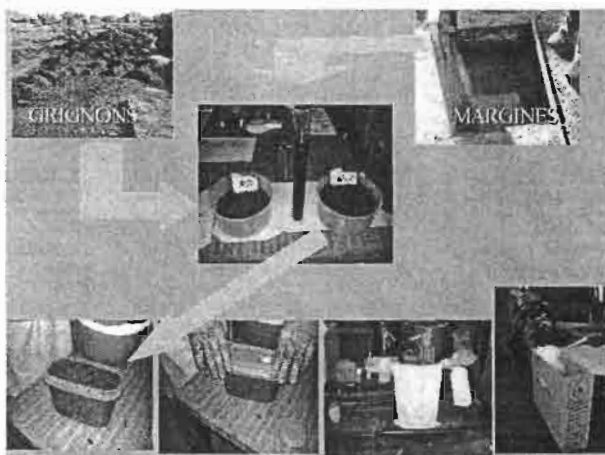


**Figure 1:** Montage des micro-silos (naturels) des sous produits issues du système par presse.

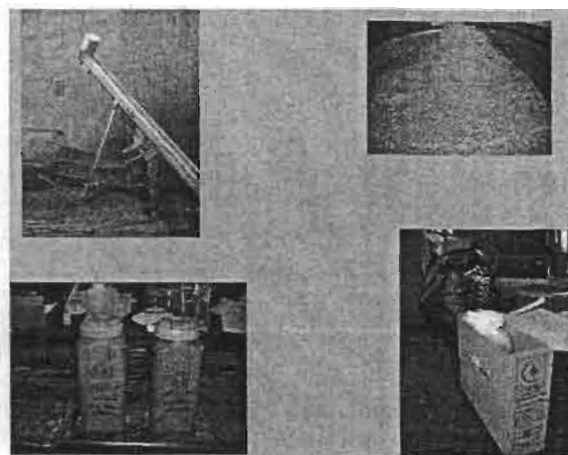


**Figure 2:** Montage des micro-silos (naturels) des margions.

**Tableau I:** Récapitulatif des microsilos réalisés au Maroc.

| Microsilos n°         | Système                     | Type sous produit | Origine          | Date    | T° Stockage |
|-----------------------|-----------------------------|-------------------|------------------|---------|-------------|
| D1BM - D2BM           | Tri-Phasique Pressage       | Grignon + Margine | Beni-Mellal      | 12/2007 | T° ambiante |
| BM1 - BM3 - BM5       | Tri-Phasique Pressage       | Grignon + Margine | Beni-Mellal      | 02/2008 | 25°C        |
| BM2 - BM4 - BM6       | Tri-Phasique Pressage       | Grignon + Margine | Beni-Mellal      | 02/2008 | T° ambiante |
| C1SBO - C2SBO - C3SBO | Tri-Phasique Centrigugation | Margion           | Sidi Bou Othmane | 12/2007 | T° ambiante |
| OTH1 - OTH3 - OTH5    | Tri-Phasique Centrigugation | Margion           | Sidi Bou Othmane | 02/2008 | 25°C        |
| OTH2 - OTH4 - OTH6    | Tri-Phasique Centrigugation | Margion           | Sidi Bou Othmane | 02/2008 | T° ambiante |

Après ouverture, des observations sur l'aspect, l'odeur et la couleur ont été réalisées pour chaque micro-silo. Le pH, l'humidité ont été déterminés.

Un isolement de la microflore endogène anaérobie a été réalisé sur milieu MRS à 30°C. Les souches pures ont été caractérisées par description morphologique et biochimique, par identification sur Galeries API 50CH et par profil fermentaire par analyse par HPLC des surnageant de culture après 24h à 30°C.

Les ensilages contrôlés par inoculation d'un ferment d'ensilage ont été réalisés dans des micro-silos de laboratoire dont la capacité est de 1300g de matière humide. En utilisant des grignons séchés et des margines congelées obtenus sur le site de Beni Mellal (structure traditionnelle par pressage) en janvier 2008 (A partir d'un lot homogène de 600 kg d'olives, il a été obtenu : 253 kg de grignon ; 220 litres de margines ; 123 litres d'huile)

Le ferment d'ensilage a été mis en culture 24 heures à 30°C dans des flacons de 150ml contenant 90ml du milieu MRS liquide. La culture a été centrifugée pendant 15 min à 14500 rpm ensuite le culot est récupéré et mélangé avec les margines.

Les grignons (conservés sec) ont été réhydratés avec les margines contenant le ferment d'ensilage à une concentration de  $2 \cdot 10^8$  bactéries par gramme MS.

Les pots d'ensilage sont remplis en tassant la pulpe afin de limiter la quantité d'air. Ils sont fermés puis scellés. Les microsilos sont incubés à l'obscurité et à l'étuve à une température de 25°C.

### 3. Résultats et discussion

Après un an de conservation, les ensilages naturels présentent des caractéristiques acceptables : pH inférieur à 5, humidité entre 50 et 60 %, (Figure III), pas d'odeur de putréfaction.

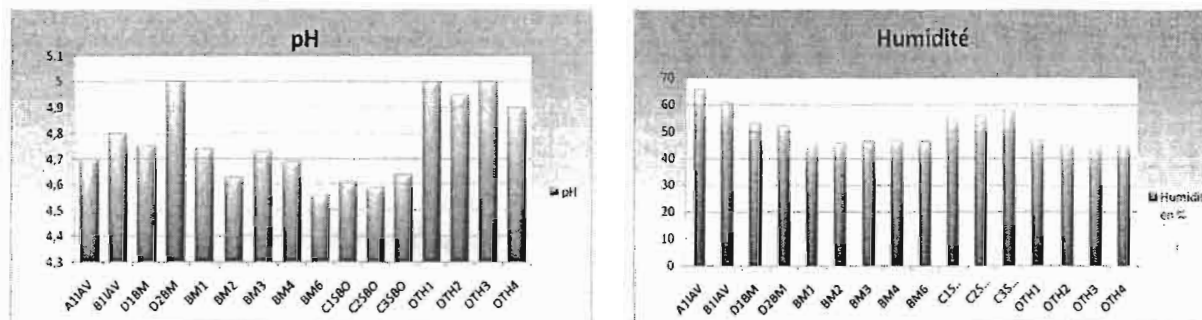


Figure 3: pH et Humidité des ensilages naturels.

L'analyse des jus d'ensilages, après centrifugation et filtration, par l'HPLC permettra d'apporter des informations complémentaires sur la concentration en sucres résiduels et sur la concentration en acide lactique et acétique. Une différence entre les ensilages à partir desquels on a pu isoler les bactéries lactiques avec ceux contenant majoritairement des levures pourra être ainsi faite et apporter des réponses plus précises sur la faisabilité des ensilages.

Ce travail a permis d'isoler 66 souches à partir des différents ensilages, 25 souches ont été identifiées comme des bactéries. Elles sont toutes Gram (+) et Catalase (-). L'analyse par HPLC des surnageants de cultures des 25 souches bactériennes isolées, a mis en évidence le métabolisme fermentaire de chaque souche, il en résulte la présence de 3 souches homofermentaires (IF 0.96/0.97) dont la production d'acide lactique est nettement supérieure à ceux des autres acides, 5 souches hétérofermentaires facultatives produisant moins d'acide lactique et plus d'acide propionique et acétique (IF 0.84/0.85), une seule souche est hétérofermentaire stricte (IF 0.68). On note l'absence de production de l'éthanol pour toutes les souches. (Tableau II).

L'isolement et la caractérisation a permis de mettre en évidence la présence des bactéries lactiques dans des ensilages des sous produit de l'olivier. Il ressort de ce travail que la présence des bactéries lactiques semble dépendre de la technique d'extraction de l'huile d'olive utilisé. En effet, les résultats d'analyses montrent que les bactéries qui produisent le plus d'acide lactique proviennent du site de

Beni Mellal où l'on utilise la méthode de pressage sur des olives non lavées pour obtenir des grignons et des margines.

Les ensilages conservés à 25°C ont permis d'isoler le plus de bactéries lactiques tandis que les levures ont été récupérées principalement sur les ensilages conservés à température ambiante. L'isolement des levures n'est pas caractéristique d'un type de résidu comme les bactéries puisqu'on les retrouve sur les grignons/margines et sur les margions.

Au vu des regroupements faits en fonction de la production d'acides organiques, de la taille des colonies, de leur aspect et de leur profil sur les galeries API, on peut supposer que l'on a 9 souches de bactéries différentes. Ces souches appartiennent aux genres : *Lactobacillus plantarum* et *Lactobacillus pentosus*.

Après une caractérisation des bactéries lactiques, la mise en place d'un ensilage a été réalisée avec une souche préalablement isolée des ensilages naturels. Après 18 jours, le processus de fermentation n'a pas été réalisé au sein des ensilages inoculés et témoin, cela a été mis en évidence par le suivi de la microflore qui a montré la prédominance des levures et les champignons (Tableau III). Les bactéries dénombrées dans l'ensilage 2 ne sont pas des bactéries lactiques.

La mise en place des ensilages inoculés avec la souche NE022 n'a pas abouti aux résultats souhaités, cela est dû à plusieurs raisons tels que l'homogénéité du mélange, la capacité et la résistance de la souche et la nature du substrat.

**Tableau II:** Détermination du métabolisme de différentes bactéries lactiques.

| Groupe | Souches | Glucose résiduel | Acide lactique g/L | Acide acétique g/L | Acide propionique g/L | Indice fermentaire | Genre et espèce     | Origine |
|--------|---------|------------------|--------------------|--------------------|-----------------------|--------------------|---------------------|---------|
| 1      | NE031   | 0,98             | 25,00              | 0,55               | 0,29                  | 0,68               | <i>L. plantarum</i> | BM1     |
| 2      | NE055   | 0,62             | 61,96              | 10,28              | 1,26                  | 0,84               | <i>L. plantarum</i> | OTH1    |
|        | NE058   | 0,59             | 61,53              | 10,34              | 1,26                  | 0,84               | <i>L. plantarum</i> | OTH1    |
|        | NE059   | 0,70             | 61,05              | 10,51              | 1,30                  | 0,84               | <i>L. plantarum</i> | OTH1    |
|        | NE053   | 0,65             | 61,25              | 10,46              | 1,26                  | 0,84               | <i>L. plantarum</i> | OTH1    |
| 3      | NE052   | 1,21             | 61,18              | 10,50              | 1,24                  | 0,84               | <i>L. plantarum</i> | BM4     |
|        | NE037   | 1,11             | 61,16              | 10,59              | 1,26                  | 0,84               | <i>L. plantarum</i> | BM3     |
|        | NE034   | 1,01             | 61,01              | 10,40              | 1,26                  | 0,84               | <i>L. plantarum</i> | BM3     |
|        | NE033   | 0,88             | 59,82              | 10,09              | 1,25                  | 0,84               | <i>L. plantarum</i> | BM3     |
|        | NE036   | 0,63             | 58,82              | 9,87               | 1,22                  | 0,84               | <i>L. plantarum</i> | BM3     |
| 4      | NE039   | 6,03             | 57,67              | 10,45              | 0,00                  | 0,85               | <i>L. pentosus</i>  | BM3     |
| 5      | NE038   | 1,50             | 61,30              | 10,52              | 1,28                  | 0,84               | <i>L. pentosus</i>  | BM3     |
| 6      | NE030   | 3,29             | 58,84              | 10,46              | 0,98                  | 0,84               | <i>L. pentosus</i>  | BM1     |
|        | NE032   | 3,00             | 60,09              | 10,62              | 0,86                  | 0,84               | <i>L. pentosus</i>  | BM1     |
|        | NE035   | 6,83             | 56,82              | 10,26              | 0,90                  | 0,84               | <i>L. pentosus</i>  | BM3     |
| 7      | NE015   | 0,64             | 25,17              | 0,43               | 0,48                  | 0,96               | <i>L. plantarum</i> | D1BM    |
|        | NE022   | 0,44             | 24,87              | 0,39               | 0,52                  | 0,96               | <i>L. plantarum</i> | D2BM    |
|        | NE018   | 0,91             | 24,73              | 0,49               | 0,50                  | 0,96               | <i>L. plantarum</i> | D1BM    |
| 8      | NE013   | 0,51             | 25,03              | 0,15               | 0,60                  | 0,97               | <i>L. plantarum</i> | D1BM    |
| 9      | NE016   | 1,20             | 24,73              | 0,69               | 0,50                  | 0,95               | <i>L. pentosus</i>  | D1BM    |
|        | NE017   | 3,22             | 23,38              | 0,47               | 0,44                  | 0,96               | <i>L. pentosus</i>  | D1BM    |
|        | NE014   | 0,54             | 25,06              | 0,65               | 0,41                  | 0,96               | <i>L. pentosus</i>  | D1BM    |
|        | NE029   | 3,85             | 22,70              | 0,55               | 0,45                  | 0,96               | <i>L. pentosus</i>  | BM1     |
|        | NE019   | 3,92             | 22,63              | 0,27               | 0,46                  | 0,97               | <i>L. pentosus</i>  | D1BM    |
|        | NE020   | 3,42             | 22,93              | 0,32               | 0,45                  | 0,97               | <i>L. pentosus</i>  | D1BM    |



**Tableau III:** Analyse de la microflore totale, du pH et de l'humidité dans les ensilages inoculés.

| Paramètres                    | Témoïn                |                       | Ensilage Inoculé 1    |                      | Ensilage Inoculé 2    |                       |
|-------------------------------|-----------------------|-----------------------|-----------------------|----------------------|-----------------------|-----------------------|
| Temps (jours)                 | T0                    | T18                   | T0                    | T18                  | T0                    | T18                   |
| Bactéries totales (ufc/g PMS) | 1,35 10 <sup>4</sup>  | <3,31 10 <sup>2</sup> | 2,54 10 <sup>4</sup>  | <3,3210 <sup>2</sup> | 2,54 10 <sup>4</sup>  | <3,92 10 <sup>2</sup> |
| Champignons (ufc/g PMS)       | 2,11 10 <sup>2</sup>  | <3,31 10 <sup>2</sup> | 5,97 10 <sup>3</sup>  | <2,3210 <sup>2</sup> | 5,97 10 <sup>3</sup>  | <2,36 10 <sup>3</sup> |
| Levures (ufc/g PMS)           | <2,11 10 <sup>2</sup> | 1,73 10 <sup>5</sup>  | <2,13 10 <sup>1</sup> | 1,5510 <sup>5</sup>  | <2,1310 <sup>1</sup>  | <2,62 10 <sup>3</sup> |
| Bactéries MRS (ufc/g PMS)     | <2,11 10 <sup>2</sup> | <3,31 10 <sup>3</sup> | 3,26 10 <sup>3</sup>  | <3,310 <sup>2</sup>  | 3,26 10 <sup>3</sup>  | 1,86 10 <sup>6</sup>  |
| Levures MRS (ufc/g PMS)       | <2,11 10 <sup>2</sup> | 1,57 10 <sup>6</sup>  | <2,13 10 <sup>1</sup> | 2,4310 <sup>5</sup>  | <2,13 10 <sup>1</sup> | <3,93 10 <sup>3</sup> |
| pH                            | 5,10                  | 4,96                  | 5,10                  | 5,04                 | 5,04                  | 5,01                  |
| Humidité %                    | 40,81                 | 35,53                 | 41,35                 | 37,59                | 41,35                 | 36,34                 |

Cependant, ces travaux préliminaires ouvrent de nouvelles perspectives quant à la valorisation des sous-produits de l'industrie oléicole par voie microbienne. En effet, ces « déchets » de l'olivier posent des problèmes de pollution car ils sont déversés dans l'environnement sans moyen de ré-utilisation et/ou de stockage adapté. L'ensilage des ces résidus saisonniers permettrait d'envisager une utilisation annuelle pour diverses valorisations tels que la lombriculture, la production de champignons supérieurs ou encore alimentation animale. L'ajout de bactéries lactiques endogènes sélectionnées permettra d'obtenir des ensilages contrôlés.

La suite de ce travail sera de continuer les essais pour arriver à avoir un ensilage correct qui répond aux critères biologiques et biochimiques d'un bon ensilage, par exemple en utilisant un ferment mixte, des substrats frais.

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## Culture des champignons comestibles et médicinaux sur un coproduit oléicole solide pour le développement durable du secteur oléicole au Maroc

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### Résumé

L'industrie oléicole, une activité concentrée dans la région méditerranéenne, connaît actuellement une évolution à tous les niveaux. Cette industrie cause, en absence de traitement de ses rejets, d'énormes problèmes environnementaux. Les perspectives actuelles s'orientent vers la valorisation de la biomasse totale de l'olivier pour assurer un développement durable du secteur oléicole au Maroc. Notre étude vise à la détermination d'une formule originale de préparation des différents composants des coproduits de l'industrie oléicole (bois d'olivier, feuilles d'olive, grignons d'olive et des margines d'olives) pour la culture des champignons comestibles et médicinaux sur un Substrat Oléicole Solide (SOS) en fermentation en milieu solide. Cette culture a pour but d'assurer à la fois la production de la biomasse fongique pour l'alimentation humaine et la détoxification des coproduits (meule) pour servir à l'alimentation animale. La saisonnalité de substrats d'oliviers (les grignons d'olives et les margines) a été remédiée par le séchage solaire sous serre, une étude qui sera présentée en détaille dans une autre présentation. Un test de criblage des souches de *Lentinula edodes* (champignons comestible et médicinal, connu sous le nom de Shiitake) a été effectué afin d'obtenir une souche efficace et capable de dégrader les polyphénols dans les margines. La préparation d'inoculum de la souche sélectionnée a été optimisée par l'induction du mycélium à la production des laccases. Les conditions de culture du champignon ont été optimisées par le développement des modèles mathématiques. La cinétique de croissance de shiitake sur le mélange ainsi que la transformation de la matière organique ont été étudiées. L'activité laccase, qui a été observée dominante pendant la croissance des champignons sur le SOS a été purifiée pour des applications biotechnologiques ultérieures.

**Mots clés :** Substrat Oléicole Solide (SOS), *Lentinula edodes*, Fermentation en Milieu Solide (FMS), Polyphénols, Enzymes, Laccases.

## Edible and medicinal mushroom cultivation on solid olive substrate (SOS) for sustainable olive field in Morocco

### Abstract

The olive industry, an activity concentrated in the Mediterranean area, is nowadays under development at all levels. This industry has, until now, lack of processing of their discharges, resulting in major environmental issue. The current research is directed towards a recovery of the total biomass of trees for a sustainable industry. Our present study aims to broaden the horizon of recovery of the so-called discharges by culturing edible and medicinal mushrooms on olive SOS biomass (olive tree wood, olive leafs, olive pomace and olive mill wastewater OMWW) to produce biomass for both food and feed, and also for the detoxification of OMWW, the main determinant of environmental pollution in the olive industry. Seasonality substrates olive (olive cake and OMWW) has been remedied by drying solar in greenhouse, a study which will be detailed in another presentation. A screening of strains of *Lentinula edodes* (edible and medicinal mushroom, known as Shiitake) was carried out to obtain an efficient strain capable of degrading the polyphenols in olive waste. The preparation of the spawn has been optimized by the induction of mycelium. Experimental plans have been developed to optimize the cultivation of shiitake by mixing of the entire biomass SOS. The kinetics of growth of shiitake was studied. The enzymes used in the detoxification of olive oil have been listed. The laccase activity which dominated during the growth of fungi on the SOS has been purified to use for further application.

**Keywords:** Solid Olive Substrate (SOS), *Lentinula edodes*, Solid State Fermentation, polyphenols, enzymes, Laccases.

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## 1. Introduction

L'oléiculture représente une des plus anciennes activités agricoles dans le bassin méditerranéen. Toutefois, elle présente l'inconvénient de générer d'énormes quantités de déchets liquides dont la fraction organique complexe leur confère un pouvoir fortement polluant (Sayadi et al., 2000). A côté des margines, l'industrie oléicole produit deux coproduits solides, les grignons d'olive et le bois de taille.

De nombreuses études ont été menées en vue de trouver des solutions pour le traitement des déchets oléicoles, en particulier la réduction de la charge phénolique des margines par la culture de champignons (Zervakis et al., 1996, Kalmis et Sargin, 2009, Lakhtar et al., 2010a). Les perspectives de ces études ont mis l'accent sur la valorisation des déchets de l'oléiculture par la culture des basidiomycètes en fermentation en milieu solide (FMS). Les basidiomycètes de pourriture blanche, en particulier *Lentinula edodes* (Berk.) Pegler, sont capables de se développer en présence de la lignine, et leur développement est associé à la production des enzymes phénoloxydases non spécifiques (Mata et Savoie, 1998). Ainsi, les basidiomycètes ont été considérés comme de bons candidats pour permettre à la fois une valorisation des coproduits oléicoles lignocellulosiques et la réduction des polyphénols des margines (Zervakis et al., 1996, Kalmis et Sargin, 2009).

L'objectif de l'étude est d'évaluer la transformation de la composition chimique et la réduction en polyphénols du mélange de coproduits de l'olivier solide (COS) constitués de bois de taille, grignons d'olive et margines durant la croissance du mycélium de *L. edodes* Le119. Une attention particulière a été consacrée à l'évaluation de la production de CO<sub>2</sub> durant la croissance mycélienne de la souche Le119. Le changement de l'abondance relative des fractions de carbone, à la fois simples et complexes analysés par la technique de la CPMAS RMN 13C soutenue par les analyses biochimiques a été étudié pour relier l'activité métabolique du champignon à la transformation des résidus oléicoles.

## 2. Matériel et méthodes

### 2.1. Origine des échantillons

Les échantillons des grignons et margines d'olive ont été prélevés dans une unité de trituration triphasique avec le système de presse. Les standards phénoliques ont été fournis par Extrasynthese. Tous les solvants utilisés pour l'extraction étaient de qualité HPLC (Sigma, France).

### 2.2. Microorganisme et préparation du spawn

La souche de *L. edodes* Le119 a été préalablement sélectionnée pour sa capacité de dégradation des polyphénols de margines (Lakhtar et al., 2010a). La souche a été régulièrement repiquée sur un milieu de PDA contenant 10% des margines. L'inoculum (spawn) a été préparé sur les gains de blé contenant 10% de margines (Lakhtar et al., 2010b). La préparation du spawn a été effectuée dans les colonnes de « Raimbault » (De Araujo et al., 1997) et l'incubation a été faite à 25°C pendant 10 jours.

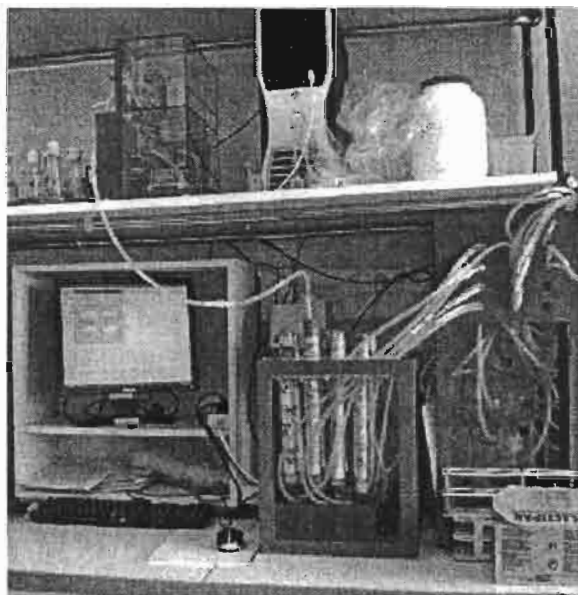


Figure 1: Dispositif de FMS connecté au Système de Respirometrie PNEO.

### 2.3. Préparation des substrats COS

Le bois de taille a été broyé et tamisé pour récupérer des particules comprises entre 2 mm et 0,8 mm. Les particules, ayant avec une taille inférieure à 0,8 mm, ont aussi été utilisées dans la composition du substrat représentant 30% du poids total du bois de taille. Le mélange du COS optimal est composé de 20% de bois de taille, 30% de grignons d'olive et 50% de margines. Il a été stérilisé à 121°C pendant 30 minutes.

## 2.4. Inoculation du COS par le spawn de *L. edodes*

L'inoculation a été faite à raison de 10% (poids humide: poids humide), et le remplissage des colonnes a été procédé en alternant des couches de substrat et de spawn, pour assurer une croissance homogène du mycélium. Les colonnes ont été incubées dans l'étuve sans aération forcée à 25°C pendant 2 jours et par la suite ont été placées dans le dispositif de la FMS (De Araujo et al., 1997) pour une durée d'incubation d'un mois. Deux colonnes témoins non inoculées ont été préparées et incubées dans les mêmes conditions.

## 2.5. Analyse des échantillons

L'activité respiratoire a été mesurée chaque jour à l'aide du PNEO, qui permet de mesurer à la fois quatre paramètres ; le débit d'aération, l'humidité de l'air, la température de l'air et la teneur de l'air en CO<sub>2</sub> à la sortie de la colonne. Le débit de l'aération a été fixé à 20 mL.min<sup>-1</sup>. La biomasse fongique a été estimée par la quantification de l'ergostérol (Lakhtar et al., 2010b). Les sucres réducteurs et sucres totaux ont été déterminés par la méthode de Miller (1959) en utilisant le réactif de DNS et celle de DuBois et al. (2002) en utilisant de l'anthrone respectivement. L'extraction de la fraction phénolique a été réalisée dans la solution eau-méthanol (40-60) ; à un gramme du substrat fermenté lyophilisé et finement broyé, on ajoute 10 ml de la solution organique. Le mélange a été homogénéisé et incubé pendant 1h à 4°C. L'extraction a été répétée trois fois. La quantification des phénols totaux a été faite suivant la méthode de Folin Ciocalteu (Bärlocher et Garça, 2005) en utilisant l'acide caféique comme standard.

## 2.6. Analyses chromatographiques

L'extraction des monomères a été effectuée en deux étapes. La première étape consiste à l'extraction de la fraction phénolique par la solution eau-méthanol (40-60). La deuxième étape consiste en la purification des monomères phénoliques par l'extraction avec l'acétate d'éthyle et l'analyse des monomères a été réalisée par l'HPLC suivant la méthode de De Marco (2007). La plateforme HPLC est composée d'un dégazeur (1200 Series Degasser, Agilent technologies), d'une pompe quaternaire (1200 Series Quaternary Pump, Agilent technologies), d'un échantillonneur liquide automatique ou passeur (1200 Series Automatic Liquid Sampler, Agilent technologies) et d'un détecteur à barrette de diodes (1200 Series Diode-Array Detector, Agilent technologies). La séparation des composés a été opérée sur la colonne Atlantis d18 (5µm, 4,6 x 250 mm) Waters gardée à une température constante (25±2°C). La détection des composés a été effectuée sur une longueur d'onde de 280 nm. Les pics ont été identifiés par comparaison avec les standards.

## 2.7. Activités enzymatiques

### 2.7.1. Extraction d'enzymes

L'extraction des enzymes a été effectuée sur 3 gr du substrat fermenté frais décongelé mélangé avec 10 ml du tampon d'acétate (0,1M, pH 5,0) contenant 5 mM de CaCl<sub>2</sub>, 0,05% du Tween 80 et 3% du polyvinylpyrrolidone insoluble (PVPP) (Sampedro et al., 2007). Le mélange a été incubé à la température ambiante pendant 1h et l'extrait aqueux a été récupéré par centrifugation (4500 rpm pendant 10 min) suivie d'une filtration sur papier Watman N°2.

### 2.7.2. Activités enzymatiques

L'activité endoglucanase (cellulase, EC 3.2.1.4) a été déterminée selon la méthode de Mandels et al. (1976). L'activité enzymatique est exprimée en unité par g du substrat poids sec (SPS) (1U= 1 µmole de sucres réducteurs formés par minute). L'activité lipase a été déterminée suivant la technique rapportée par Goujard et al., (2009), en utilisant *p*-laurate de nitrophenyl comme substrat. L'activité tannase a été mesurée suivant la technique de Sharma et al. (2000). Les activités de Mn peroxydase, Lignine peroxydase et laccase ont été mesurées suivant la technique rapportée par Lakhtar et al. (2010a). La technique décrite par Santos et Linardi (2004) a été utilisée pour la détermination de l'activité enzymatique de catéchol-1,2-dioxygénase et protocatéchuate-3,4-dioxygénase. L'activité est exprimée en unité par g SPS (1U= 1 µmole de produit formé par minute).

## 2.8. Analyses élémentaires : C et N

Les teneurs en C et N, dans les échantillons de culture de *L. edodes*, ont été déterminées à l'aide d'un analyseur élémentaire CHN (Flash EA 1212 Elemental Analyzer, France). L'analyseur CHN a été calibré avec l'acide aspartique à différentes concentrations.

## 3. Résultats et discussions

### 3.1. Croissance de *L. edodes* sur COS

Le remplissage des colonnes de Raimbault avec le substrat COS prétraité a été alterné avec l'ajout du spawn, qui permet un envahissement homogène et uniforme du substrat par le mycélium. L'incubation des colonnes pendant deux jours dans l'étuve nous a permis d'éviter les contaminations par les moisissures. Après deux jours d'incubation, on a remarqué que tous les grains du spawn inoculés ont démarré par le développement du mycélium. L'envahissement total du substrat a été remarqué après la troisième semaine d'incubation (24 jours d'incubation). Par ailleurs, aucune contamination n'a été observée sur les deux colonnes témoins non inoculées.

### 3.2. Analyses des effluents gazeux par PNEO

A la sortie des colonnes de Raimbault, les effluents gazeux ont été collectés afin de suivre l'évolution de quatre paramètres (débit d'air, humidité relative, température et la production de CO<sub>2</sub>) en fonction du temps (Fig. 1). L'humidité relative et la température de l'air à la sortie des colonnes fluctuent entre deux valeurs (50 et 58 %) pour la l'humidité et (23 et 27°C) pour la température de l'air. Avant la mesure de CO<sub>2</sub> à la sortie de la colonne, le débit de l'air traversant la colonne, a été ajusté à 20 ml/min.

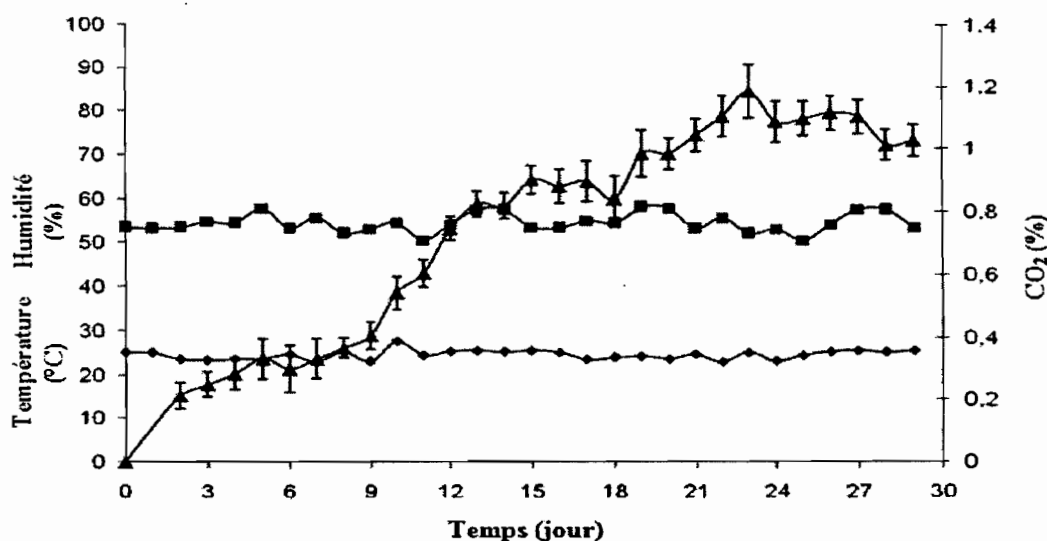


**Figure 1:** Evolution de trois paramètres; (▲) CO<sub>2</sub> produit, (◆) température de l'air, (■) humidité relative de l'air, au cours de 30 jours de culture de *L. edodes* sur COS à 25°C.

Sur la Figure 1, on remarque que l'évolution de CO<sub>2</sub> s'effectue selon quatre étapes alternées. Tout d'abord, durant les 6 premiers jours d'incubation sur le dispositif FMS, une augmentation continue de CO<sub>2</sub> a été remarquée passant de 0 à 0,23%. Ensuite, entre le 6<sup>ème</sup> et 8<sup>ème</sup> jour d'incubation, un premier plateau de stabilisation du CO<sub>2</sub> située à 0,23% de CO<sub>2</sub> produit. Par contre, entre le 8<sup>ème</sup> et le 14<sup>ème</sup> jour d'incubation, une augmentation importante de la respiration du mycélium de Le119 a été remarquée allant de 0,23 à 0,7. Un deuxième plateau de stabilisation de CO<sub>2</sub> a été observé entre le 14<sup>ème</sup> et 16<sup>ème</sup> jour de FMS. Enfin, un plateau haut de respiration a été obtenu à partir du 23<sup>ème</sup> jour de FMS et reste constant pendant une semaine avec un taux élevé de CO<sub>2</sub> obtenu (1,18%).

### 3.3. Evolution de la biomasse de *L. edodes* sur COS:

L'estimation de la biomasse a été déterminée indirectement par la quantification de l'ergostérol contenu dans le substrat fermenté par la souche Le119 de *L. edodes*. Le coefficient de conversion de l'ergostérol en biomasse de *L. edodes* a été évalué à 3.80 mg de l'ergostérol pour 1 g de biomasse pure. L'évolution de la biomasse au cours de la FMS (Fig.2) suit relativement l'allure de production de CO<sub>2</sub> mesuré dans les effluents gazeux (Fig.1).

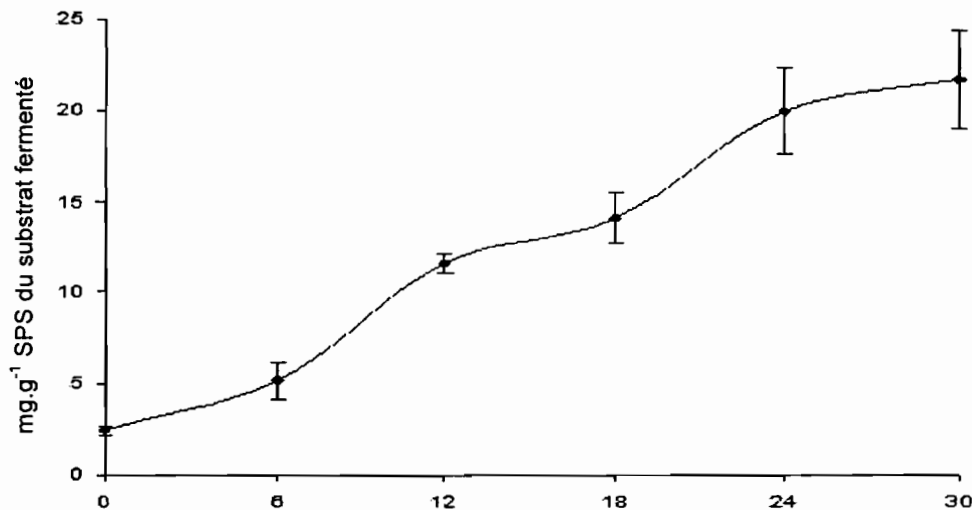


**Figure 2:** Evolution de la biomasse de la souche Le119 produite au cours de 30 jours d'incubation sur COS à 25°C.

L'évolution de la biomasse au cours de la FMS passe par trois phases de croissance rapide alternées par deux phases de croissance relativement lente. La biomasse évolue de 2.47 à 5 mg/g SPS du COS pendant les 6 premiers jours d'incubation. Ensuite entre le 6<sup>ème</sup> et le 12<sup>ème</sup> jour d'incubation une augmentation importante de la biomasse produite a été remarquée, allant de 5 à 11,4 mg.g<sup>-1</sup> SPS du COS. Par contre entre le 12 et 18<sup>ème</sup> jour d'incubation, la biomasse passe de 11,4 à 14,5 mg.g<sup>-1</sup> SPS, et cette évolution de biomasse correspond au deuxième plateau de CO<sub>2</sub> produit durant le développement du mycélium (Fig.1). Entre 18 et 24 jours d'incubation une augmentation importante a été de nouveau observée, allant de 14,5 à 20,5 mg.g<sup>-1</sup> SPS du COS. Cette augmentation correspond également à l'augmentation de CO<sub>2</sub> observé dans la Figure 1. Enfin, un plateau de biomasse a été obtenu à partir de 24 jours de FMS pour arriver à une biomasse de 21,61 mg.g<sup>-1</sup> après 30 jours d'incubation. Après un mois d'incubation, la biomasse estimée dans notre étude dépasse 2,5 fois la biomasse du *Pleurotus ostreatus* produite sur les coques de café avec le même taux d'inoculation (10%) (Sobal, 2002).

### 3.4. Evolution des sucres réducteurs, sucres totaux, pH du substrat fermenté

L'évolution de sucres réducteurs, des sucres totaux et du pH du substrat inoculé avec la souche Le119 de *L. edodes* est présentée dans la Figure 3.

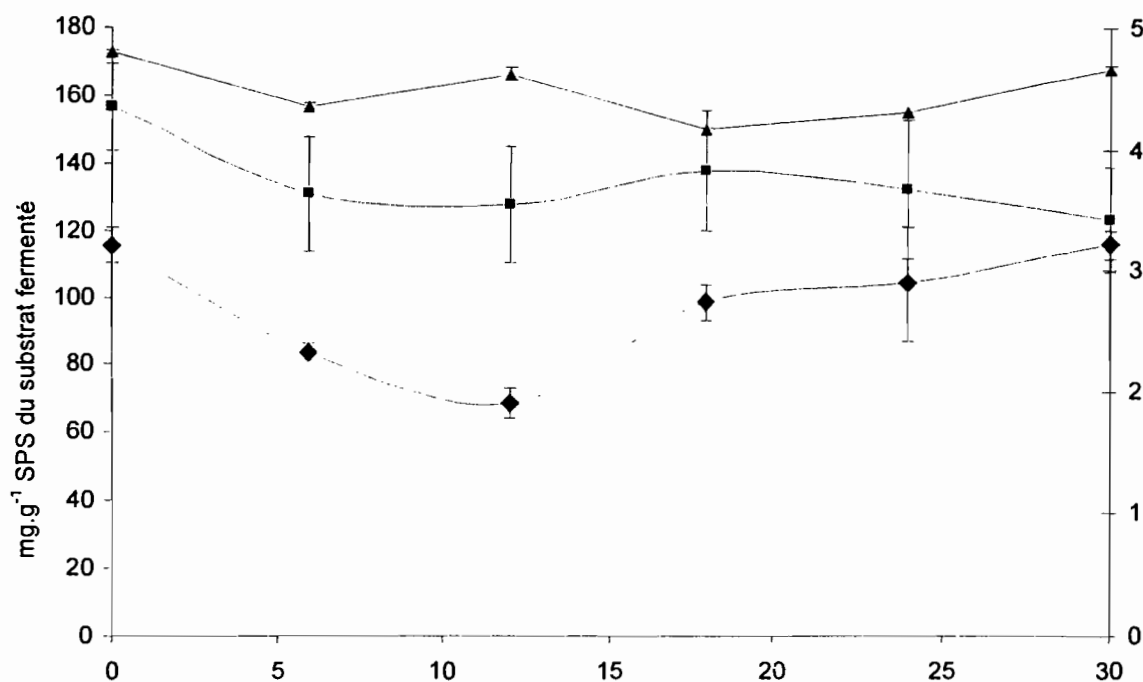


**Figure 3:** Evolution des (◆) sucres réducteurs, (■) sucres totaux et du (▲) pH du substrat fermenté au cours de 30 jours de culture de la souche Le119 sur COS à 25°C.

On remarque une diminution de la teneur en sucres réducteurs et totaux dans le milieu après 12 jours d'incubation, suivie d'une augmentation graduelle pour arriver à 108 et 120 mg.g<sup>-1</sup> SPS du SOS respectivement. Accompagné de cette évolution, on remarque que les valeurs du pH du substrat fermenté restent très stables tout au long de la FMS de la souche de *L. edodes* sur COS et fluctuent de 4,5 à 5.

### 3.5. Evolution des phénols totaux

Les phénols totaux ont été déterminés par la méthode standard de Folin Ciocalteu pour pouvoir comparer nos résultats aux ceux de la littérature. La décoloration de l'extrait organique des phénols totaux a été déterminée à 280 nm. Selon Chin et al., (1994), l'absorbance à 280 nm est une mesure approximative du degré d'aromaticité des matières organiques dissoutes. Une forte corrélation entre la dégradation des phénols et la décoloration du substrat a été observée. Le développement du champignon a permis une dégradation de 80% des phénols totaux et 85% de décoloration du substrat (Fig. 4).

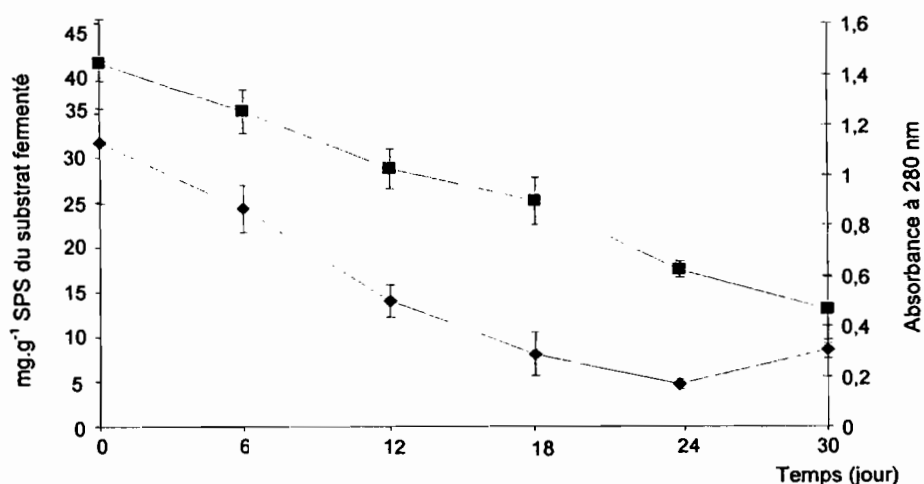


**Figure 4:** Evolution de (■) la décoloration de l'extrait phénolique et (◆) des phénols totaux du substrat fermenté au cours de 30 jours de culture de la souche Le119 sur COS à 25°C.

Les résultats confirment ceux obtenus à partir de la décoloration et montrent l'apparition des molécules absorbant aux alentours de 500 nm. Ces molécules peuvent être responsables de l'augmentation légère des phénols totaux observée après 24 jours d'incubation. Sampedro et al. (2007) ont mesuré de semblables chutes de teneurs en phénol totaux des grignons humides, issues du système de trituration à deux phases, par la culture de *Paecilomyces farinosus* après 20 semaines d'incubation. Selon Aloui et al. (2007), l'ajout de la bagasse de canne de sucre aux grignons humides favorise la dégradation des phénols totaux. En effet une réduction des phénols totaux de 60% a été observée après l'ajout de 30% de la bagasse de canne à sucre pour la culture de *Phanerochaete chrysosporium* après 6 jours d'incubation.

Les spectres UV-vis des extraits d'échantillons prélevés durant la croissance du champignon sont présentés dans la Figure 5.

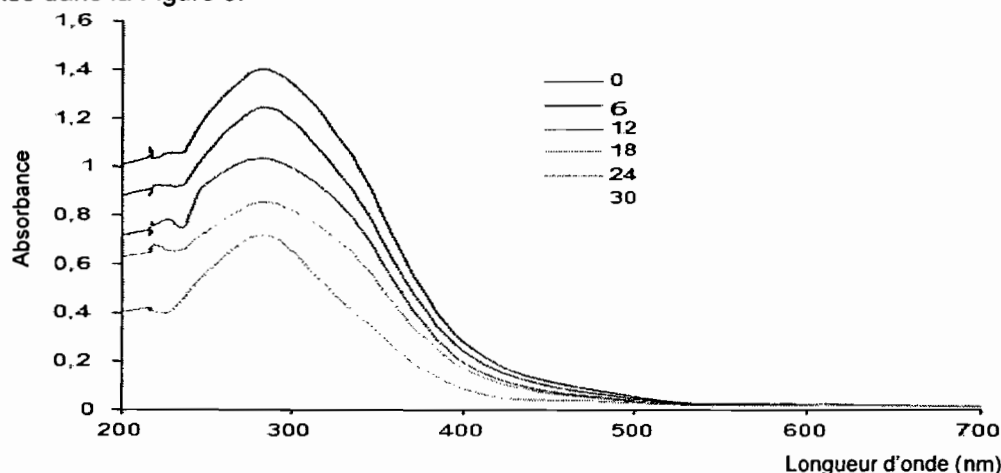


**Figure 5:** Spectres UV-visible de la fraction phénolique extraite des échantillons du substrat fermenté au cours de 30 jours de culture de la souche Le119 sur COS à 25°C.

### 3.6. Les monomères phénoliques

La disparition des monomères phénoliques est présentée dans le Tableau 1. Six monomères ont été identifiés dans l'extrait éthylique obtenu à partir du substrat fermenté. Leur quantification, déterminée par l'HPLC, a montré que l'Oleuropéine est le composé le plus abondant dans le COS avant la fermentation ( $6,54 \mu\text{mole.g}^{-1}$  SPS du SOS), suivie par l'acide *p*-coumarique et l'hydroxytyrosol ( $4,51$  et  $4,32 \mu\text{mole.g}^{-1}$  SPS du SOS respectivement). Après un mois de culture de la souche Le119, à l'exception de l'acide *trans*-cinnamique qui présente une faible diminution de sa teneur (15 %), tous les composés analysés, ont subi une réduction de leur teneur avec des rendements variables (92 et 26 % de réduction pour l'hydroxytyrosol et l'acide *p*-coumarique respectivement). Dans le COS témoin, non inoculé et incubé dans les mêmes conditions que celles de la culture, on a remarqué une réduction partielle de tous les composés analysés, après 30 jours d'incubation avec un débit d'aération de  $20 \text{ mL.min}^{-1}$ . Leur réduction peut être expliquée par l'oxydation non enzymatique qui pourrait être causée par le passage forcé de l'air dans le substrat durant son incubation dans le dispositif de la FMS. El Hajjouji et al. (2008) ont reporté qu'une augmentation de réduction des composés phénoliques est favorisée avec l'application d'une aération forcée sur les margines.

**Tableau 1:** Concentration des monomères phénoliques ( $\mu\text{mole.g}^{-1}$  SPS du COS) durant la culture de *L. edodes* pendant 1 mois d'incubation à 25°C.

| Temps (jours) | Oleuropéine | Hydroxy-tyrosol | Acide caféique | Tyrosol | Acide <i>trans</i> -cinnamique | Acide <i>p</i> -coumarique |
|---------------|-------------|-----------------|----------------|---------|--------------------------------|----------------------------|
| 0             | 6,54        | 4,32            | 3,22           | 2,32    | 2,32                           | 4,51                       |
| 6             | 4,21        | 2,35            | 3,21           | 2,12    | 2,23                           | 3,54                       |
| 12            | 3,21        | 3,21            | 3,15           | 1,63    | 2,35                           | 4,16                       |
| 18            | 2,54        | 2,32            | 2,36           | 1,33    | 2,16                           | 5,54                       |
| 24            | 1,02        | 0,32            | 1,06           | 1,03    | 2,03                           | 3,21                       |
| 30            | 0,95        | 0,32            | 1,01           | 0,03    | 1,97                           | 3,32                       |
| Témoin        | 5,98        | 4,45            | 3,11           | 2,22    | 2,25                           | 4,02                       |

L'écart type a été estimé à 5%.



### 3.7. Evolution de la composition élémentaire du substrat fermenté :

L'évolution de cinq paramètres conventionnels (humidité du substrat, C, N, C/N, Perte en matière) a été effectuée à partir des prélèvements du substrat fermenté au cours de 30 jours d'incubation à 25°C. Les résultats sont présentés dans le Tableau 2.

**Tableau 2:** Evolution de différents paramètres chimiques mesurés des échantillons du substrat fermenté au cours de 30 jours de culture de *L. edodes* sur COS à 25°C.

| Temps (jours) | C (%)     | N (%)     | C/N   | Humidité (%) | Perte en Matière (%) |
|---------------|-----------|-----------|-------|--------------|----------------------|
| 0             | 48.52±1,3 | 0,95±0,10 | 51,07 | 44.30±0.49   | -                    |
| 6             | 46.17±0,7 | 0.95±0,06 | 48,60 | 45.14±2.61   | 1.87±0.67            |
| 12            | 43.89±1,4 | 0,96±0,04 | 45,72 | 45.94±1.14   | 4.99±1.01            |
| 18            | 42.89±1,7 | 1,09±0,07 | 39,35 | 46.47±0.92   | 6.73±1.80            |
| 24            | 41.32±1,2 | 1,31±0,08 | 31,54 | 48.39±0.37   | 9.48±1.59            |
| 30            | 40.32±0,6 | 1,52±0,1  | 26,53 | 46.41±1.07   | 12.18±1.39           |

Un test d'analyse de variance a été effectué pour l'ensemble des paramètres étudiés. Les résultats montrent une différence significative entre les prélèvements ( $p=0.05$ ). Le passage de l'air humidifié à travers les colonnes (volume utile de 200 mL) avec le débit de 20 mL.min<sup>-1</sup> a permis de maintenir une humidité de 46±2 %. Les résultats des analyses élémentaires, montrent que le carbone diminue en passant de 48.52 à 40.32 % pendant la durée d'incubation, alors que l'azote présente une augmentation de 0.95 à 1.52% de la matière sèche. La diminution de carbone est caractéristique de la dégradation des matières organiques. En effet, de semblables profils de diminution de carbone ont été rapportés pour la dégradation des matières organiques (Mena et al., 2003, Huang et al., 2004). Le ralentissement de la diminution du carbone peut être expliqué par la nature lignocellulosique du substrat qui est dégradé beaucoup plus lentement. Par ailleurs, l'augmentation d'azote est due à sa concentration engendrée par la dégradation des composés carbonés réduisant la masse totale du substrat durant la culture. De ce fait, le rapport C/N diminue au cours de la culture compris entre 51,07 et 26.53. Cette diminution reflète le développement du champignon accompagné d'une part avec un dégagement de CO<sub>2</sub> et d'autre part de la décomposition de la matière organique, ainsi que l'enrichissement du substrat en matières azotées.

### 3.8. Activités enzymatiques

Les activités enzymatiques mesurées durant la croissance du champignon sur le COS sont présentées dans le Tableau 5. Parmi les activités enzymatiques impliquées dans la décomposition de la matière organique, l'activité des cellulases a été mesurée. Il y a apparition de cette activité après 6 jours d'incubation et elle atteint sa valeur maximale après 18 jours (1,13 U.g<sup>-1</sup> SPS). L'activité lipase présente une augmentation au début de la culture (de 0,01 à 0,02 U.g<sup>-1</sup> SPS), et diminue après 18<sup>ème</sup> jours de culture pour disparaître vers la fin de l'incubation. Les activités peroxydases, Mn peroxydase et Lignine peroxydase, présentent une augmentation pour arriver à sa valeur maximale (0,231 et 0,321 UI.g<sup>-1</sup> SPS du SOS après 18 et 24 jours d'incubation respectivement). Ensuite, les activités peroxydase diminuent.

Les activités laccases suivent relativement la même allure avec une augmentation de 1,52 à 8,5 UI.g<sup>-1</sup> SPS après 18 jours d'incubation, puis une diminution pour arriver à une valeur de 4,63 UI.g<sup>-1</sup> SPS. D'après les résultats, on a remarqué que l'activité laccase est l'activité majeure parmi les activités enzymatiques impliquées dans la transformation des polyphénols. A partir de l'ensemble des résultats, on a également remarqué que les activités cellulases et les deux activités phénoloxylase présentent des tendances similaires avec une augmentation au début de la culture pour atteindre un maximum puis une diminution et stabilisation jusqu'à la fin d'incubation. Cette tendance peut être expliquée par une complémentarité entre ces activités pour aboutir à la dégradation du complexe lignocellulosique. Par ailleurs, les activités catéchol-1,2-dioxygénase et protocatéchuate-3,4-dioxygénase n'ont été pas détectées dans les conditions de culture.



**Tableau 5:** Mesure des activités (UI.g<sup>-1</sup> SPS) cellulase, lipase, laccase, Mn peroxydase, lignine peroxydase et tannase de *L. edodes* Le119 durant un mois d'incubation à 25°C.

| Temps jours | Cellulase | Lipase | Laccase | Mn peroxydase | Lignine Peroxydase | Tannase |
|-------------|-----------|--------|---------|---------------|--------------------|---------|
| 0           | 0.02      | 0.001  | 1,52    | 0,04          | 0.001              | 0.004   |
| 6           | 0.4       | 0.002  | 5,8375  | 0,124         | 0,121              | 0.121   |
| 12          | 0.82      | 0.0014 | 8,055   | 0,142         | 0,245              | 0.225   |
| 18          | 1.13      | 0.0018 | 8,5175  | 0,231         | 0,214              | 0.154   |
| 24          | 0.76      | 0.010  | 7,6025  | 0,1789        | 0,321              | 0.174   |
| 30          | 0.41      | nd     | 4,63    | 0,1245        | 0,281              | 0.11    |

#### 4. Conclusion

Les résultats de l'étude ont montré une bonne croissance de *L. edodes* Le119 sur le COS en FMS. L'inoculation du COS avec un mycélium produit sur les grains de blé avec des margines à 10% (v/v) a permis d'éviter la contamination de culture par des antagonistes. Le suivi respirométrique de *L. edodes* Le119 cultivée sur le COS a mis en évidence la présence de trois phases de croissance. L'étude des paramètres de sucres réducteurs, sucres totaux, phénols totaux ainsi que les spectres RMN du <sup>13</sup>C (résultats non publiés) a montré que la première phase de croissance a été attribuée à la minéralisation des matières facilement dégradables, suivie par la phase de dégradation des polysaccharides (hémicelluloses, celluloses). La dernière phase de croissance correspondait à la dégradation légère de la lignine provenant du bois de taille. L'activité enzymatique a mis en évidence la présence d'un cocktail enzymatique qu'on pourrait utiliser pour des applications biotechnologiques.

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## Pyrolysis kinetics of olive residue/plastic mixtures by non-isothermal thermogravimetry

A. Aboulkas<sup>1,2</sup>, K. El Harfi<sup>1,2</sup>, A. El Bouadili<sup>3</sup>, M. Nadifiyine<sup>1</sup>

### Abstract

The TGA studies of a pyrolytic decomposition of mixtures of olive residue/plastic were carried out. The investigation was made at the temperature ranging from 300 to 1273 K in the nitrogen atmosphere at four heating rates  $\beta=2, 10, 20$  and  $50 \text{ K min}^{-1}$ . High density polyethylene (HDPE), low density polyethylene (LDPE), polypropylene (PP) and polystyrene (PS) were selected as plastic samples. Based on the results obtained, three thermal stages were identified during the thermal degradation. The first two were dominated by the olive residue pyrolysis, while the third was linked to the plastics pyrolysis, which occurred at much higher temperatures. Discrepancies between the experimental and calculated TG/DTG profiles were considered as a measurement of the extent of interactions occurring on pyrolysis olive residue/plastic mixtures. The maximum degradation temperatures of each component in the mixture were higher than those of the individual components. These experimental results indicate a significant synergistic effect during olive residue and plastic co-pyrolysis at the high temperature region. In addition, a kinetic analysis was performed to fit thermogravimetric data. A reasonable fit to the experimental data was obtained for all materials and their mixtures.

**Keywords:** Pyrolysis; Kinetics; Olive residue; Plastic; Olive residue/plastic mixtures.

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## **Thème 7**

**Economie et marchés oléicoles : évolution, prospective  
et stratégies d'acteurs**

## **The Global Market for Olive Oil: Trends, Policies and trade liberalisation in the Mediterranean basin**

Samir Mili

### **Abstract**

Future competitive scenario for olive oil will be shaped primarily by market dynamics (developments in production, consumption and trade), business strategies (innovation, promotion, concentration, internationalisation), and the ongoing agricultural and trade policy reforms (CAP reform, WTO negotiations, regional and bilateral trade agreements). Based on this premise, the purpose of this contribution is to address market change and policy reform processes in the world olive oil industry, pointing out the main factors associated with their present and expected evolution. It is assumed that although some of these factors act on a global level, others may vary across countries and even within countries amongst different supply chain agents. This implies that there is a wide spectrum of possible strategies and courses of action for the future.

**Keywords:** Market dynamics, business strategies, policy reforms, olive oil.

## Olive Oil Economics & Markets in Turkey

Renan Tunalioglu

### Abstract

Turkey, the Mediterranean in geography and the olive crop is grown for creating one of the lucky country. Fourth in the world olive oil production, export share of 5% to fifth place. Olive oil technology is developing and packaged and branded olive oil exports are supporting to directly in Turkey since the last decade. Olives always was presented in Ottoman Empire Republic of among the agricultural products. The first official olive policies was initiated in 1929 and has continued until today. In recent years, the existing policy of bilateral agreements with organizations such as the IMF, World Trade Organization and etc (some of the relations economic integration EU, FAO, WTO, IOC, etc) referring to some as the content has changed the scope of compliance work. In this study purpose, Changes of Turkey olive and olive oil sector will assessed as economy (production and marketing) policy developments.

**Key words:** Olive oil, Marketing, Politics, Changing, Turkey.

### 1. Introduction

Turkey has the world's olive trees number of 17%, olive areas of 9%, olive production of 8%, olive oil production and exports of 5% and 10% (Figure 1, 2, 3 and 4). Turkey is one the important producer country in the other olive producer's countries. Olive cultivation is one of the major agricultural activities in Turkey and this importance is increasing every year. It is effectived the changing agricultural policies in Turkey and the increasing the importance of olive cultivation in the world.

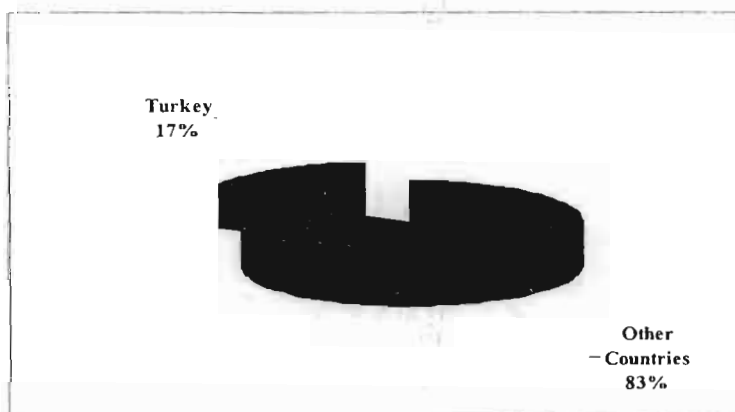


**Figure 1:** Olive Trees in the Turkey and World.

**Source:** www.fao.org,2009

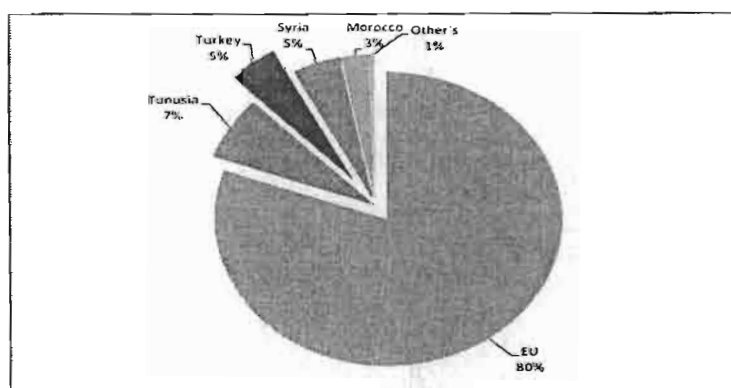


**Figure 2:** World Olive Oil Production.

**Source:** www. www.internationaloliveoil.org,2009.

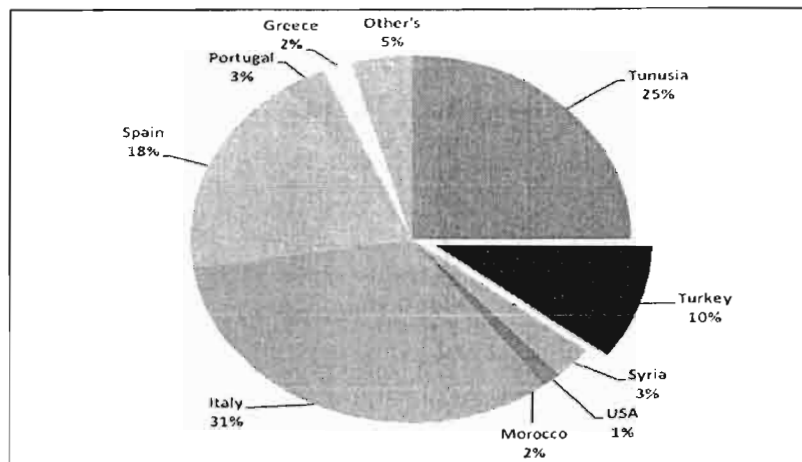


**Figure 3: World Olive oil Exportations.**  
**Source:** www.internationaloliveoil.org,2009

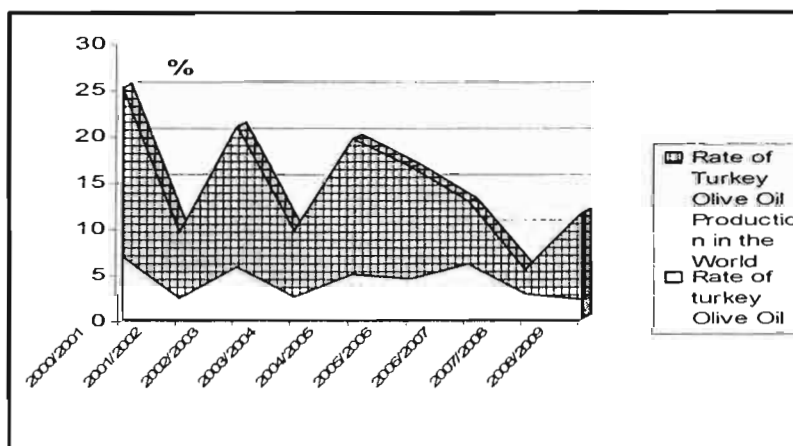


**Figure 4: Rate of Turkey's Olive Oil Production and Export in the World**  
**Source:** is prepared by Aegean Exporter Union's datas in Turkey, 2008.

Olive cultivation were taken into culture in ancient Mesopotamia in BC 4000's which belongs to the Republic of Turkey in Anatolia today. Olives and olive oil, when Turks come to Anatolia 11. century and then established Ottoman Empire 12. century and of the Republic of Turkey has always been important product (Blázquez, 1997) (TGNA, 2008) (Unsal, 2003).

A large number of mono-species olive orchards established during the Ottoman Empire and even some benevolents donated them to foundations. And after then young Republic of Turkey was founded in 1923, leader and founder Atatürk was started a big olive cultivation campaign

## 2. Olive Oil Economics and Marketing Policies in Turkey

### 2.1. Economics

Olives is always a valuable sector in Turkey. This value has increased that applied through agricultural policy since 2000. Olive areas has increased 21% in the last decade in Turkey. Olive areas has reached of agricultural land within 3.4% (www.tuik.gov.tr) (Figure 5).

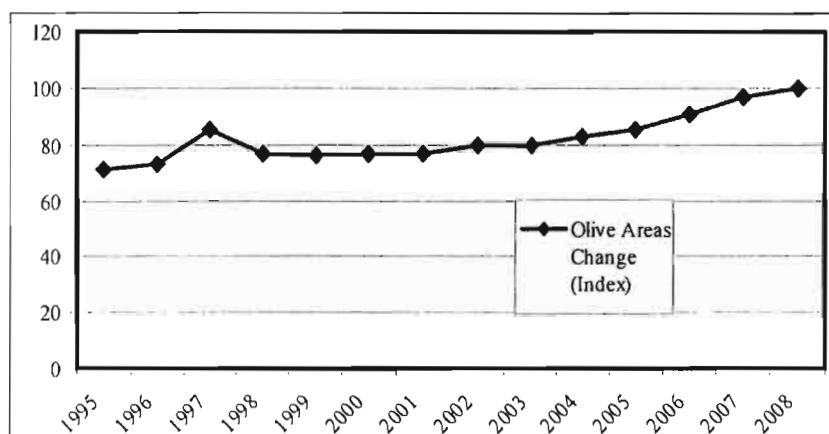


**Figure 5:** Turkey's Olive Growing Area in the Agricultural Area (Thousand ha)

**Source:** [www.tuik.gov.tr](http://www.tuik.gov.tr)

There are 774 thousand hectares olive areas, 151 million olive trees in Turkey. This tree has occurred 70% of bearing, 30% non bearing. The total olive production's 62% is olive for oil and rest into table olive (Figure 6 and 7). Turkey is produced approximately 112 thousand tons of olive oil. Today, Turkey's olive and olive oil net trade volume is \$ 250 million. This is 5.7% of world olive oil net trade volumes ([www.tim.org.tr](http://www.tim.org.tr), 2009) (Net Trade Volume is calculated Exports + Imports (value or quantity))

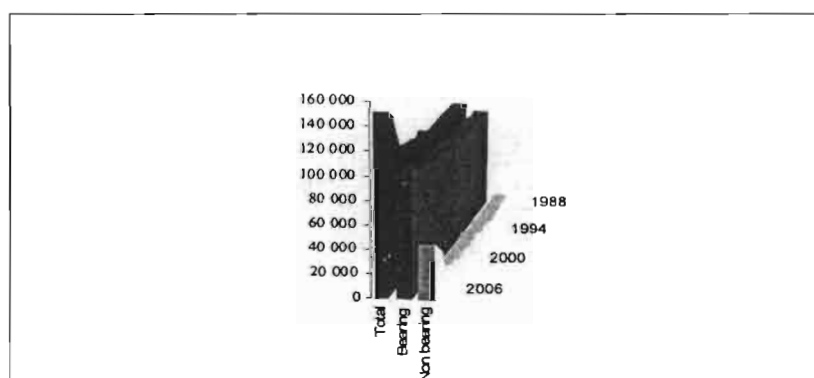


**Figure 6:** Olive Trees Number in Turkey. **Source:** [www.tuik.org.tr](http://www.tuik.org.tr)

### 2.2.1. Policies and Marketing

Private sector is more effective than cooperatives in olive oil marketing in Turkey because olive oil production and exports of 80% belongs to private sector (Tunalioglu and Ozdogan, 2008).

#### Production:

Republic of Rural Affairs and Ministry of Agriculture is responsible for agricultural and olive cultivation policy in Turkey. But in practice, there are many responsible authorities; Ministry of Agriculture and Rural Affairs, Industry and Trade Ministry and Interior Ministry. That is experiencing confusion.

In Turkey, the price of olive oil is the most important agricultural policy instruments for production. Apart from input and orchard plant support are also important in recent years.

- 1929-1939 period: olive campaign and in 1939 "a special olive law" was removed and breeding efforts begin.
- 1939 - 1960 period: olives in the new planting and maintenance of provincial support.
- 1960-1966 period: It was taken place in first Development Planning and exported olive oil in the first time.
- 1966 - 1972: period: olive oil was supported



- in 1973: olive oil was not supported
- 1974 - 1978 period: olive oil was supported.
- in 1979 year: olive oil was not supported
- 1980 - 1986 period: olive oil was supported
- 1987 - 1990 period: olive oil was not supported
- 1991 - 1993 period: olive oil was supported.
- 1994 - 1997 period: olive oil was not supported
- in 1998: Premium support was applied.
- In 1999: olive oil was not supported
- From 2000 until today: premium support and stability was achieved in changing agricultural policies (Tunalioglu, 2006 and TGNA, 2008) (Figure 1).

Applied olive oil production policies in Turkey is stable with two main reasons in recent years. Firstly, same negotiations are made to the EU, WTO, IMF, World Bank agreements. Second is affect by Grand National Assembly of Turkey's (TGNA) agricultural and marketing policies laws (in 2004 Food, in 2004 Organic Agriculture, in 2005 Agricultural Insurance, in 2006 Agriculture Law) Turkey's agriculture policies, in January 1996 Customs Union Agreement with the EU has entered a new process. The production and marketing policies have changed in this process.

- 2001 May, with the IMF stand-by agreement to be signed,
- 2001 July, World Bank International Bank for Reconstruction and Development (IBRD) was initiated with the approval of the Agricultural Reform Implementation Project (ARIP)
- 2004 July, adoption of the text for the WTO framework,
- October 2005, begin to negotiations with the EU and the opening up of the agricultural chapter,
- December 2008, end of the Agricultural Reform Implementation Project (ARIP)

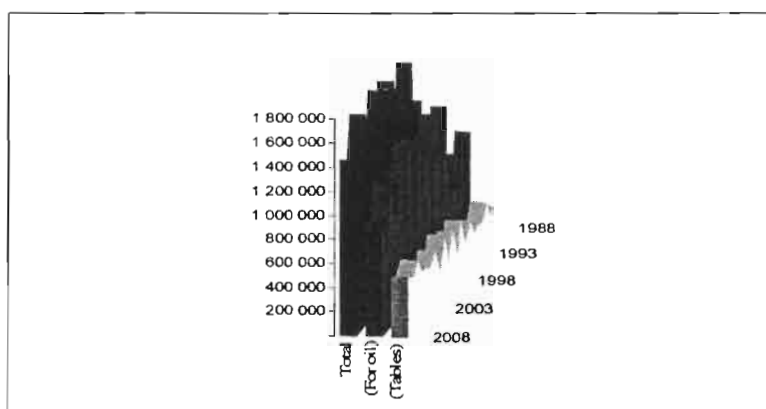


**Figure 7:** Olive Production in Turkey (Thousand tons). **Source:** [www.tuik.org.tr](http://www.tuik.org.tr)

This project was carried out by the Ministry of Agriculture and Rural Affairs. Industry and Trade Ministry has also provided support. ARIP is a project which was affecting agricultural and olive policies in Turkey. This project has been registered with the producer, restructured of cooperatives and have been changed production support policies. Olives in this context, input, drip irrigation, orchard plant, R & D and machine renewal has benefited from support (Table 1).

**Table 1: Applications of Support Prices in Olive Oil in Turkey.**

| 1960 |     |     |     |     |     |     |     |     |     |
|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 0    | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   |
| -    | -   | -   | -   | -   | -   | SPP | SPP | SPP | SPP |
| 1970 |     |     |     |     |     |     |     |     |     |
| 0    | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   |
| SPP  | SPP | SPP | PAP | SPP | SPP | SPP | SPP | SPP | PAP |
| 1980 |     |     |     |     |     |     |     |     |     |
| 0    | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   |
| SPP  | SPP | SPP | SPP | SPP | SPP | SPP | PAP | PAP | PAP |
| 1990 |     |     |     |     |     |     |     |     |     |
| 0    | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   |
| PAP  | SPP | SPP | SPP | PAP | PAP | PAP | PAP | PS  | PAP |
| 2000 |     |     |     |     |     |     |     |     |     |
| 0    | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   |
| PS   | PS  | PS  | PS  | PS  | PS  | PS  | PS  | PS  | PS  |

Source: Tunalıoglu, 2006, SPP= Support of the Purchase Price, PAP=Producer Association's Price, PS=Premium Support

### 2.2.2. Trade:

Foreign trade policies are carried out by Undersecretariat of the Prime Ministry for Foreign Trade. These policies of WTO and EU negotiations continued commitment to innovation will be open as long (Table 2).

**Table 2: Subsidies of Olive and Olive Oil Production and Exports.**

|                                        |
|----------------------------------------|
| • <b>PRODUCTION's support</b>          |
| • <b>Olive Oil Premium Support</b>     |
| • Input (fertilizer, pesticide etc.)   |
| • Agricultural Research Development    |
| • Machinery Equipment                  |
| • Drip Irrigation                      |
| • Environmental Protection             |
| • Plant Insurance                      |
| • Certified Nursery with Orchard Plant |
| • Agricultural Loans                   |
| • <b>EXPORT's and IMPORT's support</b> |
| • Trade Fairs                          |
| • TURKQUALITY                          |
| • Customs Tax                          |

Source .is prepared to [www.tarim.gov.tr](http://www.tarim.gov.tr) and [www.egebirlik.org.tr](http://www.egebirlik.org.tr)

Olive Oil Marketing policy in Turkey;

- Between 1972-1994: Bulk and barrels of olive oil with export is prohibited.
- From 1994 - till today: Bulk and barrels of olive oil exports is free.
- From 1997-till today: As part of export refunds for agricultural products are packaged olive oil subsidies.
- From 2006-till today: are given to both branded and packaged olive oil exports (Table 1).

To support exports in Turkey, within the framework of the WTO commitments and the quality of packaged classes are based. Turkey also introduced the world to olive oils "Turkquality" program under the "Olive to live" and "Olive and Olive oil from Turkey" studies have been initiated. On the other hand Turkey has still a big problem today. Turkey's olive oil can not be exported which is the largest market to the European Union, because of high customs duties branded-packaged olive oil. Therefore, Turkey's exports of oil barrels and bulk is higher than packaged (Figure 8).

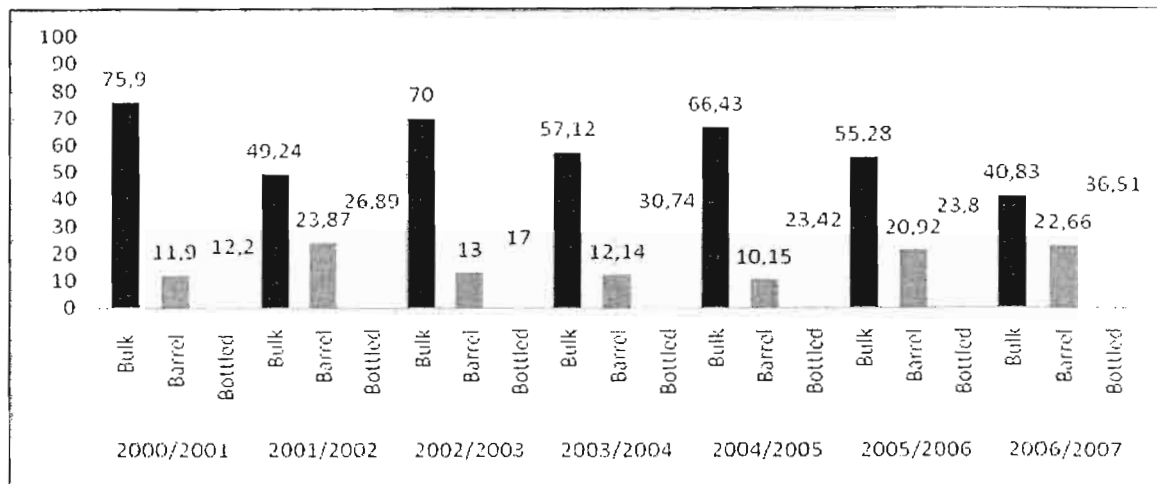


**Figure 8:** Olive Oil Exportations of Turkey. **Source:** Aegean Exporter Unions, 2008.

### 3. Results and Summarize

Olive oil economy and marketing have changed and developed since last ten years in Turkey. Because agricultural policies based on olive policy also has changed. This change, the National Olive Council to leave some inaccuracies and omissions, despite are positive.

Prices is preferred olive policies most the way in all methods. In Turkey, price supports increase to olive production and exports but unfortunately structural problems can not solve. Completely. Olive genetic problems (alternans), small size olive holding, cultural applications, and lack of organization of producers enable to continuity and quality in production difficulties. These difficulties, also negatively affects the exports and exporters.

Turkey can be solved genetic, structural and marketing problems in olive sector but in the long term and permanent policies. These situations is directly related to directly policies of Turkey's changing and renewed agricultural policies

#### 3.1. Current Situation in Turkish Olive Oil Sector

##### 3.1.1. Increase

- Mono-olive species orchards and olive areas,
- Olives production quantity,
- Olive oil production and quality,
- Packaged and branded olive oil exports,

##### 3.1.2. Development

- Developments of olive oil technology process,
- Turkish Food Codex, FAO and EU legislation in compliance with the olive oil production,
- Geographical indications in olive oil,
- Organization of olive oil tasting panels,
- Supported with laws (legislation and regulations) (Tunalıoglu, 2009)

#### 3.2. Trends and Strategies in Turkish Olive Oil Sector

- The continuity of production support,
- Enable effectiveness of cooperatives,
- Training licensed trade exchange in olive oil
- Increasing of boutique olive oil production,
- Increasing of the consumption Turkish people's,
- Membership of IOC again,
- Increasing packaged and branded olive oil exports.

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## Compétitivité des exportations tunisiennes de l'huile d'olive face à la nouvelle concurrence sur le marché mondial : Analyse par l'approche *shift-share*

Aicha Mokrani<sup>1</sup>, Mohamed Béchir Sai<sup>2</sup>, Boubaker Dhehibi<sup>3</sup>

### Résumé

L'exportation de l'huile d'olive occupe une place très importante dans l'économie de la Tunisie en maintenant l'équilibre de la balance commerciale agricole. En effet, elle représente 3,5% des exportations totales du pays en 2005. En effet, nous constatons qu'au cours de ces dernières années l'émergence de nouveaux pays producteurs/ exportateurs concurrents à la Tunisie et qui essaient de conquérir le marché de l'huile d'olive en se développant à une croissance et un rythme important. A cet égard, il est impératif d'étudier le positionnement de la Tunisie et de la situer parmi ces nouveaux pays émergents qui menacent sa position sur le marché international. L'objectif, donc de cet travail, s'insère dans le cadre de cette préoccupation et s'intéresse à l'évaluation de la situation actuelle du positionnement de la Tunisie sur le marché mondial de l'huile d'olive afin d'aider les orientations du pays à établir une stratégie plus efficace face à cette nouvelle tendance. Dans cette optique, il s'agit d'évaluer la position compétitive des exportations tunisiennes de l'huile d'olive par rapport à ses nouveaux concurrents moyennant l'approche d'analyse *shift-share* sur deux périodes 1997-2001 et 2002-2006. Les résultats empiriques montrent que la Tunisie a su maintenir sa première place sur le marché américain et canadien en comparaison avec les autres concurrents qui sont la Syrie, l'Argentine, l'Australie et le Chili, tout en augmentant sa part du marché. Pour le marché européen, la Tunisie reste toujours le premier fournisseur de l'UE mais avec une part du marché qui a diminuée au cours des années et qui est compensée par l'augmentation de la part de la Syrie.

**Mots clés :** Huile d'olive, compétitivité, analyse *shift-share*, marché mondial, Tunisie.

### Competitiveness of Tunisian olive oil exports against the new competitors on the international market: A *shift-share* analysis approach

### Abstract

The olive oil export sector occupies an important place in the Tunisian economy by maintaining agricultural trade balance. In 2005, it account for 3.5 % of total country exports. However, during these last years, we observe the emergence of new competitors for Tunisia which try to conquer the olive oil market by an important development growth rhythm. For this reason, it is imperative to study the position and competitiveness of Tunisian olive oil exports among these new emerging countries in the international market.

The main objective of this research is to evaluate the current position of Tunisian olive oil sector exports among these new competitors in the international market in order to draw an appropriate strategies to this new trend. To this end, the method used is based on the *shift share* approach analysis for the periods 1997-2001 and 2002-2006.

Empirical results show that Tunisia continue increasing its market share and maintaining its leader position in the American and Canadian market on comparison with the new competitors such as Syria, Argentina, Australia and Chile. In the European Union market, Tunisia always remains the first supplier but with a decreasing market share during the last years which is compensated by the increasing Syrian market share.

**Key-words:** Olive oil, competitiveness, *shift-share* analysis, international market, Tunisia.

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## **La signalisation de la qualité est elle une solution pour la valorisation de l'huile d'olive tunisienne sur les marchés extérieurs ?**

Oueslati Meriem, Rastoin Jean-Louis ; Daviron Benoit ; Khaldi Raoudha

### **Résumé**

En Tunisie, la compétitivité de l'huile d'olive à l'exportation n'est plus à prouver. Cependant, ce produit n'est évalué que sur ses attributs matériels alors que les attributs symboliques sont accaparés par les pays importateurs. Cet article a pour objectif de mettre en évidence les principales contraintes à la valorisation de l'huile d'olive tunisienne en termes de signes de qualité (appellation d'origine ; indication géographique) à travers une enquête réalisée auprès des exportateurs. L'absence d'une action collective pour la promotion de l'huile d'olive, l'inexistence d'appellation d'origine reconnue, la faible notoriété de la qualité de ce produit, le manque d'unités de conditionnement sont autant d'obstacles évoqués par les exportateurs. Les politiques quantitatives adoptées devraient être accompagnées par des mesures spécifiques tout au long de la filière pour la valorisation du produit.

**Mots clés:** Signalisation- Qualité-Huile d'olive-Indications d'origine.

## **Is signaling quality a good argument for promoting Tunisian olive oil on foreign markets?**

### **Abstract**

Exported Tunisian olive oil has proven itself to be highly competitive. It is however promoted solely on the basis of its physical characteristics, and it is only importing countries that point out its symbolic or intangible advantages. This article seeks to put forth the main problems that need to be solved before Tunisian olive oil can effectively use signaling of quality [designation of origin and geographical indication (GI)] as a selling point. These constraints have been established on the basis of information gathered through a survey of exporters. Indeed, exporters agree that the absence of a joint effort to promote Tunisian olive oil, the lack of an acknowledged system for designation of origin, the low level of awareness of the quality of this product, and the scarcity of processing units are all obstacles to signaling the quality of Tunisian olive oil. Policies that focus on quantity will need to be complemented by specific measures based on quality at all levels of the industry if export of this key product is to reach its full potential.

**Keywords:** constraints to export, quality, olive oil, valorization

## Filière oleicole au Maroc : formes et développements alternatifs du monde rural

Pr. Ismaili Alaoui Mustapha<sup>1</sup> & Ali Heddoun<sup>2</sup>

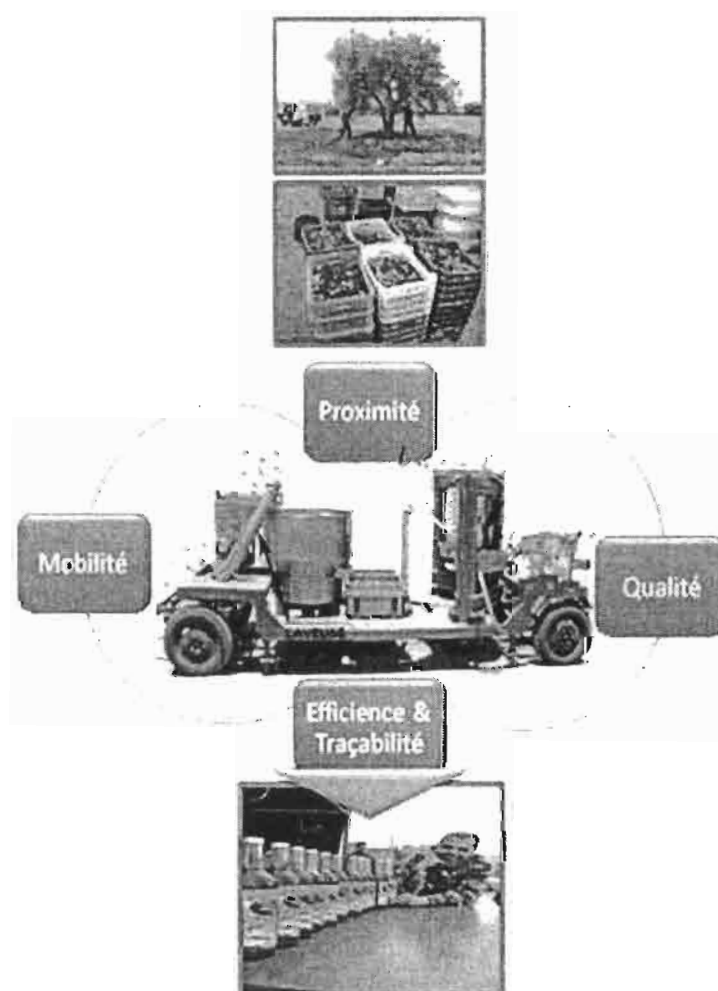
### Résumé

L'Oléiculture Marocaine participe à l'autosuffisance du pays en huiles alimentaires à près de 18 % des besoins. La production marocaine est d'environ 600 000 tonnes d'olives dont 60% de la production est réalisée par des petits producteurs pratiquant l'extraction dans des unités artisanales (maasra). Elles produisent de l'huile d'olive dans des conditions d'extraction fastidieuses avec un faible rendement qui ne dépassent pas 14 % contre 21 à 23 % du potentiel de la variété « Picholine marocaine », ce qui engendre une perte qualitative et quantitative pouvant atteindre 25 % de la production nationale, soit environ 10 000 t/an. Les raisons qui expliquent une telle situation ont trait aux multiples opérations dans la chaîne de production (récolte, transport, stockage, Hygiène, conduites des opérations, qualification etc.) au niveau des maasras.

L'objectif du projet est d'apporter de nouveaux outils de compétitivité et d'innovation capables de produire des huiles d'olives de bonnes qualité sur les lieux de production, d'annuler les coûts et les effets de transport, de stockage, d'Hygiène et de conduites des opérations par la mise en place d'une unité mobile adaptée aux réalités socio-économique du milieu rural marocain. Cette nouvelle technologie devrait permettre le soutien du pilier II du plan Maroc Vert, la réhabilitation de la filière oléicole dans les zones enclavées et le développement d'entreprises rurales innovantes qui vont s'approprier des technologies nationales modernes développées sur la base du savoir faire local.

**Mots clés :** Filière oléicole, Maroc, maasra, unité mobile, réhabilitation.

Archivage Labellisation



Modérnisation savoir faire

## Abstract

Moroccan olive culturing participates in the self-sufficiency of countries'edible oils of about 18% of the needs. Moroccan production is about 600 000 tons of olives in which 60% of the production is realised by small producers practicing extraction in craft units (maasra).

They produce olive oil in tedious extraction conditions with a weak yield which does not exceed 14% versus 21 to 23 % of the variety potential "Moroccan Picholine", which generates qualitative and quantitative loss which can reach 25% of national production (crop, transport, storage, hygiene, operations passages, qualification etc.) on the level of maasras.

This project goal is to bring new tools of competitiveness and innovation capable of producing olive oils of good quality in the production areas, to cancel the costs and effects of transport, storage, hygiene and operations conduct by the implementation of a mobile unity adapted to the socio-economic realities of the Moroccan rural area. This new technology should enable the support of the pillar II of the Green Morocco plan, the rehabilitation of the olive chain in the landlocked areas et the development of the innovative rural companies which will appropriate modern developed national technologies on the base of the local know-how.

**Key words:** olive chain, Morocco, maasra, mobile unit, rehabilitation.

## 1. Introduction

Le Maroc cherche depuis longtemps à travers des plans stratégiques à promouvoir et à moderniser son agriculture pour subvenir aux besoins alimentaires des populations. Le patrimoine oléicole national s'étend sur une superficie totale de 600.000 Ha répartie sur 400.000 exploitations agricoles. La "Picholine Marocaine" représente plus de 90% des plantations. Le secteur permet de générer une valeur ajoutée de l'ordre de 1,5 Milliard de Dirhams et contribue à la création d'environ 15 Millions de journées de travail/an, soit l'équivalent de 70.000 emplois permanents. Elle permet de garantir l'approvisionnement de 300 unités industrielles ou semi industrielles de transformation des olives et de 15.591 unités traditionnelles de trituration (maâsras) détenant des capacités de près de 700.000 T et 170.000 T, respectivement (DPV 2008) (Fig. 1). En plus de son rôle nutritionnel (résorption de plus de 16 %, du déficit du pays en matière d'huiles alimentaires particulièrement en milieu rural), le secteur joue un rôle déterminant dans la consolidation de l'équilibre de la balance commerciale et permet de placer le Maroc au 3ème rang des pays exportateur des olives de table, après l'Espagne et la Grèce, avec une moyenne annuelle de près de 60.000 T. Le secteur génère en chaque fin de campagne environ 250.000 m<sup>3</sup> de margine et 200.000 tonnes de grignons d'olive.

L'objectif du présent travail et d'apporter de nouveaux outils de compétitivité et d'innovation capables de produire des huiles d'olives de bonnes qualité sur les lieux de production, d'annuler les coûts et les effets de récolte, de transport, de stockage, d'hygiène et de conduites des opérations par la mise en place d'une unité mobile adaptée aux réalités socio-économique du milieu rural marocain. Cette nouvelle technologie devrait permettre le soutien du pilier II du plan Maroc Vert, la réhabilitation de la filière oléicole dans les zones enclavées et le développement d'entreprises rurales innovantes qui vont s'approprier des technologies nationales modernes développées sur la base du savoir faire local.



## 2. Etat des lieux de l'oléiculture en milieu rural au Maroc

- Grande biodiversité, climats, végétale et humaine
- Population agricole oléicole en croissance extension des oliveraies
- Activité oléicole liée aux aléas climatiques
- Persistance d'une petite agriculture familiale (peu compétitive).
- Existence d'exploitations et industries familiales sous dimensionnées et sous exploitées
- Micro exploitation sous forme de clôture
- Faible valorisation de produits oléicole
- Grande capacité de trituration des unités dans les grandes villes (Fig. 1)
- Le milieu rural à besoin de plus d'infra structures « plusieurs chaînes sont coupées : Routes, Electrification, Froid, Encadrement technique .etc.

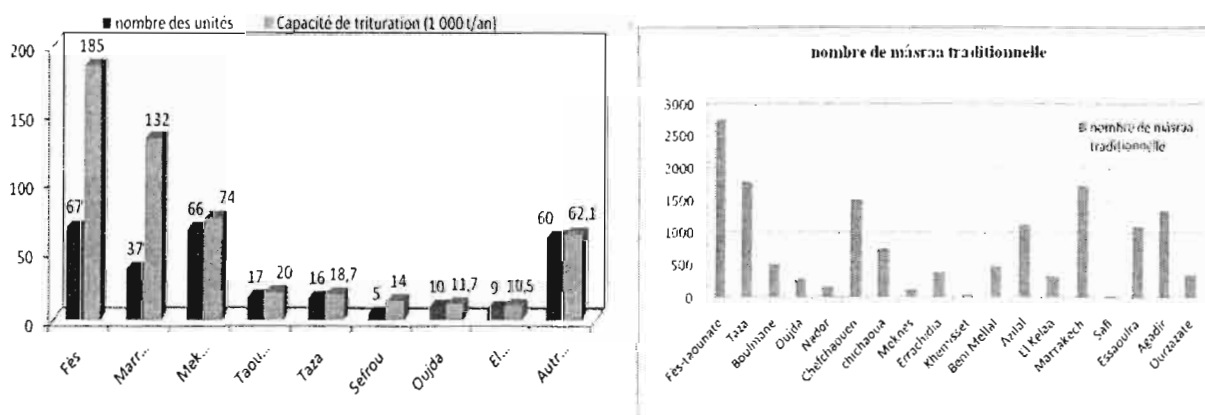


**Figure 1:** Répartition des unités de trituration industrielles et traditionnelles au niveau des principales zones oléicoles (DPV 2008).

L'analyse des prix des olives et des huiles d'olives, mises à part les fluctuations annuelles constatées durant les années 90 (Tableau 1), ont amorcé une tendance marquée à la hausse et particulièrement durant la période 1998-2006 où les prix ont pratiquement doublé (Rapport MCC 2009).

**Tableau 1:** Evolution des prix des olives et huiles d'olive pendant les campagnes 1998/99 et 2005/06.

| Produits                      | 1998/99 | 2005/06 |
|-------------------------------|---------|---------|
| Olives de trituration (DH/Kg) | 2       | 4,75    |
| Olives de conserverie (DH/Kg) | 3       | 5,7     |
| Huile d'olive (DH/L)          | 22,5    | 37,5    |

Source : Rapport MCC 2009.

## 3. Contraintes de la filière oléicole

Nombreuses sont les contraintes endogènes et exogènes qui pèsent sur l'oléiculture marocaine :

- La vulnérabilité de la production vis-à-vis des variétés retenues (90 % de la picholine marocaine), de la conduite culturale, de la rareté des ressources hydriques et de l'accompagnement professionnelle des oléiculteurs.
- La faible productivité au niveau des exploitations (1,5 tonnes à l'hectare) en raison de l'inadéquation du système de production, de l'exiguïté des exploitations oléicole, de la variété employée dans la majorité des exploitations, et de formation aux exigences de bonnes pratiques de production et de transformation en matière de maîtrise des technologies modernes et leur mise au service du développement de la filière.
- La faiblesse de l'organisation professionnelle
- Les mauvaises conditions de récolte, de collecte et de transformation
- La faible maîtrise des paramètres régissant la maturité des produits
- L'inadaptation du matériel utilisé pour le ramassage et le transport des olives
- L'omniprésence du secteur traditionnel de trituration qui triture de 30-40% de la production nationale.

- La concentration de l'activité des unités de trituration sur la prestation de service
- La concentration des grandes unités dans les grandes villes, alors que la production est en montagnes.
- La vétusté des équipements et la non conformité aux règles de bonnes pratiques de production de fabrication au niveau de plusieurs unités industrielles
- La faible valorisation du savoir-faire traditionnel en matière de la conservation des olives.
- Faible productivité qualitative au niveau des unités de trituration traditionnelles (maasra)

#### 4. Opportunités

Plusieurs opportunités réelles sont présentement à saisir notamment par l'application des formes alternatives adaptées aux réalités socio-économiques des petits oléiculteurs à l'amont et à l'aval de la filière. Ces formes doivent tenir compte des contraintes ci-dessus mentionnées et des programmes de développement du gouvernement en la matière notamment ; le Plan National Oléicole (PNO) et le plan Maroc Vert (MAMVA, 1998 et 2009).

En effet, le plan National élaboré en 1998 avait comme objectifs essentiels de doubler les superficies des plantations oléicoles pour les faire passer de 500.000 ha à 1.000.000 ha en 2010 et restructurer la filière sur le plan culturelle et technologique. Malgré les efforts déployés, ce dernier n'a pu augmenter la superficie que de 100.000 à 150.000 ha soit 20% à 30 % de l'objectif initial, alors qu'on matière de restructuration des maasras traditionnelles le PNO n'a restructuré que 2.5 % des unités traditionnelles. En montagne et oasis l'olivier continue d'être utilisé clôture. Le plan vert est venu pour palier ces contraintes avec deux piliers.

Le Plan Maroc Vert table sur la création d'un million d'entreprises agricoles et s'attend à un PIB agricole supplémentaire de 70 à 100 milliards DH. Tout en diagnostiquant le secteur, il oriente vers des pistes de relance après l'élaboration de plans régionaux de développement agricole (MAMVA, 1998).

Ces plans permettront d'asseoir les bases d'une utilisation optimale des ressources naturelles qui varient selon les régions et d'où découlent diverses possibilités de production. Ils sont fondés sur des paramètres dictés par le potentiel et les contraintes du milieu naturel, les acquis de l'expérience agricole régionale et l'environnement humain.

Le programme d'action pour l'optimisation de la mise en valeur agricole dans chaque région retenue à l'horizon 2020 est décliné sous formes de deux piliers à mettre en œuvre.

L'objectif du pilier I est de développer une agriculture performante, adaptée aux règles du marché, grâce à une nouvelle vague d'investissements privés, organisés autour de nouveaux modèles d'agrégation équitables.

Par contre, l'objectif du pilier II est de développer une approche orientée vers la lutte contre la pauvreté, en augmentant significativement le revenu agricole des exploitants les plus fragiles, notamment dans les zones périphériques (par exemple en bour défavorable). En termes de chiffres, le pilier I a pour but de générer un maximum d'investissements privés sur ces filières avec 10 milliards de dirhams par an pour les relancer, autour de nouveaux modèles d'agrégation, portés par des investisseurs à forte capacité managériale.

Ces modèles d'agrégation seront équitables, par le biais d'une double contractualisation, entre l'Etat et l'agrégateur d'une part, et entre l'agrégateur et l'exploitant agrégé d'autre part.

Le pilier II, vise également la mise en œuvre de projets sociaux autour de trois programmes. Ce pilier prévoit un volet financement innovant. Il s'agit ainsi de traiter les bailleurs des fonds sociaux comme des investisseurs à part entière, au moyen d'une «Offre sociale Maroc».

Par ailleurs, des opportunités réelles sont également à saisir notamment pour les créneaux suivants :

- Les produits de terroir labellisés, étant donné que l'oléiculture marocaine présente la caractéristique d'être pratiquée dans des contextes de production très diversifiés (montagne, plaines, oasis, bour, irrigué) (Fig. 2) et travers des signes distinctifs d'origines et de qualité (SDOQ loi 25.06) ;

- les produits biologiques, sachant qu'une part importante de l'oliveraie nationale s'apprête parfaitement à ce mode de production avec le faible recours des agriculteurs à l'usage des produits chimiques pour la conduite et l'entretien de leurs plantations ;
- les produits de haute gamme dans la mesure où la qualité de la production oléicole nationale est intrinsèquement supérieure pouvant facilement être valorisée à travers l'instauration d'un système de production sauvegardant cette qualité et la valorisant par l'accroissement de sa valeur ajoutée et la promotion de son image de marque auprès des consommateurs par le recours notamment aux techniques de marketing.

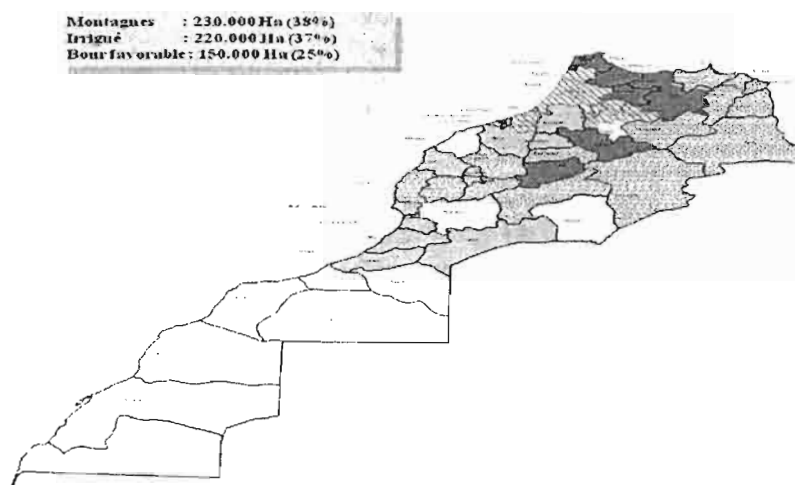


**Figure 2:** Répartition des zones oléicole à l'échelle nationale au Maroc (Ismaili-Alaoui, 2006) .

## 5. Mesures d'accompagnement de la filière

Les mesures d'accompagnement proposés pour les développements alternatifs sont basés sur la mise en place des économies de qualité qui sont supposées se fonder sur l'utilisation durable des ressources naturelles et humaines de la région, promouvoir une perspective à long terme et tendre vers une stabilité économique basée sur l'efficacité, la diversité et l'équité. Elles sont « douces » envers la nature et les gens, respectent les connaissances et les cultures traditionnelles et apportent une valeur ajoutée aux populations. Il s'agit de :

- Améliorer les technologies de récolte, de stockage et de transformation des matières premières
- Appuyer les plans et la stratégie de l'état en matière de développement : PNO et Plan Maroc Vert
- Appuyer le management de la qualité dans les IAA
- Chercher la maîtrise de la qualité (40 % des pertes) : la maîtrise de la qualité passe par la maîtrise des opérations production et de transformation
- Spécialiser les ressources humaines et la terre sur les choses qu'on sait faire.

Appliquer les systèmes de traçabilité : La traçabilité et la labellisation dans le monde rural passe par le transfert et l'adaptation des réglementations, des innovations technologiques et biotechnologiques auprès des gardiens de ce savoir. En effet, les itinéraires techniques des produits de terroirs sont bien adaptés aux réalités socio-économiques des milieux, tous les segments s'y retrouvent.

Les développements alternatifs proposés visent de redresser voir annuler les contraintes qui handicapent le développement de la filière et valoriser au mieux les ressources disponibles et les opportunités offertes. Elles intéressent l'ensemble des maillons, depuis la production, la commercialisation et la valorisation des sous produits de l'olivier, ce qui permettra d'une part l'accroissement de la productivité et ainsi de la valeur ajoutée sur les lieux de production et d'autre part une élimination des contraintes liées à la mauvaise gestion au sein des petites exploitations et unités de trituration (maasra), notamment, la qualification des agriculteurs (an alphabétisation professionnelle), le gaulage, le mode de transport des olives, le stockage sur les lieux de production et au niveau des unités de trituration, le mode de trituration, les conditions d'hygiène et la labellisation etc.

## 6. Unité mobile d'extraction des huiles d'olive

Les unités artisanales de trituration au nombre 15591 unités, ne valorisent que partiellement la production des huiles d'olive. En effet, avec des rendements en huiles d'olive qui ne dépassent guère 14 % contre un rendement potentiel de 23 % pour la picholine marocaine en pleine maturité. La perte en huile dans les grignons d'olives est estimée de 8000 à 10.000 t/an. Cette perte représente entre 18 à 25 % de la production nationale en huile d'olive dans ces unités, sans tenir compte des pertes en huile dans les margines<sup>1</sup>.

La conception des unités mobiles est basée sur le savoir faire local est sur le principe de la mobilité et de la proximité. Le principe part de l'idée de la moissonneuse-batteuse. Concrètement, l'idée est que c'est la machine qui va vers le fruit et non l'inverse. Car, «la qualité dépend du temps passé entre la cueillette et la trituration. Il ne faut pas qu'elle dépasse les 24 heures. La Figure 3 résume le schéma de réalisation du prototype. Deux modèles ont été réalisés/ unité mobile sur roues et plate forme transportable en monobloc. Cette unité d'extraction d'huile d'olive devrait réduire la durée de stockage et la fréquence de broyage des olives 2 h au lieu de 2 mois parfois au niveau des unités artisanales, ce qui contribuera à l'amélioration de la qualité du produit, du rendement de l'environnement écologique des oliveraies.

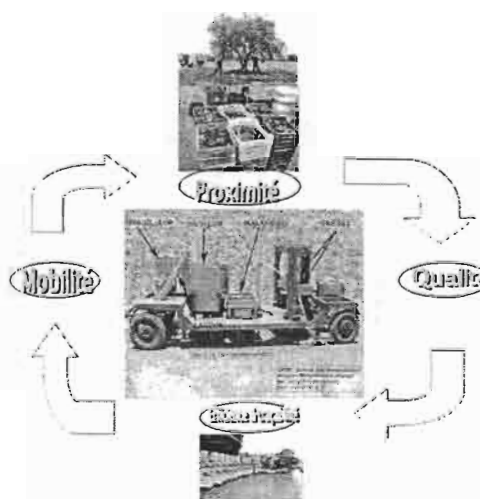


**Figure 3:** La mobilité et la proximité par les unités mobiles d'extraction des huiles d'olive (Ismaili Alaoui, 2004).

## 7. Enrichissement en protéines des grignons d'olive

Les grignons générés pendant l'extraction du système à trois phases sont des matières organiques riches en fibre, cellulose en matières grasses et pauvres en protéines (Tableau 2).

**Tableau 2:** Composition chimique indicative des différents types de grignons en % de matières sèches (Ismaili-Alaoui et al. 2003).

| Type                | Matière sèche | Matières minérales | Matière azotée totale | Cellulose brute | Matières grasses |
|---------------------|---------------|--------------------|-----------------------|-----------------|------------------|
| Grignon brut        | 75 - 80       | 3 - 5              | 5 - 10                | 35 - 50         | 8 - 15           |
| Grignon gras p. d.* | 80 - 95       | 6 - 7              | 9 - 12                | 20 - 30         | 15 - 30          |
| Grignons épuisés    | 85 - 90       | 7 - 10             | 8 - 10                | 35 - 40         | 4 - 6            |
| Grignon épuisé p.d. | 85 - 90       | 6 - 8              | 9 - 14                | 15 - 35         | 4 - 6            |
| Pulpe grasse        | 35 - 40       | 5 - 8              | 9 - 13                | 16 - 26         | 26 - 33          |

\* p.d. = partiellement dénoyauté

Leur enrichissement par fermentation en milieu solide a permis d'augmenter leur taux protéiques de l'ordre de 14 % (Ismaili Alaoui et Cheheb, 2004). La Figure 4, résume le procédé de Fermentation en milieu solide sur plateaux à l'échelle pilote pour la préparation d'aliment pour bétail à partir des grignons d'olive.

<sup>1</sup> Transfert de technologie en agriculture (2001), Qualité des huiles d'olive au Maroc, Rabat, avril.



**Figure 4:** Enrichissement des grignons d'olive par Fermentation en Milieu Solide (FMS). Préparation du substrat (A) ; Inoculation avec une suspension de spores de champignons filamenteux (B) ; Répartition du substrat inoculé sur des plateaux (C) et Transport des plateaux dans le fermenteur pilote (D).

## 8. Conclusion:

Ce Travail a permis d'apporter de nouveaux outils de compétitivité et d'innovation capables de produire des huiles d'olives de bonnes qualité sur les lieux de production, d'annuler les coûts et les effets de récolte, de transport, de stockage, d'hygiène et de conduites des opérations par la mise en place d'une unité mobile adaptée aux réalités socio-économique du milieu rural marocain. Cette nouvelle technologie devrait permettre le soutien du pilier II du plan Maroc Vert, la réhabilitation de la filière oléicole dans les zones enclavées et le développement d'entreprises rurales innovantes qui vont s'approprier des technologies nationales modernes développées sur la base du savoir faire local.

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## **Prospective de la production et de l'exportation de l'huile d'olive biologique et de l'huile d'olive conditionnée**

**Fatma Kanoun Kchaou, Boubaker Karay**

### **Résumé**

Cette recherche, entamée en mai 2008, fournit une estimation de la production et de l'exportation tunisiennes de l'huile d'olive biologique et de l'huile d'olive conditionnée à l'horizon 2020 et procure une évaluation globale des facteurs internes (forces et faiblesses) et externes (opportunités et menaces) qui régissent les exportations tunisiennes. Elle utilise la méthode Delphi qui constitue un outil courant populaire de prospective à moyen et long terme et d'aide à la décision et la méthode SWOT qui constitue un cadre de diagnostic et de réflexion stratégique et un outil universel, simple et populaire d'aide à la décision. Les informations utilisées proviennent des résultats d'une enquête effectuée en face à face auprès d'un échantillon de 20 experts qui assurent des fonctions différentes au niveau de la filière huile d'olive en Tunisie.

**Mots clefs:** Huile d'olive, production, exportation, expert, DELPHI, SWOT

### **Abstract**

This research started on May 2008, provides an estimate about the production and the Tunisian exportation of biologic olive oil conditioned by 2020, and it provides a global evaluation of the internal factors (strengths and weaknesses) and external ones (opportunities and threats) which govern Tunisian exports. It uses Delphi method which constitutes a popular tool of medium and long term perspective helping the decision and SWOT method which constitutes a frame of diagnostic and strategic reflexion and a universal, simple and popular tool helping the decision. The used information coming from the results of an investigation carried out with a sample of 20 experts who ensure different functions at the Tunisian olive oil chain.

**Key words:** olive oil, production, exportation, expert, DELPHI, SWOT

## **L'importance de l'origine géographique dans le choix des produits agroalimentaires : le cas d'huile d'olive en Espagne**

Erraach Yamna, Sayadi Samir

### **Résumé**

La référence à l'origine du produit est devenue un facteur de différenciation et de valeur ajoutée dans le secteur agroalimentaire, où les nouvelles exigences vis à vis la sécurité et la qualité alimentaires provoquent, de plus en plus, des changements dans les habitudes d'achat et le comportement de consommation. Le secteur oléicole ne fait pas une exception à cette tendance. Ainsi, il paraît intéressant estimer la structure des préférences des consommateurs en ce qui concerne l'huile d'olive, en mettant l'accent sur l'importance relative de l'origine géographique et / ou de sa certification. L'information utilisée dans cette recherche proviennent d'un sondage dirigé à 500 consommateurs d'huile d'olive en Andalousie (Sud de l'Espagne), en appliquant la technique de l'Analyse Conjointe (AC) dont on a considéré quatre attributs d'huile d'olive: Le type l'emballage, le prix d'huile d'olive, sa couleur et son origine géographique.

**Mots clés:** Huile d'olive, origine géographique, structure des préférences, Analyse Conjointe, Espagne.

## **The importance of origin in the choice of agro-food products: the case of Spanish olive oil**

### **Abstract**

The reference to the origin of the product has become actually a factor of differentiation and added value in the Agro-food industry, where the new requirements of consumer to safety and quality are increasingly causing a change in the habits of purchasing and consuming. The olive oil sector is not an exception to this tendency. In this context, it is of great interest to estimate the consumer preferences structure regarding olive oil, paying special attention to the relative importance of geographic origin and / or its certification. The information handled in this research were obtained from a survey performed to 500 olive oil consumer's in Andalusia (South of Spain), applying the Conjoint Analysis method (CA) and considering four attributes of olive oil: The type of package, the price of olive oil, its color and the geographic origin.

**Key words:** Olive oil, geographic origin, preference structure, Conjoint Analysis, Spain.

### **1. Introduction**

L'évolution des productions agroalimentaires au cours des dernières décennies, surtout dans les pays développés, ainsi que la stagnation qui marque la demande alimentaire ont engendré une certaine saturation des marchés (Sánchez, 2006 ; Ulloa et Gil, 2007). Par conséquent, les consommateurs et les entrepreneurs se trouvent immergés dans un processus de croissance et de développement global de l'économie, qui leurs impose à son tour un rythme d'adaptation à tous les niveaux (Dawar et Frost, 1999 ; Espejel et al., 2007). Parallèlement à tous ce qui précèdent, les consommateurs sont à chaque fois plus exigeants et expriment leurs préférences à la consommation et l'achat des produits innovants, de qualité et surtout avec des garanties sanitaires. Ceci rend les producteurs et les agents des différentes filières agroalimentaires obligés à adapter leurs manières d'organisation, stimuler la demande existante, de la créer dans des nouveaux marchés potentiels et se différencier de la compétence et acquérir des avantages compétitifs dans ce contexte de mondialisation.



Dans le cas espagnol, une des stratégies de différenciation la plus soutenue, par le secteur de la production et les administrations locales, régionales, nationales et voir même européennes, est l'emploi des Appellations d'Origine Protégée (AOP) ou les Indications Géographiques Protégées (IGP). Il s'agit d'un ensemble des marques génériques attribuées à des produits spécifiques avec une identité propre soutenue par la qualité et l'origine de la matière primaire et tout de même par la spécification de la méthode de production employée (ou le savoir-faire) (Haucap et al., 1997 ; Ulloa et Gil, 2007).

Les Appellations d'Origine Protégée, comme stratégies d'organisation de la qualité différentielle liée au territoire, ont pour objectif la création de produits alimentaires, comme le cas d'huile d'olive, avec une forte composante patrimoniale et de typicité par rapport à des attributs de qualité différentielle (Bonnet et Simioni, 2001 ; Van der Lans et al., 2001 ; Steiner, 2004 ; Sanz Cañada, 2009).

Le secteur d'huile d'olive espagnol jouit d'une place importante aussi bien à l'échelle nationale qu'internationale, étant donné que l'Espagne se classe en premier rang de point de vue production et exportation d'huile d'olive sur le marché international. Ainsi qu'à partir d'une production annuelle moyenne de 800.000 tonnes, une moyenne de 300.000 tonnes est destinée à l'exportation à plus de 100 pays durant la dernière décennie (ICEX, 2009). En dehors de son rôle économique, le secteur d'huile d'olive joue un rôle vital dans l'articulation du milieu rural (création des postes de travail et maintien de la communauté locale dans son lieu d'origine) ainsi que dans le maintien du patrimoine culturel, régional et environnemental du pays. On dénombre actuellement en Espagne environ 31 AOP d'huile d'olive (MARM, 2009) parmi lesquelles environ la moitié (13 AOP) est accaparée par l'Andalousie. Avec 1,5 million d'hectares d'olivier l'Andalousie est considéré le majeur producteur d'olives et d'huile d'olive du pays ainsi que dans le monde. Environ la moitié du territoire olivier andalou, est protégé avec des AOP, lui procurant la possibilité de commercialiser une grande partie des huiles d'olive extra vierge.

L'objectif de ce travail est d'estimer l'importance relative de la certification liée à l'origine géographique et au territoire, plus concrètement les Appellations d'Origine Protégée, par rapport à d'autres attributs comme l'emballage, la couleur et le prix, dans la formation des préférences des consommateurs andalous vis à vis l'huile d'olive.

## 2. Méthodologie

Dans cette étude nous avons fait recours à la méthodologie de sondages auprès des consommateurs ou enquêtes. Pour ce fait, on a réalisé durant les mois de mai à juillet de 2009 des entretiens avec un échantillon représentatif des habitants de l'Andalousie choisis d'une manière aléatoire et stratifiés selon le genre, les groupes d'âge et le lieu de résidence pour un niveau d'erreur de 3,10%. L'échantillon enquêté regroupe 439 consommateurs d'huile d'olive. Le questionnaire se compose de deux grandes parties. La première est le test de préférence ou la preuve d'Analyse Conjointe (AC), où les personnes interrogées évaluent les profils d'huile d'olive par rapport à leurs préférences. La seconde présente des données sur les caractéristiques sociales et démographiques et les modes de vie des personnes interrogées (genre, âge, niveau d'études, revenus, etc.).

Pour traiter les données obtenues à travers le questionnaire et estimer l'importance relative de «l'origine territoriale» par rapport à d'autres attributs d'huile d'olive, nous avons fait recours à la méthode de l'Analyse Conjointe (AC). Cette méthode est devenue un outil de grande importance pour l'évaluation des préférences exprimées par un consommateur aux différents attributs qui composent un bien (Ruiz de Maya et Munuera, 1993). Elle est très utilisée dans le champ de la psychologie commerciale et le marketing (Luce et Tukey, 1964 ; Green et Rao, 1971 ; Cunhal-Sendim et al., 1999 ; Van der Lans et al., 2001 ; Brugarolas et al., 2003 ; Krystallis et Ness, 2005 ; Rodríguez et Sayadi, 2008 ; Mesías et al., 2009) et plus récemment dans l'économie environnementale (Sánchez et Pérez, 2000 ; Sayadi et al., 2005 y 2009).

La principale hypothèse de cette méthodologie stipule que la demande d'un produit répond à la perception et à l'évaluation de ses attributs, plus qu'à sa conception intégrale (Múgica, 1989), étant donné que, d'après Lancaster (1971), l'utilité reçue par un individu, le consommateur dans notre cas, dépend de l'utilité fournie par les différents attributs.



Dans le processus de prise de décision, l'individu évalue l'utilité de chaque combinaison, et son choix manifeste sa préférence relative aux différentes combinaisons des attributs. On accepte que l'utilité totale, résultat du choix du produit, soit déterminée par les différentes utilités partielles (part-worths) de chaque niveau d'attribut (Steekamp, 1987). On assume ici, la règle de la décomposition additive que d'après Hair et al., (1992 y 1999) ce modèle explique, dans presque tous les cas, un pourcentage très élevé (entre le 80% et le 90%) de la variance de la préférence des individus.

Pour appliquer la méthode AC, il est fondamental de bien choisir les attributs les plus explicatifs du comportement d'achat des produits ainsi que leurs niveaux respectifs. Dans notre cas, ce choix a été fait sur la base de la consultation de la bibliographie existante, des entretiens avec des experts, d'un focus-group et d'un sondage préliminaire avec des mères au foyer des consommateurs, etc. A travers les informations préalables et en considérant les objectifs de cette étude, les attributs et leurs niveaux respectifs sont : « Origine » (certifiée avec AOP ; mentionnée sans AOP et non mentionnée), le « Prix » (3€ ; 4,5€ et 6€), la « Couleur de l'huile » (Jaune-verdâtre et jaune-doré) et finalement « Emballage » (Plastique ; verre normal ; verre de design). Avec ces quatre attributs et leurs niveaux on a obtenu 54 profils possibles ou stimulus (combinaison d'attributs - niveaux). Etant donné le grand nombre de produits à évaluer par les consommateurs on a utilisé un modèle orthogonal qui nous a permis de réduire les combinaisons à seulement 9 produits finaux (Tableau 1).

**Tableau 1:** Conception fractionnée incomplète orthogonale.

| Huiles  | Couleur        | Prix (€/l) | Type d'emballage | Origine              |
|---------|----------------|------------|------------------|----------------------|
| Huile 1 | Jaune-verdâtre | 3          | Plastique        | Certifiée avec AOP   |
| Huile 2 | Jaune-doré     | 3          | Plastique        | Mentionnée sans AOP  |
| Huile 3 | Jaune-doré     | 4,5        | Verre de design  | Mentionnée sans AOP  |
| Huile 4 | Jaune-doré     | 4,5        | Verre normal     | Certifiée avec AOP ? |
| Huile 5 | Jaune-verdâtre | 4,5        | Plastique        | Non mentionnée       |
| Huile 6 | Jaune-doré     | 6          | Verre de design  | Non mentionnée       |
| Huile 7 | Jaune-doré     | 6          | Verre de design  | Certifiée avec AOP   |
| Huile 8 | Jaune-doré     | 3          | Verre normal     | Non mentionnée       |
| Huile 9 | Jaune-verdâtre | 6          | Verre normal     | Mentionnée sans AOP  |

Source: Elaboration personnelle.

Concernant la quantification des préférences relatives aux huiles d'olive, on a utilisé la technique d'évaluation en échelle "Rating", où les consommateurs donnent une ponctuation aux huiles d'olive de 0 (ils n'aiment pas du tout cette huile) à 10 (ils aiment beaucoup cette huile). La présentation du stimulus aux personnes interrogées a été réalisée à partir de l'élaboration de neuf cartes à base de la conception orthogonale qui se montre dans le Tableau 1. Le choix d'une conception orthogonale par rapport à la présentation de toutes les combinaisons possibles d'huile limite l'obtention d'informations aux principales effets des attributs, en ignorant les interactions entre eux mais au même temps elle présente l'avantage d'offrir seulement neuf huiles à chaque personne interrogée, en estimant que cet avantage est supérieur aux inconvénients mentionnés (Kirk, 1982 ; Braña et al., 1995 ; Sayadi et al., 2005).

### 3. Résultats

D'après, les résultats de l'estimation du modèle de l'Analyse Conjointe (Tableau 2), on peut observer l'importance relative accordée aux différents attributs par le groupe de personnes interrogées ainsi que les utilités partielles (Part-Worths) de leurs niveaux correspondants.

**Tableau 2:** Résultats agrégés de l'importance des attributs et les utilités partielles de leurs niveaux respectifs.

| Attributs d'huile                                                        | Importance Relative (%) | Niveaux                        | Utilités partielles (Part-Worth) |
|--------------------------------------------------------------------------|-------------------------|--------------------------------|----------------------------------|
| Prix                                                                     | 36,66                   | 6€                             | -1,167                           |
|                                                                          |                         | 4,5€                           | -0,050                           |
|                                                                          |                         | 3€                             | <b>1,217</b>                     |
| Couleur                                                                  | 16,57                   | Jaune-doré                     | -0,157                           |
|                                                                          |                         | Jaune-verdâtre                 | <b>0,157</b>                     |
| Emballage                                                                | 18,67                   | Verre de design                | -0,050                           |
|                                                                          |                         | Verre normal                   | -0,257                           |
|                                                                          |                         | Plastique                      | <b>0,307</b>                     |
| Origine                                                                  | 28,10                   | Certifiée avec AOP             | <b>0,971</b>                     |
|                                                                          |                         | Mentionnée sans certificat AOP | -0,188                           |
|                                                                          |                         | Non mentionnée                 | -0,784                           |
| Constante : 4,516<br>R de Pearson : 0,923***    Tau de Kendall : 0,995** |                         |                                |                                  |

\*\*\*: Niveau de signification ( $p \leq 0,001$ )

Source: Elaboration personnelle. Sondage sur les préférences des consommateurs par rapport à l'huile d'olive, (2009).

Pour vérifier l'adéquation de l'ajustement, on a utilisé des indicateurs qui mesurent le coefficient de corrélation existant entre les valeurs manifestées par les consommateurs et les valeurs pré-estimées par le modèle (le taux de Kendall et le coefficient de corrélation de Pearson). Dans ce cas, les deux coefficients sont très significatifs ( $p \leq 0,001$ ).

A partir des résultats obtenus sur l'importance relative des attributs, on peut affirmer que le « Prix » est le critère le plus important dans la formation des préférences des consommateurs avec une importance relative du 36,66%, en second rang on trouve l'attribut « Origine » (28,10%), suivi par l'attribut « Emballage » (18,67%) et en dernier rang figure l'attribut « Couleur » (avec une importance relative du 16,57%).

Pour l'attribut « Prix », le niveau le plus bas (3€) est le plus déterminant dans la formation des préférences d'huile. La valeur de son utilité moyenne relative est positive (1,217). Les deux niveaux de prix moyen (4,5€) et élevé (6€) ont une importance secondaire dans la formation des préférences vis à vis l'huile et leurs utilités moyennes relatives sont négatives (respectivement : -0,050 y -1,167). Plus le prix est élevé pour les consommateurs, moindres sont leurs utilités et par conséquent les préférences d'une alternative déterminé. En somme, l'utilité marginale d'une alternative diminue lorsque les niveaux de prix augmentent.

En ce qui concerne « l'Origine » territoriale de la production d'huile d'olive, la « Certification avec une AOP » est le niveau majeur dans la formation des préférences des consommateurs, suivi par « l'Origine mentionnée » et finalement par « l'Origine non mentionnée ». Les utilités négatives de ces derniers niveaux sont très inférieures à l'utilité de la « Certification avec AOP ». Alors, lorsque l'origine territoriale d'huile d'olive est à la fois connue, et garantie avec une certification, l'utilité et la satisfaction sont majeures pour les consommateurs d'huile d'olive. L'importance de la certification relative au territoire et son utilité pour les consommateurs est aussi confirmée par l'application de l'Analyse Conjointe en huiles (Krystallis et Ness, 2005 ; Van der Lans et al., 2001) et dans le cas d'autres produits agroalimentaires comme la pomme avec AOP (Fotopoulos et Krystallis, 2003), le fromage avec AOP (Mojardino et Ventura, 2001), le vin avec AOP (Bernabéu et al., 2001) et la viande bovine avec IGP (Ulloa et Gil, 2007).

En ce qui concerne « l'Emballage », l'huile emballée en « Plastique » est plus appréciée que celles en « Verre de design » et en « Verre normal » étant donné qu'elle a une utilité partielle positive (0,307). Les huiles emballées en bouteilles de verre normal ou de design ont été les moins valorisées, ainsi que leurs utilités étaient négatives de l'ordre de -0,257 y -0,050 respectivement. Ce fait paraît très logique vue que les consommateurs associent mentalement ces deux types d'emballage avec des produits de haute gamme et de prix élevés.

Finalement, on a pu authentifier que l'attribut qui a la moindre influence dans la formation des préférences des consommateurs est la « Couleur d'huile », et que le niveau « Jaune-verdâtre » est plus valorisé que le niveau « Jaune-doré » avec une utilité partielle positive (0,157).

A travers les résultats ci-dessus, on peut souligner que « l'huile d'olive la plus préférée » pour les consommateurs est celle de couleur jaune-verdâtre, vendu à un prix de 3€/l, avec un emballage en plastique et avec certification AOP. Par contre, « l'huile d'olive la moins préférée » est celle de couleur jaune-doré, sans information sur son origine territoriale de production, commercialisée avec un emballage de verre de design et à un prix élevé (6€/l).

#### 4. Conclusions

Cette étude tente à analyser l'importance relative de l'origine géographique dans la formation des préférences des consommateurs d'huile d'olive en Andalousie à travers une Analyse Conjointe entre neuf alternatives qui combinent quatre attributs considérés les plus pertinents lors de l'achat du produit: Prix, Origine, Emballage et Couleur d'huile.

Une des premières conclusions dérivées de l'analyse de la structure des préférences des consommateurs d'huile en Andalousie, est la grande importance accordée au « Prix » et à « l'Origine » territoriale d'huile, étant les deux attributs ayant la majeure importance relative dans la formation des préférences des consommateurs. On doit souligner que les attributs « Couleur » et « Emballage » d'huile ne semblent pas avoir trop d'importance dans la fonction d'utilité des consommateurs interrogés.

En ce qui concerne « l'Origine » territoriale de la production d'huile, la « Certification AOP » est le niveau le plus pertinent dans la formation des préférences des consommateurs, suivi de « l'Origine mentionnée » et finalement par « l'Origine non mentionnée ». Partant de ce constat on peut déduire que plus l'origine territoriale d'huile d'olive est connue, et encore plus elle est garantie par une certification, majeure sont son utilité et sa satisfaction pour les consommateurs.

Par rapport aux conclusions mentionnées ci-dessus on peut conclure que la différenciation des huiles d'olive espagnoles à travers l'emploi des alternatives de certification liées avec le territoire, plus concrètement les Appellations d'Origine Protégée, peuvent être un exemple d'outil efficace pour acquérir des avantages compétitifs sur les marchés, en profitant des caractéristiques différentielles du milieu naturel et la méthode d'élaboration propre à chaque territoire.

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## **Les politiques de l'huile d'olive, la Pauvreté Rurale et le Développement Economique en Méditerranée**

Elabed Ghada, Travis Lybbert

### **Résumé**

Plusieurs pays de la méditerranée favorisent la production d'huile d'olive comme facteur de développement. Ces politiques sont la conséquence de l'essor de la demande grâce à l'adoption du régime méditerranéen à travers le monde et grâce à l'émergence de marchés de produits à haute valeur. En outre, ces politiques s'inscrivent dans le cadre du renforcement des politiques de développement rural ainsi que de l'émergence des préoccupations environnementales. Nous décrivons les dernières tendances de ces marchés et les nouvelles orientations des politiques gouvernementales de l'huile d'olive comme outil de développement. Nous documentons et comparons ces politiques en Tunisie, au Maroc, en Syrie et au Liban. Nous évaluons par ailleurs leurs impacts potentiels sur la pauvreté ainsi que les décalages entre ces politiques et la réalité du marché international.

**Mots clés:** développement économique, pauvreté rurale, MENA, marché de l'huile d'olive, politiques de l'huile d'olive.

## **Olive oil policies, Rural Poverty & Economics Development in the Mediterranean**

### **Abstract**

Many Mediterranean countries are actively promoting olive oil as a promising vector of rural development and poverty reduction. These policies are a response to a booming olive oil market characterized by the spread of the Mediterranean diet and the emergence of high value markets, reinvigorated agricultural and rural development policies, and concerns about water availability, soil preservation and natural resources. We describe the recent market and policy trends responsible for casting olive oil as a rural development tool. We then document and compare olive oil policies in Tunisia, Morocco, Lebanon and Syria. We assess their likely poverty impacts, including limitations such as credit constraints among smallholders and inconsistencies between these policies and the realities of the current international olive oil market.

**Key words:** economic development, rural poverty, MENA, olive oil policies & markets

## **L'intégration de la demande sociale dans l'évaluation et la conception des pratiques d'innovation dans le secteur agro-alimentaire : le cas d'huile d'olive en Espagne**

Samir Sayadi, Yamna Erraach, Carlos Perra-Lopez & Ascensión Hinojosa-Rodríguez

### **Résumé**

La compétitivité et la durabilité du système oléicole sont fortement liées à la satisfaction de la demande sociale des consommateurs ainsi que leurs préférences vis-à-vis la qualité, la sécurité et les issues environnementaux. L'adoption des 'bonnes pratiques agroalimentaires' innovatrices, doit engendrer une maximisation du bien être social et une plus grande diversification, compétitivité et rentabilité financière pour les agents producteurs. Dans cette recherche, la relation entre la production oléicole, la transformation, les pratiques de gestion et d'organisation et la demande sociale du consommateur est explorée à travers l'application de la méthode 'Quality Function Deployment' (QFD) dans le cas de l'Espagne. Cette méthodologie permet d'identifier les 'bonnes pratiques agro-alimentaires' innovatrices, qui optimisent mieux la demande sociale du consommateur et les bénéfices des agents producteurs. Les résultats seront de grande utilité pour la conception des politiques publiques et des stratégies économiques permettant la durabilité et la compétitivité du système oléicole Espagnol.

**Mots clés :** Les pratiques agro-alimentaires durables, compétitivité, politiques publiques et économiques, système oléicole, QFD.

### **Integrating social demands in the evaluation and design of innovative practices in the agro-food system: The case of Spanish olive oil sector**

#### **Abstract**

The competitiveness and sustainability of the olive agro-food system are strongly related to the satisfaction of social and consumers' demands and preferences for quality, security and environmental issues. The adoption of innovative 'good agro-food practices' would entail the maximization of social welfare and a greater differentiation, competitiveness and financial profit for productive agents. In this research, the relationships among olive farming, processing, management and organisation practices and the social and consumers' demands are explored by using the Quality Function Deployment (QFD) method in the case of Spain. It allows identifying innovative 'good agro-food practices' that optimally satisfy social demands, and consumers' and productive agents' benefits. These results are aimed to be useful for the design of public policies and business strategies towards the sustainability and competitiveness of the Spanish olive system.

**Key words:** Agro-food practices for sustainability, competitiveness, public and business policies, olive system, QFD.

#### **1. Introduction**

La croissance de la concurrence dans le secteur agro-alimentaire, accentuée par la mondialisation et la globalisation économique, oblige les entreprises à promouvoir et à mettre en œuvre des politiques et des stratégies innovantes de mise en valeur de leurs productions, en les différenciant selon des signes distinctifs de qualité, durabilité, territorialité, modes de productions alternatifs, etc. L'innovation paraît être une excellente opportunité de différenciation, diversification et de positionnement des produits dans un marché de plus en plus dynamique et exigeant. Aussi, la croissante préoccupation et l'intérêt des consommateurs envers les produits qu'ils consomment, la sécurité et la salubrité des aliments et le respect de l'environnement dans les processus de production, sont à l'origine de



l'imposition des nouvelles demandes pour l'industrie alimentaire (Van der Valk et Wynstra, 2005). Un des facteurs clés de réussite dans le marché actuel, consiste à comprendre le comportement du consommateur, d'identifier ses besoins et exigences, et les intégrer dans la planification stratégique de toutes les étapes du processus de production, transformation et commercialisation des produits et/ou services.

Cela présente un grand défi pour un nombre considérable d'industries agro-alimentaires, en général, et pour l'industrie oléicole, en particulier. Plus concrètement, en Andalousie, le Système Oléicole (SO) est de grande importance économique, socioculturelle et territoriale. Le choix d'huile d'olive comme objet de cette étude revient au fait qu'il est considéré par les consommateurs comme un produit peu différencié et ainsi les résultats dégagés peuvent être une opportunité pour le développement d'un produit plus orienté au consommateur et pour des futures segmentations du marché.

L'objectif de cette étude est de développer et de mettre en œuvre un cadre méthodologique qui intègre les exigences du consommateur vis-à-vis les attributs de qualité d'huile (organoleptiques, socioculturelles, environnementales, etc.) et les pratiques agricoles potentielles pour les satisfaire. En définitive, il s'agit de développer une approximation complète du concept de la qualité d'huile d'olive, qui inclue les demandes du consommateur et les techniques susceptibles d'être appliquées dans la production d'olives.

## **2. Méthodologie**

Le processus d'intégration de la « Voix du consommateur » avec les possibilités technologiques du secteur ou le « Déploiement Fonctionnel de la Qualité » a été mis en œuvre selon quatre étapes chronologiques que se détaillent ci-dessous.

### **2.1. Identification, définition et quantification des exigences de qualité des consommateurs d'huile d'olive (QUOI – WHATS)**

La première étape du travail consiste à identifier les caractéristiques perçues par le consommateur (*Voix du consommateur*) d'huile d'olive de qualité. A cet effet, on a fait recours à des techniques qualitatives basées sur des entretiens informels et de discussions en groupe pour la collecte des informations relatives aux aspects, besoins ou exigences des personnes interrogées par rapport à l'huile d'olive, en obtenant une large liste des demandes des clients (QUOI). Postérieurement, on a effectué une *purification* de l'information, en évitant les répétitions et les manifestations ambiguës qui ne peuvent pas être mesurées et par conséquent classées comme besoins. Finalement, on a élaboré une liste définitive de 8 besoins ou demandes (Tableau 1).

A partir des résultats extraits dans l'étape qualitative, on a développé une étude quantitative approfondie basée sur l'emploi d'une enquête à 439 consommateurs pour quantifier et pondérer les demandes et les exigences des consommateurs ( $W_d$ ) et qui seront utilisés postérieurement dans la matrice du QFD (Tableau 2). La pondération des besoins a été effectuée selon une échelle allant de 1 (peu important) à 5 (très important).

### **2.2. Identification et définition des pratiques dans le secteur de la production (COMMENT – HOWs)**

Au-delà de la révision documentaire (Junta de Andalucía, 2006 ; Parra-López, 2006 ; Jiménez et Carpio, 2008 ; Alba et al., 2009 ; Humanes et Humanes, 2009 ; Uceda, 2009 ; Vega et al., 2009, entre autres), on a réalisé plusieurs discussions en groupe pendant le mois de septembre 2009 avec des experts du secteur (agriculteurs, chercheurs, techniciens et responsables des huileries, laboratoires de qualité, etc.) pour identifier et définir les pratiques potentielles ou les « Attributs de Conception » potentiellement utilisables par les agriculteurs pour satisfaire les nécessités sociales préalablement établies, étant certaines pratiques très innovantes et d'autres plus étendues. Ce processus n'a pas été seulement qualitatif mais également quantitatif pour pouvoir sélectionner uniquement les pratiques qui peuvent techniquement avoir des relations potentielles avec les demandes identifiées ultérieurement. Finalement on a choisi 47 pratiques agronomiques qui ont été classées en sous-niveaux (Tableau 3).



### 2.3. Déploiement des demandes du consommateur (QFD): Corrélation entre QUOI - COMMENT

Le principe de base de la méthodologie QFD consiste dans la construction de la matrice de la qualité ou « la maison de qualité » (Rudolph, 1995 ; Bech et al., 1997 ; Viaene et Januszewka, 1999 ; Benner et al., 2003) qui est essentiellement, une matrice qui met en rapport la voix du client (QUOI), avec les exigences techniques qui la satisfont (COMMENT). Dans ce cas, pour déterminer la contribution des pratiques (exigences techniques) dans la satisfaction de la demande du consommateur envers la qualité d'huile d'olive, on a construit la matrice de relations entre les « QUOI » et les « COMMENT » au niveau des pratiques individuelles et au niveau de pratiques agrégées. La *matrice stratégique ou matrice de production* ( $W_{pj,di}$ ) est obtenue en pondérant les relations au niveau désagrégé (par exemple : rapport entre « saveur fruitée » et « variété picual » du Tableau I) avec le rapport au niveau agrégé (par exemple : dans ce cas rapport entre « saveur » et « variété »). Pour ce fait, on a interrogé 10 experts dans l'oléiculture (oléiculteurs, techniciens des huileries, chercheurs, distributeurs, etc.) pour décrire et quantifier les relations, en obtenant une matrice pour chacun d'entre eux. L'échelle de quantification employée a été de 0 (il n'existe pas une relation) à 9 (relation très forte) (voir Ramanathan et Ganesh, 1994 ; Parra-López et al., 2008). Ainsi on obtient une pondération pour chaque expert ou agent décideur ( $W_{pj,di(e)}$ ). La contribution relative moyenne correspondante de chaque pratique (j) à chaque demande (i) est calculée comme la moyenne arithmétique des pondérations de tous les experts :

$$W_{pj,di(\text{groupeexp})} = \sum_{e=1}^E W_{pj,di(e)} / E$$

Étant E le nombre d'expert.

### 2.4. Pondération de la contribution des pratiques pour satisfaire l'ensemble des demandes

La contribution totale d'une pratique ( $W_{pj}$ ) est déterminée par la somme des contributions relatives moyennes de cette pratique pour chaque demande (i) ( $W_{pj,di(\text{groupeexp})}$ ) multipliées par l'importance relative de chaque demande selon les consommateurs ( $W_{di}$ ):

$$W_{pj} = \sum_{i=1}^n W_{pj,di(\text{groupeexp})} * W_{di}$$

Dans notre cas: n = 8 demandes ; j = 47 pratiques

## 3. Résultats

Le consommateur évalue une série de caractéristiques de qualité dans l'huile d'olive qui peuvent être identifiées comme *qualité totale* perçue. On remarque que cette qualité inclue, non seulement des caractéristiques organoleptiques d'huile (saveur, couleur, acidité, etc.), mais aussi d'autre du genre socioculturelles (maintenir la population locale, créer des postes du travail, etc.), et y compris territoriales et environnementales (compatibilité avec l'environnement, etc.) (Tableau 1). Parmi ces caractéristiques se distinguent : la saveur fruitée, le faible degré d'acidité d'huile, la couleur jaune-verdâtre, le prix raisonnable et la production selon les normes de l'agriculture écologique, comme étant les demandes les plus prioritaires pour le consommateur d'huile d'olive (Tableau 3).

En ce qui concerne les pratiques agronomiques considérées dans cette étude, plus du 60% s'avèrent significatives pour mieux satisfaire les exigences et les désirs d'une huile d'olive de qualité. Ces alternatives constituent les pratiques critiques qu'il faut prendre en considération lors de la production selon les indiquent la demande du consommateur.

Selon les résultats obtenus, on peut souligner que les pratiques agronomiques les plus significatives et déterminantes de la qualité d'huile d'olive selon la demande sociale sont: La séparation des olives du sol et du vol ; les critères pour la récolte (selon le taux de maturation) ; le mode de récolte de sol (non récolte du sol) ; le mode de récolte de vol (gauler) ; le traitement des infections et des maladies ; le mode de transport depuis l'exploitation aux huileries (en caisses) ; les substances utilisées pour fertiliser (engrais organiques qui incluent les restes de la taille, grignon d'olive, etc.) ; le contrôle des infections-Mouche (lutte biologique avec pulvérisation terrestre) ; la variété (Picual) ; le contrôle des infections-Mites (lutte biologique *Bacillus thuringiensis* en floraison) ; la gestion du sol (sol couvert) ; la fertilisation (non fertilisation) ; le moment de l'irrigation (selon les recommandations des techniciens).

Dans le Tableau 1, on détaille la contribution relative de chacune des pratiques agronomiques considérées. Par exemple, « la séparation de l'olive du sol et du vol » est la pratique qui satisfait plus « le bas degré d'acidité » et l'ensemble des exigences du consommateur vers la qualité exigée d'huile d'olive.

#### **4. Conclusions**

Le travail présente une approche méthodologique d'intégration des demandes des consommateurs et des citoyens vis-à-vis la qualité d'huile d'olive et les externalités de son processus de production avec les pratiques potentielles les plus adéquates pour satisfaire ces demandes.

La qualité d'huile d'olive la plus demandée par les consommateurs incorpore des aspects liés aux caractéristiques organoleptiques (l'acidité, la saveur, la couleur, etc.) ainsi que socioculturelles (la création des postes du travail, le maintien de la population, etc.) et environnementales (le respect environnemental, etc.).

La séparation des olives du sol et du vol ; les critères pour la récolte ; le mode de récolte-sol ; le mode de récolte-vol ; le traitement des infections et des maladies ; le mode de transport depuis l'exploitation aux huileries ; les substances utilisées pour fertiliser ; le contrôle des infections-Mouche ; la variété ; le contrôle des infections-Mites ; la gestion du sol ; la fertilisation et le moment d'irrigation, sont les pratiques agronomiques optimales selon la perspective sociale (optimum technico-social).

Toute politique agricole dans le secteur oléicole qui vise être efficace et prendre en compte les préférences de la société devrait être orientée à favoriser la mise en œuvre de ces pratiques au niveau des exploitations oléicoles. Ceci permettrait d'améliorer la légitimité et l'appui social aux aides de la PAC pour le secteur agricole en général et le secteur oléicole en particulier.

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Tableau 1: Matrice de Déploiement de la conception des pratiques agricoles et des demandes du consommateur d'huile d'olive

| Pratiques agronomiques                     | Importance relative ( $W_{di}$ ) | Variétés |            | Gestion des sols   |                                                                    |                           | Irrigation  |       | Systèmes d'irrigation |                               | Moment irrigation                          |                 | Analyse de la qualité de l'eau            |       |      |
|--------------------------------------------|----------------------------------|----------|------------|--------------------|--------------------------------------------------------------------|---------------------------|-------------|-------|-----------------------|-------------------------------|--------------------------------------------|-----------------|-------------------------------------------|-------|------|
|                                            |                                  | Piquet   | Hojiblanca | Sol nu avec labour | Sol nu avec labour (contrôle des mauvaises herbes avec herbicides) | Sol nu avec labour réduit | Sol couvert | Oui   | Non                   | Irrigation au goutte-à-goutte | Autres systèmes (aspersion, a mante, etc.) | Calendrier fixe | Selon les recommandations des techniciens | Oui   | Non  |
| Demande du consommateur                    |                                  |          |            |                    |                                                                    |                           |             |       |                       |                               |                                            |                 |                                           |       |      |
| Saveur fruitée                             | 4,3                              | 54,7     | 58,0       | 0,0                | 0,0                                                                | 0,0                       | 0,0         | 28,6  | 11,1                  | 0,0                           | 0,0                                        | 0,0             | 0,0                                       | 0,0   | 0,0  |
| Couleur jaune-verdâtre                     | 3,3                              | 48,0     | 25,3       | 0,0                | 0,0                                                                | 0,0                       | 0,0         | 1,3   | 1,1                   | 0,0                           | 0,0                                        | 0,0             | 0,0                                       | 0,0   | 0,0  |
| Bas degré d'acidité                        | 4,1                              | 4,9      | 5,8        | 0,0                | 0,0                                                                | 0,0                       | 0,0         | 3,8   | 2,3                   | 0,0                           | 0,0                                        | 3,9             | 6,0                                       | 0,0   | 0,0  |
| Prix                                       | 3,8                              | 0,0      | 0,0        | 0,0                | 0,0                                                                | 0,0                       | 0,0         | 5,0   | 6,8                   | 0,0                           | 0,0                                        | 0,0             | 0,0                                       | 3,7   | 1,3  |
| Système de Production Ecologique           | 2,6                              | 0,0      | 0,0        | 34,1               | 17,5                                                               | 17,5                      | 49,8        | 0,0   | 0,0                   | 0,0                           | 0,0                                        | 23,0            | 30,8                                      | 25,9  | 6,2  |
| Production respectueuse de l'environnement | 2,4                              | 0,0      | 0,0        | 13,8               | 19,1                                                               | 32,9                      | 62,7        | 28,8  | 35,2                  | 66,9                          | 34,0                                       | 22,9            | 61,0                                      | 30,5  | 12,2 |
| Création d'emploi en milieu rural          | 1,8                              | 0,0      | 0,0        | 0,0                | 0,0                                                                | 0,0                       | 0,0         | 13,1  | 8,2                   | 0,0                           | 0,0                                        | 0,0             | 0,0                                       | 0,0   | 0,0  |
| Maintient de la population rurale          | 1,8                              | 0,0      | 0,0        | 0,0                | 0,0                                                                | 0,0                       | 0,0         | 9,4   | 6,7                   | 0,0                           | 0,0                                        | 0,0             | 0,0                                       | 0,0   | 0,0  |
| Contribution totale ( $W_{pj}$ )           |                                  | 413,6    | 356,7      | 121,9              | 91,5                                                               | 124,6                     | 280         | 271,1 | 197,7                 | 160,7                         | 81,6                                       | 130,5           | 251,1                                     | 154,6 | 50,4 |

| Pratiques<br>agronomiques                     | Importance relative<br>( $W_{di}$ ) | Fertilisation |       | Méthode de fertilisation             |                         | Substances pour la<br>fertilisation |                                                                       |             | Analyses<br>préalables à la<br>fertilisation |                                  |
|-----------------------------------------------|-------------------------------------|---------------|-------|--------------------------------------|-------------------------|-------------------------------------|-----------------------------------------------------------------------|-------------|----------------------------------------------|----------------------------------|
|                                               |                                     | Oui           | Non   | Application<br>directe sur le<br>sol | Application<br>foliaire | Autre<br>méthodes                   | Engrais<br>organiques<br>(restes<br>élagage,<br>l'alpeorajo,<br>etc.) | Engrais NPK | Analyse<br>foliaire ou du<br>sol             | Analyse<br>foliaire ou du<br>sol |
| Saveur fruitée                                | 4,3                                 | 0,0           | 0,0   | 0,0                                  | 0,0                     | 0,0                                 | 0,0                                                                   | 0,0         | 0,0                                          | 0,0                              |
| Couleur jaune-verdâtre                        | 3,3                                 | 0,0           | 0,0   | 0,0                                  | 0,0                     | 0,0                                 | 0,0                                                                   | 0,0         | 0,0                                          | 0,0                              |
| Bas degré d'acidité                           | 4,1                                 | 1,3           | 3,0   | 0,0                                  | 0,0                     | 0,0                                 | 0,0                                                                   | 0,0         | 0,0                                          | 0,0                              |
| Prix                                          | 3,8                                 | 3,5           | 4,5   | 0,0                                  | 0,0                     | 0,0                                 | 0,0                                                                   | 5,9         | 2,3                                          | 2,3                              |
| Système de Production<br>Ecologique           | 2,6                                 | 29,0          | 39,0  | 25,3                                 | 28,7                    | 16,9                                | 71,0                                                                  | 11,0        | 40,7                                         | 9,7                              |
| Production respectueuse<br>de l'environnement | 2,4                                 | 22,4          | 46,7  | 24,0                                 | 49,8                    | 36,0                                | 69,0                                                                  | 17,0        | 55,8                                         | 21,8                             |
| Création d'emploi en<br>milieu rural          | 1,8                                 | 13,6          | 5,7   | 0,0                                  | 0,0                     | 0,0                                 | 0,0                                                                   | 0,0         | 0,0                                          | 0,0                              |
| Maintient de la<br>population rurale          | 1,8                                 | 5,6           | 2,3   | 0,0                                  | 0,0                     | 0,0                                 | 0,0                                                                   | 0,0         | 0,0                                          | 0,0                              |
| Contribution totale ( $W_{pi}$ )              |                                     | 181,9         | 257,2 | 123,4                                | 194,1                   | 130,2                               | 350,2                                                                 | 91,9        | 248,5                                        | 86,3                             |

**Tableau 2:** Matrice de Déploiement de la conception des pratiques agricoles et des demandes du consommateur d'huile d'olive (suite)

| Pratiques<br>agronomiques                        | Importance relative<br>( $W_d$ ) | Traitement<br>des infections<br>et des<br>maladies |       | Contrôle des infections et maladies<br>- Mouches |                                                           | Contrôle des infections<br>et des maladies - Mites        |                                                                       | Moment du<br>traitement |                 | Localisation des<br>traitements<br>phytosanitaires         |                          |                             |
|--------------------------------------------------|----------------------------------|----------------------------------------------------|-------|--------------------------------------------------|-----------------------------------------------------------|-----------------------------------------------------------|-----------------------------------------------------------------------|-------------------------|-----------------|------------------------------------------------------------|--------------------------|-----------------------------|
|                                                  |                                  | Oui                                                | Non   | Trempage<br>massif                               | Lutte<br>biologique<br>avec<br>pulvérisation<br>terrestre | Lutte<br>biologique<br>avec<br>pulvérisation<br>terrestre | Contrôle<br>biologique<br>(Bacillus<br>thuringiensis<br>en floraison) | Traitement<br>chimique  | Calendrier fixe | Quand il<br>dépasse un<br>seuil déterminé<br>de population | Sur toute la<br>parcelle | Sur le foyer<br>d'infection |
| Saveur fruitée                                   | 4,3                              | 20,3                                               | 5,3   | 0,0                                              | 0,0                                                       | 0,0                                                       | 0,0                                                                   | 0,0                     | 0,0             | 0,0                                                        | 0,0                      | 0,0                         |
| Couleur jaune-<br>verdâtre                       | 3,3                              | 0,0                                                | 0,0   | 0,0                                              | 0,0                                                       | 0,0                                                       | 0,0                                                                   | 0,0                     | 0,0             | 0,0                                                        | 0,0                      | 0,0                         |
| Bas degré d'acidité                              | 4,1                              | 37,5                                               | 3,9   | 0,0                                              | 0,0                                                       | 0,0                                                       | 0,0                                                                   | 0,0                     | 0,0             | 0,0                                                        | 0,0                      | 0,0                         |
| Prix                                             | 3,8                              | 3,8                                                | 4,4   | 0,0                                              | 0,0                                                       | 0,0                                                       | 0,0                                                                   | 0,0                     | 1,9             | 1,4                                                        | 1,7                      | 1,8                         |
| Système de<br>Production<br>Ecologique           | 2,6                              | 43,2                                               | 13,6  | 63,8                                             | 74,4                                                      | 11,3                                                      | 67,0                                                                  | 11,3                    | 11,1            | 39,7                                                       | 16,4                     | 28,1                        |
| Production<br>respectueuse de<br>l'environnement | 2,4                              | 29,0                                               | 36,0  | 41,0                                             | 62,0                                                      | 26,7                                                      | 64,7                                                                  | 17,8                    | 16,3            | 46,4                                                       | 23,3                     | 61,0                        |
| Création d'emploi en<br>milieu rural             | 1,8                              | 11,8                                               | 2,7   | 0,0                                              | 0,0                                                       | 0,0                                                       | 0,0                                                                   | 0,0                     | 0,0             | 0,0                                                        | 0,0                      | 0,0                         |
| Maintient de la<br>population rurale             | 1,8                              | 7,2                                                | 1,7   | 0,0                                              | 0,0                                                       | 0,0                                                       | 0,0                                                                   | 0,0                     | 0,0             | 0,0                                                        | 0,0                      | 0,0                         |
| Contribution totale ( $W_p$ )                    |                                  | 471,4                                              | 184,8 | 264,2                                            | 342,2                                                     | 93,3                                                      | 329,5                                                                 | 72,2                    | 75,1            | 220,0                                                      | 104,9                    | 226,6                       |

Tableau 3: Matrice de Déploiement de la conception des pratiques agricoles et des demandes du consommateur d'huile d'olive (suite)

| Pratiques agronomiques                     | Importance relative ( $W_{di}$ ) | Critères de récolte     |                 | Mode récolte - Sol |                   |                    | Mode de récolte - Vol |                                     |                  | Séparation des olives du sol et du vol |                | Mode de transport de l'exploitation au moulin d'huile |         |                                |
|--------------------------------------------|----------------------------------|-------------------------|-----------------|--------------------|-------------------|--------------------|-----------------------|-------------------------------------|------------------|----------------------------------------|----------------|-------------------------------------------------------|---------|--------------------------------|
|                                            |                                  | Selon degré de maturité | Calendrier fixe | Récolte à main     | Récolte mécanique | Non récolte du sol | Gaulage               | Vibreurs (de branches ou de troncs) | Le peigne manuel | Séparation                             | Non séparation | Sacs                                                  | Caisses | Remorque du tracteur ou camion |
| Saveur fruitée                             | 4,3                              | 48,6                    | 33,4            | 13,4               | 7,6               | 33,4               | 12,3                  | 4,4                                 | 14,0             | 65,8                                   | 11,9           | 15,1                                                  | 26,8    | 24,3                           |
| Couleur jaune-verdâtre                     | 3,3                              | 53,5                    | 36,0            | 7,2                | 7,2               | 27,1               | 3,3                   | 3,3                                 | 3,3              | 45,3                                   | 7,0            | 9,9                                                   | 6,7     | 7,3                            |
| Bas degré d'acidité                        | 4,1                              | 55,7                    | 16,5            | 39,1               | 18,1              | 50,5               | 34,9                  | 39,7                                | 51,3             | 75,4                                   | 8,5            | 14,1                                                  | 52,3    | 34,4                           |
| Prix                                       | 3,8                              | 6,7                     | 12,8            | 15,3               | 14,3              | 19,6               | 12,2                  | 15,4                                | 12,2             | 8,3                                    | 17,5           | 2,8                                                   | 4,1     | 4,1                            |
| Système de Production Ecologique           | 2,6                              | 28,1                    | 27,3            | 18,9               | 22,8              | 39,3               | 26,0                  | 16,5                                | 21,8             | 53,1                                   | 18,8           | 22,5                                                  | 29,6    | 18,0                           |
| Production respectueuse de l'environnement | 2,4                              | 2,9                     | 1,0             | 0,0                | 0,0               | 0,0                | 0,0                   | 0,0                                 | 0,0              | 0,0                                    | 0,0            | 0,0                                                   | 0,0     | 0,0                            |
| Création d'emploi en milieu rural          | 1,8                              | 0,0                     | 0,0             | 37,2               | 18,9              | 12,2               | 60,1                  | 37,4                                | 54,2             | 0,0                                    | 0,0            | 0,0                                                   | 0,0     | 0,0                            |
| Maintient de la population rurale          | 1,8                              | 0,0                     | 0,0             | 29,3               | 13,5              | 10,7               | 51,7                  | 31,7                                | 44,4             | 0,0                                    | 0,0            | 0,0                                                   | 0,0     | 0,0                            |
| Contribution totale ( $W_{pj}$ )           |                                  | 719,6                   | 452,0           | 468,1              | 302,6             | 658,1              | 521,4                 | 418,6                               | 562,1            | 911,5                                  | 224,4          | 224,2                                                 | 444,4   | 331,6                          |

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## **Le secteur de l'huile d'olive biologique en Tunisie et les différentes structures de production: une analyse comparative**

Saida Elfkhi, Boubaker Karrray

### **Résumé**

En Tunisie l'agriculture biologique remonte aux années 90 avec une domination majeure de l'activité oléicole représentant actuellement environ 85% de la superficie totale des cultures biologiques. Ce secteur est régi par une gamme assez diversifiée de structures de production où les plus importantes sont: Sociétés d'agriculteur, agro-combinats, et agriculteurs privés. Ces structures se distinguent par leurs différentes logiques de fonctionnement, d'organisation et de décision, dégagant ainsi des différences au niveau de leurs performances économiques, techniques et sociales. Ce travail consiste alors en une analyse comparative des fonctionnements et des performances de ces structures, synthétisant leurs points forts et leurs points faibles. Les données qui ont servi à cette étude ont été collectées à travers des enquêtes et des entretiens auprès des producteurs de la zone de Sfax; la plus importante en termes de superficie, de production de l'huile d'olive biologique du pays et en termes de nombre d'opérateurs.

**Mots Clés:** Oléiculture biologiques, structures de production, performances économiques techniques et sociales.

## **The Tunisian organic olive oil sector and the different production structures: A comparative analysis**

### **Abstract**

The Tunisian organic agriculture dates back to the end of the 90 th years where the main activity is the olive oil sector with 85% of the total area of organic crops. This sector is controlled by diversified production structures, where the more important are: Farmers associations, public farms and private farmers. These structures differed by its functional, organisational, and decision making logics. This situation leads to different levels of economics, techniques and social performances. This work aims to make a comparative analysis of the function and the performances of several production structures, focusing the synthesis on the strong and the weak issues. To reach the study objectives, we used data provided by questionnaires and interviews from the producers of Sfax region, the most important in Tunisia by its production area, its interesting number of producers and its diversified production structures.

**Keywords:** Organic olive oil sector; production structures; economics, social and techniques performances.

### **1. Introduction**

L'agriculture biologique qui a surgi au début comme résultante d'une raison de dimension environnementale et spirituelle a fini pour se convertir en une alternative de valorisation des produits agricoles et une opportunité aux producteurs pour apporter une valeur ajoutée a leurs outputs. Réellement, il ne s'agit pas d'une déviation des objectifs fondamentaux de la philosophie de Rudolf Steiner<sup>1</sup> sur l'agriculture écologique, mais il s'agit d'une nouvelle conception de la même vision mais dans un contexte plus organisé et régi par des règlements et une législation claire. Ce n'est pas l'agriculture romantique comme pense beaucoup mais c'est l'agriculture responsable et compromis avec l'environnement et la durabilité des systèmes agricoles. L'importance environnementale de ce système est une réalité qui a été contrastée par la simple logique d'être l'alternative la plus viable pour faire face aux modes intensifs voire agressifs de production. Reste à démontrer sa viabilité économique, surtout dans un monde régi par une économie ouverte, de plus en plus globalisée et de plus en plus compétitive.



Plusieurs études ont traité la thématique de l'agriculture biologique et sa viabilité économique. La littérature disponible est relativement abondante où les études les plus importantes pour le présent travail sont: Le Travail de Laajimi et Ben Nasr (2009) qui compare la durabilité des exploitations oléicoles biologiques et conventionnelles en Tunisie traitant le cas particulier de la région de Sfax. Le travail de Ben Salah et Hammami (2006) qui a porté sur l'étude technico-économique des exploitations oléicoles biologiques à Mahdia (Tunisie). Le travail de Alonso et Guzman (2006), où une analyse comparative des exploitations oléicoles en mode biologique et conventionnel était entamé, ceci en utilisant 17 indicateurs de durabilité (socio-économiques et environnementaux). Le travail de Parra López et Calatrava Requena (2005), où une analyse comparative de l'oléiculture biologique et conventionnelle de la région d'Andalousie (Espagne) était établie. Le travail de Parra López et Calatrava Requena (2004) où était traitée la diffusion des techniques de production biologique au niveau des exploitations oléicoles en Andalousie comme un processus de diffusion technologique. Del campo Tejedor et al. (2001) ont traité à l'agriculture biologique comme alternative de développement rural. Chinchilla fernandez (1999) a effectué une analyse du rôle des différents acteurs impliqués dans la diffusion de l'oléiculture biologique dans la zone d'Andalousie (Espagne). Lampkin et Padel (1994) ont analysé les caractéristiques, les avantages et les barrières que l'adoption de l'agriculture biologique peut représenter, ceci dès une perspective internationale. Diebel et al. (1993) ont analysé les barrières économiques à l'adoption de l'agriculture biologique.

Au niveau de la présente étude, une analyse comparative des performances techniques économiques et sociales des différentes structures de production du secteur oléicole biologique tunisien sera effectuée. Ceci afin de dégager leurs atouts et leurs contraintes, en mettant l'accent sur les liens entre elles et les différents acteurs de la filière. La présente communication est organisée de la manière suivante: i) introduction, ii) le secteur de l'oléiculture biologique en Tunisie: législation, importance et organisation, iii) Objectifs et méthodologie du travail, iv) Résultats, v) Conclusions et réflexions finales.

## **2. L'oléiculture biologique en Tunisie: législation, importance économique et organisation**

L'agriculture biologique est une activité qui remonte à la fin des années 90 en Tunisie. Cette activité a évolué considérablement cette dernière décennie en matière législative, économique et organisationnelle.

A niveau législatif, il faut signaler que la Tunisie a sa propre législation et son propre cahier des charges en matière de production biologique (loi N° 99-30 de 5 Avril 1999). Après huit ans de négociation, cette législation a été reconnue par un régime d'équivalence pour l'agriculture biologique par la Réglementation de la Commission Européenne (CE) N° 537/2009, ceci en conformité à la Réglementation (CE) N° 834/2007. Le régime d'équivalence reconnaît les normes de production et de contrôle établies par la législation Tunisienne en matière d'agriculture biologique. Ce régime couvre les produits végétaux non transformés et ceux transformés. Ceci facilite les opérations de commerce international en évitant les procédures d'autorisation d'importation exigées auparavant aux importateurs de la Communauté qui désirent importés des produits biologiques de la Tunisie.

Quant à son importance économique, l'oléiculture représente la composante principale de l'agriculture biologique en Tunisie. Sa superficie a augmenté de 12.666 ha en 2002 à 115.000 ha en 2008 représentant 85% de la superficie des cultures biologiques. La production de l'huile d'olive biologique a connu une évolution très importante ces dernières années, passant de 400 tonnes en 2002 à 10.000 tonnes en 2007. Les exportations ont évolué dans le même sens, de 400 tonnes en 2002 à 6061 tonnes en 2007. L'oléiculture biologique est concentrée principalement dans la région de Sfax avec 55,5% de la superficie totale, occupant le premier rang en matière de production de l'huile d'olive biologique. (CTAB, 2008)

Plusieurs acteurs contribuent à l'organisation du secteur. Ces acteurs sont principalement, les opérateurs de la filière, les organisations des Agriculteurs Biologiques, les organismes certificateurs et l'administration. Les opérateurs de la filière huile d'olive biologique ce sont principalement les agriculteurs, les transformateurs et les exportateurs. Les organismes de certification sont au nombre de 5: Ecocert SA en Tunisie, L'Institut Méditerranéen de Certification IMC,

<sup>1</sup> Au début des années 20 l'autrichien Rudolf Steiner (1860-1925) a travers un cours de formation dirigé aux agriculteurs, il a mis les bases de l'agriculture biodynamique qui se base sur une philosophie antimatérialiste (l'anthroposophie). Un groupe de sympathisants de Steiner a mis en pratique les techniques culturales de l'agriculture biodynamique, commercialisant en 1928 les premiers aliments sous une marque de qualité avec le nom de la déesse grecque Déméter.

BCS et Lacon et ICEA. La Fédération Nationale des Agriculteurs Biologiques représente l'unique organisation des agriculteurs biologiques Tunisiens. Les administrations engagées sont principalement: la Direction Générale de la Production Agricole (DGPA), le Centre Technique de l'Agriculture Biologique (CTAB) et Les Commissariats Régionales de Développement Agricole (CRDA).

Le secteur oléicole biologique dans la région de Sfax est caractérisé par la diversité de ses structures de production. En effet, il y a la présence de plusieurs structures de production qui diffèrent essentiellement par leurs statuts, leurs modes de gestions et leurs logiques de fonctionnement. Ces structures de production sont principalement: les agro-combinats issus de l'Office des Terres Domaniales, les sociétés ou groupements d'agriculteurs et les agriculteurs privés.

### 3. Objectifs et Méthodologie

L'objectif principal du travail est d'effectuer une analyse comparative des performances socio-économiques et techniques des différentes structures de production qui régies le secteur d'oléiculture biologique, partant de l'hypothèse que le système biologique est environnementalement durable. Cette étude était entamée dans la région de Sfax, région plus importante en termes de superficie, de production et de diversité des structures de production. Des enquêtes auprès des producteurs et des interviews auprès du reste des acteurs étaient établis. Les enquêtes comportent plusieurs rubriques: des données démographiques et de structure, Application des normes du cahier des charges, données économiques sur les productions et les charges de production, données techniques sur la maîtrise des opérations culturales et les opérations d'aménagement et des données sur la relation des producteurs avec les services de développement. Les interviews portent principalement sur le rôle de chaque intervenant de la filière et son interaction avec les opérateurs de la filière.

On a travaillé avec les agriculteurs (qui sont parfois des transformateurs et (ou) des exportateurs). L'objectif était d'effectuer une étude exhaustive auprès des différents opérateurs certifiés biologiques ayant les caractéristiques déjà mentionnées. Les opérateurs enquêtés sont les: quatre agro-combinats de la région de Sfax (Chaâl, Bouzouita, Essalama et Bir Ali), qui sont des fermes de l'état gérées par l'Office des Terres Domaniales. La catégorie société d'agriculteurs vient représentée par la société Ezzayatine, qui est une société d'oléiculteurs techniciens qui ont émergé suite à la restructuration des fermes de l'état. Par agriculteurs privés on désigne les agriculteurs qui travaillent ou individuellement ou en unités familiales. Des interviews étaient effectuées auprès du reste des acteurs, organisme certificateur qui est dans notre cas Ecocert SA en Tunisie, la Fédération Nationale des Producteurs Biologiques, La DGPA et le CRDA et le CTAB. A part les données quantitatives qui ont été collectées, des données qualitatives et des appréciations ont été obtenues à travers de tout le processus de collecte des données et de contact avec les opérateurs ainsi que le reste des acteurs.

Des indicateurs étaient étudiés, qui sont de nature technique et socio-économique. Les indicateurs techniques sont les suivants: date de début du projet biologique, Superficie olivier-biologique, importance de l'oléiculture biologique par rapport aux restes des spéculations de l'exploitation, densité de l'olivier biologique, pourcentage des oliviers biologiques sénescents (>70ans), pourcentage des oliviers biologiques jeunes (<10ans), degrés d'implication dans les processus de rénovation [Utilisation de compostage, Opérations d'aménagement (Arrachage, Replantation, augmentation des densités par nouvelles plantations)] et finalement le rendement moyen des trois dernières campagnes. Les indicateurs économiques sont les coûts moyens de production par Kg d'olive à huile et par Kg d'huile d'olive, prix de vente moyen par Kg d'olive à huile et par Kg d'huile d'olive et résultat par Kg d'olive à huile et par Kg d'huile d'olive. Les aspects sociaux qui vont être étudiés portent essentiellement sur des constatations qualitatives dégagées le long du processus de travail du terrain.

### 4. Résultats

#### 4.1. Indicateurs techniques

L'adoption du mode de production oléiculture biologique avait ses origines dans la région de Sfax en 1997-98 par des exploitants de la société des agriculteurs et par l'exploitant privé numéro 16. Cette initiative avait un succès ce qui se traduisait par l'augmentation du nombre des opérateurs dans ce secteur et par l'adhésion dernièrement (2004-05) des agro-combinats issus de l'office des Terres

Domaniales. Le taux de spécialisation en oléiculture biologique par les agriculteurs est élevé, en effet la superficie oléicole biologique occupe presque la totalité de la SAU chez la plupart des agriculteurs.

Les agro-combinats malgré qu'ils occupent de grandes superficies oléicoles biologiques (85% de la superficie totale oléicole biologique de la région de Sfax), sont moins spécialisés; ce sont des fermes de l'état de grandes superficies qui présentent des spéculations diversifiées. Les oléiculteurs biologiques de la région de Sfax sont tous des grands agriculteurs (des superficies en générale supérieures à 100ha). Les densités sont généralement plus élevées au niveau des exploitations des agriculteurs de la société des agriculteurs, ces derniers ont entamé des programmes d'augmentation des densités par de nouvelles plantations en intercalaire. Les agro-combinats sont les uniques producteurs qui ont entamé un programme d'arrachage d'oliviers sénescents et de replantation, ceci entre dans le cadre d'un programme national. Le compost est plus utilisé par les agriculteurs de la société d'agriculteurs. Plusieurs exploitants de la société d'agriculteurs ont introduit le mode irrigué (soit irrigation d'appoint pour les anciennes plantations, ou par de nouvelles plantation en gouttes à gouttes). Les rendements en tonnes d'olivier par hectares sont variables d'une exploitation à une autre, en moyenne, la société des agriculteurs présente la valeur la plus élevée (0,7 Tn/ha) suivi par les agro-combinats (0,5 Tn/ha) et les privés (0,47 Tn/ha). On doit signaler qu'au niveau de la société des agriculteurs étaient enregistrés les rendements les plus élevés et les plus faibles (les faibles rendements sont dus essentiellement d'une part au phénomène de sénescence des oliviers et d'autre part à l'existence de jeunes plantations qui ne sont pas en pleine production)

#### **4.2. Indicateurs économiques**

Observant les indicateurs économiques (tableau 2), on constate que tous les exploitants dégagent des résultats moyens positifs de leurs activités oléicoles. Ces résultats sont variables dépendant des exploitations enregistrant des différences entre les différentes structures de production.

Certains agriculteurs ont optés par la vente des olives à huile, les prix de vente sont assez élevés variant entre 0.910 DT à 1.17 DT. Les bénéfices enregistrés sont variables dépendant des coûts de production. Le reste des agriculteurs et qui représentent la majorité ont opté par la vente de l'huile d'olive.

Les prix de ventes moyens de l'huile d'olive sont plus élevés, en premier lieu chez l'exploitant 15 (qui est un exportateurs) et chez la plupart des exploitants de la société des agriculteurs, les agro combinats présentent les prix de ventes les plus faibles.

Des coûts de production d'huile d'olive élevés sont enregistrés chez l'exploitant 15 ainsi que la plupart des exploitants de la société des agriculteurs. Les coûts les plus faibles sont enregistrés au niveau des agro-combinats.

Concernant les résultats par Kg d'huile d'olive, les plus élevés sont enregistrés chez l'agro-combinat 3, chez l'exploitant 15 et chez quelques exploitants de la société d'agriculteurs (exploitants 3, 4 et 10).

Ces résultats montrent d'une part, une compétitivité prix chez les agriculteurs présentant une forme d'intégration (verticale ou horizontale) et une compétitivité coût de production des agro-combinats.

#### **4.3. Observations et implications sociales**

Au niveau de la région de Sfax on a constaté que l'agriculture biologique peut être considérée comme une forme d'innovation adoptée généralement par les grands agriculteurs plus qu'une forme de développement rural. Les agriculteurs qui sont impliqués dans l'oléiculture biologique dans la région démontrent une attitude positive et réceptive ayant une relation interactive avec son environnement, surtout avec les services de développement et le reste des acteurs. Malgré les avantages qui caractérisent le secteur de l'agriculture biologique, les agriculteurs souffrent de beaucoup de problèmes qui sont liés essentiellement à la commercialisation de leurs produits. Des efforts considérables sont déployés par les producteurs dans la valorisation de l'huile d'olive biologique produite, travaillant sur la qualité et sur le conditionnement (des projets en cours).

Tableau 1: Indicateurs Techniques au niveau des différentes structures de production

|                                           | Début projet | Superficie<br>Olivier<br>bio | % olive<br>bio/SAUT | Densité<br>Olivier<br>organique | % oliviers<br>bio> 70ans | % oliviers<br>bio<10a<br>ns | Arrachage et<br>replantation | Nouvelle plant <sup>a</sup><br>et augment <sup>a</sup> des<br>densités | Utilisation du<br>compost | Rendement –<br>moyen en bio:<br>tn/ha |
|-------------------------------------------|--------------|------------------------------|---------------------|---------------------------------|--------------------------|-----------------------------|------------------------------|------------------------------------------------------------------------|---------------------------|---------------------------------------|
| <b>Agriculteurs en société de service</b> |              |                              |                     |                                 |                          |                             |                              |                                                                        |                           |                                       |
| Exploitant 1                              | 1997         | 131                          | 92                  | 27                              | 60                       | 40                          | 0                            | 1                                                                      | 1                         | 0,38                                  |
| Exploitant 2                              | 1999         | 104                          | 99                  | 21                              | 87                       | 13                          | 0                            | 1                                                                      | 1                         | 0,74                                  |
| Exploitant 3                              | 1997         | 120                          | 80                  | 33                              | 50                       | 50                          | 0                            | 1                                                                      | 1                         | 1,47                                  |
| Exploitant 4                              | 1998         | 150                          | 98                  | 43                              | 28                       | 19                          | 0                            | 1                                                                      | 1                         | 0,92                                  |
| Exploitant 5                              | 1999         | 117                          | 91                  | 19                              | 100                      | 0                           | 0                            | 1                                                                      | 1                         | 0,38                                  |
| Exploitant 6                              | 1999         | 98                           | 100                 | 23                              | 88                       | 12                          | 0                            | 1                                                                      | 1                         | 0,71                                  |
| Exploitant 7                              | 1998         | 109                          | 100                 | 21                              | 87                       | 13                          | 0                            | 1                                                                      | 1                         | 0,33                                  |
| Exploitant 8                              | 1998         | 93,5                         | 100                 | 18                              | 100                      | 0                           | 0                            | 0                                                                      | 1                         | 0,72                                  |
| Exploitant 9                              | 1999         | 104                          | 100                 | 19                              | 100                      | 0                           | 0                            | 0                                                                      | 1                         | 0,35                                  |
| Exploitant 10                             | 2000         | 145                          | 100                 | 17                              | 100                      | 0                           | 0                            | 0                                                                      | 1                         | 0,57                                  |
| <b>Agriculteurs privés</b>                |              |                              |                     |                                 |                          |                             |                              |                                                                        |                           |                                       |
| Exploitant 11                             | 1998         | 135                          | 100                 | 17                              | 100                      | 0                           | 0                            | 0                                                                      | 0                         | 0,62                                  |
| Exploitant 12                             | 2000         | 235                          | 100                 | 17                              | 50                       | 0                           | 0                            | 0                                                                      | 0                         | 0,52                                  |
| Exploitant 13                             | 2003         | 250                          | 89                  | 17                              | 0                        | 0                           | 0                            | 0                                                                      | 1                         | 0,55                                  |
| Exploitant 14                             | 1998         | 400                          | 84                  | 20                              | 100                      | 0                           | 0                            | 0                                                                      | 0                         | 0,22                                  |
| Exploitant 15                             | 2004         | 1500                         | 100                 | 17                              | 10                       | 0                           | 0                            | 0                                                                      | 0                         | 0,40                                  |
| Exploitant 16                             | 1997         | 200                          | 50                  | 31                              | 44,5                     | 55,5                        | 0                            | 1                                                                      | 0                         | 0,54                                  |
| <b>Agro-combinats</b>                     |              |                              |                     |                                 |                          |                             |                              |                                                                        |                           |                                       |
| Agro-combinat 1                           | 2004         | 7209                         | 39                  | 23                              | 91                       | 9                           | 1                            | 0                                                                      | 1                         | 0,65                                  |
| Agro-combinat 2                           | 2004         | 2784                         | 69                  | 17                              | 50                       | 0                           | 0                            | 1                                                                      | 0                         | 0,32                                  |
| Agro-combinat 3                           | 2004         | 17125                        | 5                   | 17                              | 100                      | 0                           | 1                            | 0                                                                      | 1                         | 0,68                                  |
| Agro-combinat 4                           | 2005         | 12150                        | 81                  | 17                              | 100                      | 0                           | 1                            | 0                                                                      | 0                         | 0,36                                  |

Tableau 2: Résultats économiques<sup>2</sup>

| Indicateurs Economiques (Valeurs moyennes des campagnes 2006/07, 07/08 et 08/09) | Prix de vente moyen / kg Olive à huile | Coût moyen de production / Kg Olive à Huile | Résultat moyen/ kg Olive à Huile | Prix e Vente Moyen / Kg Huile d'Olive | Coût moyen de production / Kg Huile d'Olive | Résultat moyen / kg Huile d'olive |
|----------------------------------------------------------------------------------|----------------------------------------|---------------------------------------------|----------------------------------|---------------------------------------|---------------------------------------------|-----------------------------------|
| <b>Société agriculteurs</b>                                                      |                                        |                                             |                                  |                                       |                                             |                                   |
| Exploitant 1                                                                     | 1,008                                  | 0,584                                       | 0,424                            | ***                                   | ***                                         | ***                               |
| Exploitant 2                                                                     | 0,927                                  | 0,356                                       | 0,572                            | ***                                   | ***                                         | ***                               |
| Exploitant 3                                                                     | ***                                    | 0,347                                       | ***                              | 4,756                                 | 2,296                                       | 2,460                             |
| Exploitant 4                                                                     | ***                                    | 0,572                                       | ***                              | 4,849                                 | 2,536                                       | 2,313                             |
| Exploitant 5                                                                     | ***                                    | 0,586                                       | ***                              | 4,141                                 | 3,034                                       | 1,107                             |
| Exploitant 6                                                                     | ***                                    | 0,522                                       | ***                              | 4,533                                 | 2,783                                       | 1,750                             |
| Exploitant 7                                                                     | ***                                    | 0,815                                       | ***                              | 4,318                                 | 4,128                                       | 0,189                             |
| Exploitant 8                                                                     | 0,910                                  | 0,858                                       | 0,052                            |                                       |                                             |                                   |
| Exploitant 9                                                                     | ***                                    | 0,636                                       | ***                              | 3,785                                 | 3,375                                       | 0,410                             |
| Exploitant 10                                                                    | ***                                    | 0,440                                       | ***                              | 4,392                                 | 2,305                                       | 2,086                             |
| <b>Agriculteurs Privés</b>                                                       |                                        |                                             |                                  |                                       |                                             |                                   |
| Exploitant 11                                                                    | ***                                    | 0,540                                       | ***                              | 4,028                                 | 2,573                                       | 1,455                             |
| Exploitant 12                                                                    | ***                                    | 0,700                                       | ***                              | 4,584                                 | 2,798                                       | 1,786                             |
| Exploitant 13                                                                    | ***                                    | 0,551                                       | ***                              | 4,049                                 | 2,503                                       | 2,211                             |
| Exploitant 14                                                                    | ***                                    | 0,775                                       | ***                              | 3,903                                 | 2,982                                       | 1,871                             |
| Exploitant 15                                                                    | ***                                    | 0,391                                       | ***                              | 5,812                                 | 3,113                                       | 2,699                             |
| Exploitant 16                                                                    | 1,170                                  | 0,619                                       | 0,551                            |                                       |                                             |                                   |
| <b>Agro-combinats</b>                                                            |                                        |                                             |                                  |                                       |                                             |                                   |
| Agro-combinat 1                                                                  | ***                                    | 0,4207                                      | ***                              | 4,140                                 | 2,245                                       | 1,898                             |
| Agro-combinat 3                                                                  | ***                                    | 0,267                                       | ***                              | 4,129                                 | 1,240                                       | 2,889                             |
| Agro-combinat 4                                                                  | ***                                    | 0,386                                       | ***                              | 3,500                                 | 2,194                                       | 1,381                             |

<sup>2</sup> Pour manque d'information on n'a pas prix en considération les résultats économiques de l'Agro-combinat 2.

## 5. Conclusions

Cette communication a pour objectif d'étudier la rentabilité économique au niveau du secteur oléicole biologique partant de l'hypothèse qu'il est environnementalement viable. Les différentes structures de production qui régies le secteur ainsi qu'aux organismes intervenants ont été pris en considération.

A partir des résultats obtenus, on peut conclure que le secteur oléicole biologique malgré quelques difficultés observées surtout au niveau de la commercialisation des produits, c'est un secteur prépondérant. Les agriculteurs présentent des attitudes positives, d'une part une bonne relation avec les organismes d'encadrement, d'autre part une tendance innovatrice, évolutionniste (volonté de contrôle de toute la chaîne, recherche de clients à l'étranger, des projets de conditionnement en cours, etc.).

Au niveau des structures de production, une compétitivité prix était enregistrée par les agriculteurs ayant opté par une forme d'intégration et une compétitivité coût par les fermes étatiques. Cette compétitivité coût de production engendre des ventes à des prix moins élevés que le reste des producteurs, ce qui peut provoquer une situation de compétition déloyale au niveau du marché.

Finalement, à travers cette simple lecture du secteur oléicole biologique dans la région de Sfax, on peut affirmer la nécessité d'une intégration horizontale et verticale au niveau du secteur afin de pouvoir évoluer vers des structures de production plus compétitives.

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# Posters

## **Thème 1**

**Patrimoine oléicole méditerranéen :**

**Biodiversité, potentialités et perspectives de préservation  
et d'amélioration**



## **Caractérisation morphologique et agronomique *in situ* des oliviers sauvages (*Olea europaea* subsp. *europaea* var. *sylvestris*)**

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### **Résumé**

L'évaluation *in situ* de la variabilité morphologique et agronomique a été effectuée sur 48 génotypes sauvages échantillonnés dans 4 localisations de deux provinces en Andalousie (Cadix et Jaen) au Sud de l'Espagne. Les résultats préliminaires indiquent un haut degré de la variabilité pour tous les caractères évalués. On a observé une forte corrélation entre les mesures morphologiques ( $r : 0.56-0.81$ ) et agronomiques  $0.50 < r < 0.78$ ) effectuées dans deux campagnes (2007-2008 ; 2008-2009). Les niveaux les plus élevés et significatifs de la variabilité, se trouve dans chaque localisation qu'entre eux. Seulement trois caractères, la largeur des feuilles, la longueur du fruit et la proportion longueur/largeur de l'endocarpe ont montré des différences significatives entre les localisation. Ces résultats indiquent la richesse génétique d'oliviers sauvages en Andalousie.

Mots clés: Richesse génétique, Oliviers, Andalousie, caractères de variabilité.

## ***In situ* morphological and agronomical characterization of wild olives (*Olea europaea* subsp. *europaea* var. *sylvestris*)**

### **Abstract**

*In situ* evaluation of morphological and agronomical variability was carried out on 48 wild genotypes from 4 different sites from two provinces of Andalusia (Cadiz and Jaen), Southern Spain. Preliminary results indicate high levels of variability for all the characters evaluated and highlight the differences with cultivated olive. High correlation values were observed between morphological ( $r: 0.56-0.81$ ) and agronomical measures ( $r: 0.50-0.78$ ) taken in two different prospecting campaigns (2007-2008; 2008-2009). For all the characters under study higher and significant levels of variability were found within each site than between sites. And only three characters, leaf blade width, fruit length and stone length/width ratio showed significant differences between sites. These results indicate the richness of wild olive genetic resources in Andalusia.

**Key words:** olive characters, leaf blade width, fruit length and stone length/width ratio, agronomical variability, Andalusia.

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## Expression of genes involved in water transport and photosynthesis in leaves of olive plants subjected to drought stress

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### Abstract

Olive tree (*Olea europaea* L.) is a typical Mediterranean species adapted to drought seasons. To understand some of the molecular mechanisms of drought resistance in olive we studied the expression of three genes involved in water transport and photosynthesis: *OePIP2.1*, coding for an olive aquaporin, carbonic anhydrase and Rubisco. Plants were subjected to drought treatment and the twig water potential was monitored regularly; the expression levels of the genes were determined by real-time PCR analysis. The drought treatment resulted in diminished transcript levels for *OePIP2.1* and carbonic anhydrase, and such a down-regulation showed a similar trend for both genes. By contrast, Rubisco expression was not influenced by the water potential decline, neither at the strongest stress stage.

**Keywords:** *Olea europaea* L., water stress, aquaporin, carbonic anhydrase, Rubisco.

### Résumé

L'olivier (*Olea europaea* L.) est une espèce typique du bassin méditerranéen qui est bien adapté aux saisons sèches. Avec le but de comprendre les mécanismes moléculaires de résistance à la sécheresse de cette plante, nous avons étudié l'expression de trois gènes de l'olivier impliqués dans le transport hydrique et la photosynthèse: *OePIP2.1*, qui encode pour une aquaporine, anhydrase carbonique et RubisCO. Après avoir subi un période de sécheresse, le potentiel hydraulique des plantes a été surveillé et le niveau d'expression des gènes a été établi par PCR real-time. Le niveau d'expression des gènes *OePIP2.1* et anhydrase carbonique a réduit dans les plantes qui avaient subi la sécheresse; au contraire le niveau de transcription du gène RubisCO n'a pas été influencé par la réduction du potentiel hydraulique, aux stades plus forts de sécheresse non plus.

**Mot-clés:** *Olea europaea* L., sécheresse, aquaporine, anhydrase carbonique, RubisCO.

## Caractéristiques de juvénilité et végétativité dans dix variétés d'oliviers

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### Résumé

Les caractéristiques de juvénilité et végétativité sont souvent confondues. Les deux phases peuvent être présentes à même temps sur les oliviers adultes, mais elles représentent deux phases physiologiques différentes dans la vie de l'arbre. La juvénilité est une phase de longue durée, où l'arbre n'est pas fertile, caractéristique des jeunes plantes issues des graines, tandis que la phase de croissance végétative représente une courte période de croissance (un à trois ans) d'une pousse, qui précède la phase reproductive. La juvénilité se limite, sur des arbres adultes, à la partie inférieure et intérieure de la frondaison et en général on retrouve les caractéristiques typiques de cette phase sur les gourmands adventifs produits à la suite d'une taille sévère. Pour ce travail expérimental, des pousses, avec des caractéristiques différentes et situées dans des positions différentes sur l'arbre, ont été collectées sur des oliviers adultes, pour évaluer l'attitude à la juvénilité de dix variétés très répandues. Une classification des caractéristiques de juvénilité et végétativité a aussi été faite selon la position des pousses sur l'arbre. Cette approche permet d'avoir des informations sur le comportement naturel des variétés étudiées et sur leur possible rajeunissement pendant la multiplication in vitro.

**Mots clefs:** Phase physiologique, juvénilité, maturité, reproduction, pousse.

## Juvenile and vegetative characteristics in ten olive varieties

### Abstract

Juvenility and vegetative growth characteristics are often confused. Both may be present at the same time in adult olives, but they represent two well differentiated physiological stages in the life of the plant. Juvenility is a not-fertile and long-lasting stage in young olive trees coming from seed, while vegetative growth phase represents a relatively short growing period (one to three years) of a shoot preceding the reproduction. Juvenility in mature olives is limited to the inner-lower portion of the canopy and generally its characters appear in adventitious watersprouts induced by severe pruning. In the present work, different shoots were collected from diverse portions of the canopy of mature olives to investigate the juvenile attitude of ten widespread varieties. A classification of the juvenile and vegetative characteristics was performed also according to the position in the tree. This approach gives interesting information about the natural behaviour of the studied varieties and their possible rejuvenation when multiplied by in vitro culture.

**Key words:** Physiological stage, juvenility, maturity, reproduction, shoot.

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## Performances agronomiques d'hybrides de la variété d'olive à huile Chemlali Sfax

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### Résumé

Dans le cadre de l'amélioration génétique de l'olivier, des croisements dirigés ont été effectués pour la variété Chemlali Sfax en 1993 pour améliorer sa composition acide déséquilibrée. Une présélection d'hybrides a été effectuée en 2005 sur la base de la teneur en acide oléique (>65%). Ces hybrides sont en cours de caractérisation agronomique. Ce travail consiste à caractériser ces hybrides répartis en deux sites dans la région de Sfax sur le plan agronomique durant deux années. Le suivi a concerné la phénologie florale, le taux de floraison, le nombre de fleurs par inflorescence, la longueur de l'entre-nœud, le poids du fruit, le rapport pulpe/noyau et la production en olives. La variabilité constatée met en évidence l'effet du croisement et permettra ainsi d'effectuer une sélection efficace.

**Mots clés:** Olivier, hybridation, performance, variabilité, sélection, acide oléique.

## Agronomic characterization of Chemlali Sfax to improve the composition in fatty acids

### Abstract

In the framework of the olive genetic improvement, directed crosses were carried out for the variety Chemlali Sfax in 1993 to improve its composition in fatty acids. A preliminary selection of hybrids was carried out in 2005 on the basis of the oleic acid rate (>65%). These hybrids are now in stage of agronomic characterization. This work aims to characterize these hybrids located in two sites in the region of Sfax on the agronomic plan during two years. The follow-up concerned the floral phenology, the flowering rate, the number of flowers by inflorescence, the internode length, the fruit weight, the ratio pit/kernel and the olive production. The observed variability makes obvious the effect of the cross and will allow us to carry out an effective selection.

**Key words:** Olive genetic improvement, Chemlali, oleic acids, agronomic characterization.

### 1. Introduction

Aujourd'hui, le patrimoine oléicole tunisien est estimé à plus de 65 millions d'arbres qui couvrent une superficie de 1,7 millions d'hectares, soit le tiers des terres labourables (Kheireddine, 2005). La Tunisie occupe ainsi la quatrième position au niveau mondial en nombre d'arbres et le second en superficie (Kheireddine, 2005).

Au niveau du marché oléicole mondial, on assiste à une orientation stricte vers la qualité de l'huile et l'émergence de nouveaux pays oléicoles. La compétitivité est ainsi de plus en plus acharnée sur le plan de la production en olives et la qualité de l'huile.

La production de la Tunisie en huile d'olives connaît des fluctuations énormes qui varient de 35.000 tonnes en 2001 à 280.000 tonnes en 2004 (Kheireddine, 2005). Elle est marquée par la dominance de deux variétés qui sont Chemlali Sfax dans le centre et le sud et Chétoui dans le nord (Trigui, 1996). La variété à huile la plus cultivée en Tunisie Chemlali Sfax se caractérise par sa vigueur, son adaptation à différents environnements et sa productivité (Trigui et Msallem, 2002). Cependant, elle connaît des problèmes au niveau de la composition acide de son huile : taux d'acide oléique faible et celui d'acide palmitique élevé (Grati-Kamoun et Khlif, 2001).

Un programme d'amélioration génétique par croisements dirigés a été initié en 1993 et qui a pour objectif l'obtention des nouveaux génotypes performants au niveau de la composition acide tout en préservant les bonnes qualités de cette variété (Trigui et Msallem, 2001).

Les hybrides obtenus de ce travail d'hybridation sont plantés en collection dans la région de Sfax depuis 1997 dans deux sites, la station de l'Institut de « Taous » et le siège de l'Institut (I.O, 2000). Une sélection préliminaire d'une centaine d'hybrides sur la base de leur composition en acide oléique (> 65 %) a été opérée (I.O, 2005). Un travail de caractérisation de ces hybrides est entamé depuis 2008 pour une sélection finale.

Dans cet article, on se propose de présenter les performances agronomiques de 71 hybrides relatives à la morphologie de l'arbre, la biologie florale et la production.

## 2. Matériel et méthodes

Le matériel végétal de cette étude se compose de 71 hybrides présélectionnés et qui se répartissent entre deux sites dans la région de Sfax, à savoir le siège de l'Institut de l'Olivier (IOS, 24 hybrides) et la station Taous (Taous, 47 hybrides). A chaque année, on a repéré 4 pousses âgées d'un an au démarrage des bourgeons (début mars) répartis sur la frondaison de l'arbre et on a noté le nombre bourgeons et la longueur.

Le suivi agronomique de ces hybrides a concerné les paramètres suivants :

- \* Le port de l'arbre noté à la phase de maturation de l'année 2008 et qui peut être étalé, érigé ou retombant (Trigui et Msallem, 2002)

- \* La production en olives en kg estimée en novembre des années 2007 et 2008.

- \* Le taux de floraison pris pour chacune des 4 pousses par la formule : (nombre d'inflorescences/nombre de bourgeons)\*100. La valeur de l'hybride est la moyenne des pousses.

- \* Le nombre de fleurs par inflorescence est la moyenne de celui de 20 inflorescences prises de chaque hybride au stade bouton blanc (juste avant le début de floraison).

La comparaison des moyennes est faite par le test de comparaison de deux moyennes à variances inégales et au seuil de 5 % sur Microsoft Excel. Chaque moyenne est accompagnée par l'écart type qui représente la variabilité intra variétale. Cette variabilité est appréciée aussi par le coefficient de variation (%) :  $CV = (\text{écartype}/\text{moyenne}) \times 100$

## 3. Résultats et discussions

### 3.1. Port de l'arbre

Le port des hybrides au siège est à dominance partagée des types érigé et étalé avec 44 et 48 % (Fig 1). A Taous, le port étalé est le plus dominant avec 34 hybrides (72 %). Le port retombant est faiblement représenté dans les deux sites avec au total 4 hybrides (12 %). Le type étalé est dominant et représente le port du parent Chemlali Sfax rapporté dans la littérature. L'apparition du port érigé peut s'expliquer par la réponse de certains hybrides à l'effet de la densité de plantation hyperintensive. Ces hybrides peuvent être étudiés pour leur adaptation à ce mode de conduite.

### 3.2. Production en olives

Au siège, la production moyenne des hybrides est plus élevée en 2008 (8 kg) et plus variable avec un écartype de 12 et un coefficient de variation de 120 % (Fig 2). A Taous, ce paramètre est au contraire plus élevé et plus variable en 2007. La moyenne sur deux années est plus élevée et plus variable chez les hybrides du siège. La variabilité entre les hybrides dans les deux sites fait ressortir un effet génétique qui fait intervenir le type de croisements et la ségrégation entre les hybrides. La variabilité entre les années fait apparaître plutôt un effet d'alternance et une sélection au niveau du seuil de ce facteur permettra de retenir des hybrides moins alternants dans leur production en olives.

### 3.3. Biologie florale

Le taux de floraison est toujours plus élevé en 2008 pour les deux sites (Fig 3). En effet, il est de 42 % au siège contre 58 % à Taous. La variabilité entre les hybrides est toujours assez large avec un écartype de 21 à 41 et un coefficient de variation pouvant atteindre 100 %. Cette variabilité peut permettre une sélection efficace d'hybrides ayant en moyenne un taux élevé et une alternance de floraison plus réduite surtout si ce paramètre est significativement lié à la production en olives.

Le nombre de fleurs par inflorescence ne varie pas de façon significative entre les années pour chaque site (Fig 4). En effet, ce paramètre oscille entre 16 et 18 % pour les hybrides du siège et entre 14 et 17 % pour ceux de Taous. La variabilité entre les hybrides est assez faible avec un écartype de 3 à 5 et un coefficient de variation de 35 % au maximum. La collection des hybrides étudiés conserve un nombre de fleurs par inflorescence presque constant qui rappelle sûrement la performance du parent commun Chemlali Sfax. La sélection au niveau de ce paramètre paraît être ainsi aisée et efficace.

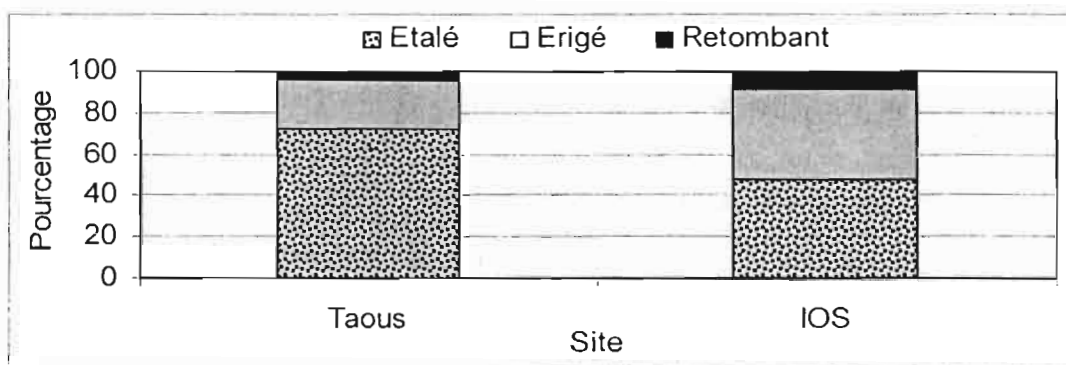


Figure 1: Répartition des hybrides (%) des sites du siège et de Taous selon le port de l'arbre.

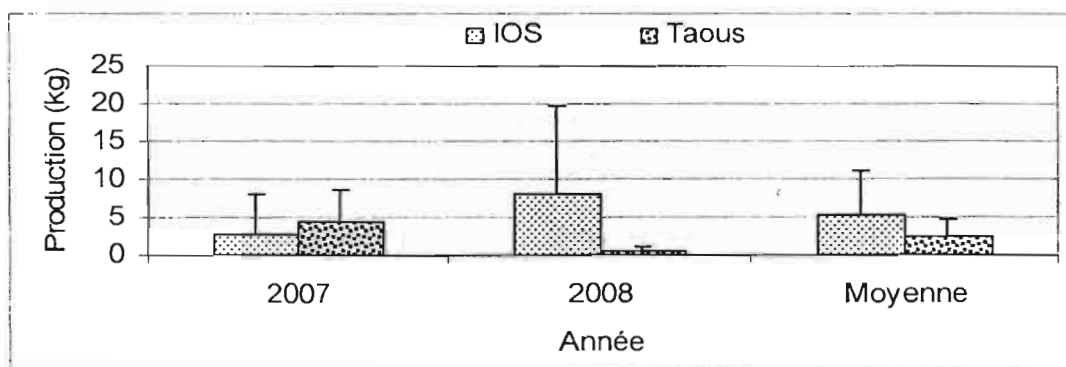


Figure 2: Production en olives (kg) des hybrides des sites du siège et de Taous en 2007 et 2008 et moyenne des deux années (barre verticale : écartype).

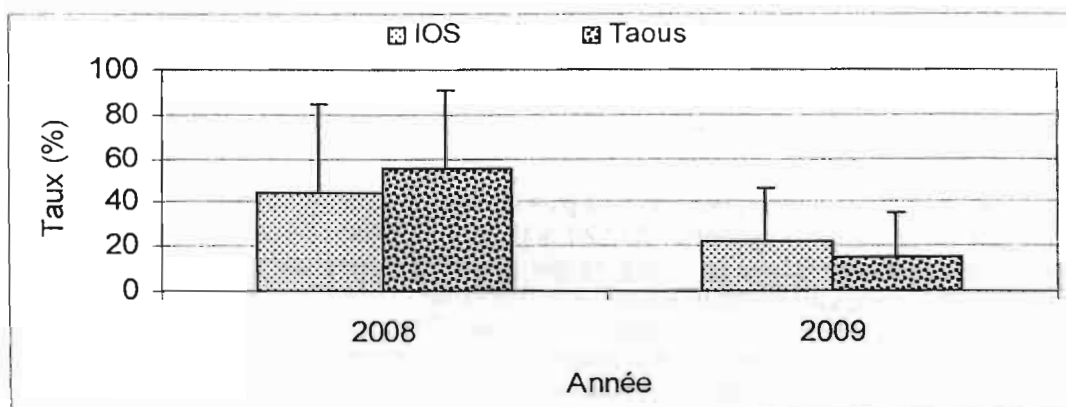


Figure 3: Taux de floraison des hybrides (%) des sites du siège et de Taous en 2008 et 2009 (barre verticale : écartype).

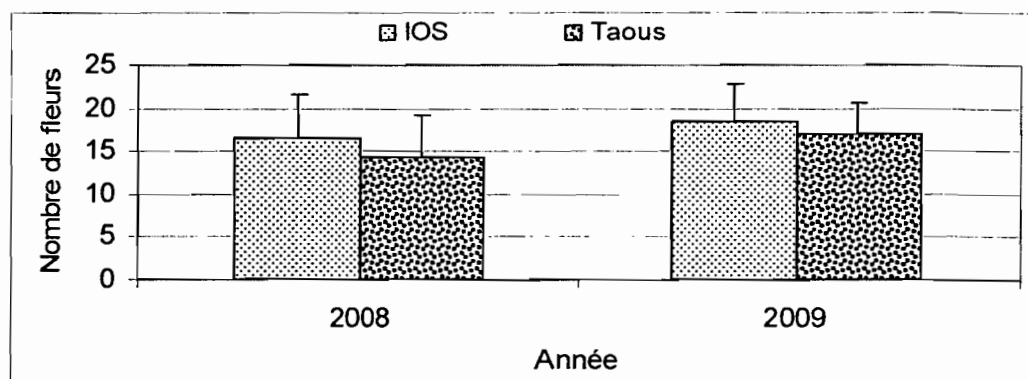


**Figure 4:** Nombre de fleurs par inflorescence des hybrides (%) des sites du siège et de Taous en 2008 et 2009 (barre verticale : écartype)

#### 4. Conclusion

L'étude agronomique d'hybrides de la variété Chemlali Sfax fait apparaître une variabilité assez large dans les deux sites dans la région de Sfax. Une sélection efficace peut être entreprise au sein de ces hybrides au niveau des paramètres de productivité et de floraison. Cette sélection doit tenir compte des facteurs de l'environnement et surtout l'effet année qui témoigne de l'alternance. Ainsi, on doit poursuivre l'étude agronomique sur plusieurs années et différents sites pour pouvoir déterminer les performances de chaque hybride et sélectionner des hybrides bien adaptés.

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## Etude de la répartition quantitative des phénols totaux dans l'olivier en fonction des organes et leur degré de maturité

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### Résumé

L'effet bénéfique de l'huile d'olive sur la santé humaine est attribué, entre autres, à sa teneur en composés phénoliques. L'intérêt croissant aux composés phénoliques de l'huile d'olive est étendu à tous les produits de l'olivier qui peuvent être consommés comme aliments (olive de table, pâte d'olive) ou qui sont engendrés comme sous-produit de l'industrie oléicole (margine, grignon, feuille, bois). Ce travail vise l'étude de la distribution quantitative en phénols totaux dans les différents organes de l'olivier (pulpe d'olive, noyau d'olive, feuilles et rameau). Les pourcentages de répartition de la concentration en phénols totaux dans les feuilles, le rameau, la pulpe et le noyau d'olive sont respectivement 35%, 26%, 25% et 14%. Ce résultat montre un élargissement des sources potentielles de composés phénoliques.

**Mots clés:** Olive ; feuilles ; noyaux ; rameau ; phénols totaux.

### Abstract

An increasing interest in phenolic compounds is due to their antioxidant and health enhancing properties.

Phenolic compounds contents in the different olive tree parts (leaves, stone, branch) weren't negligible comparing to that of olive pulp, which has been taken as reference plant material.

All olive tree parts (leaves, stone, branch and olive pulp) could therefore serve as a promising, cheap source of natural antioxidants, like other by-products from the olive oil agroindustry.

**Key words:** Olives ; feuilles ; noyaux ; rameaux ; composés

### 1. Introduction

L'effet bénéfique de l'huile d'olive sur la santé humaine est attribué, entre autres, à sa teneur en composés phénoliques. Ces derniers ne cessent de prendre une importance croissante à cause de leurs vertus sur la santé. Leur pouvoir antioxydant naturel suscite de plus en plus d'intérêt pour la prévention et le traitement du cancer, des maladies inflammatoires et cardiovasculaires. Ils sont également utilisés comme additifs pour l'industrie agroalimentaire, pharmaceutique et cosmétique.

L'intérêt croissant aux composés phénoliques de l'huile d'olive a été renvoyé au fruit (olives) (Romero *et al.*, 2004) et aussi à ces dérivés (margines, grignons, pâtes d'olive, olives fermentées, poudre d'olive) (De Marco *et al.*, 2007 ; Winkelhausen *et al.*, 2005).

Ce travail vise à quantifier les composés phénoliques dans différents tissus de l'olivier avec l'objectif d'évaluer la potentialité des différents organes d'olivier comme sources de composés phénoliques.

### 2. Matériel et Méthodes

Les échantillons de feuilles, d'olives et rameau ont été collectés fin novembre sur un olivier de variété française cultivé à la Faculté des Sciences et Technique à Marseille (Sud France). Après lyophilisation, la pulpe d'olive a été séparée du noyau.

Les échantillons déshydratés ont été broyés afin d'obtenir des échantillons homogènes de pâte de pulpe d'olive et des échantillons en poudre fine pour les feuilles, le rameau et les noyaux d'olives.

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Dans un objectif de faire une extraction des composés phénoliques, une masse de 1 g d'échantillon lyophilisé et broyé est macérée dans un solvant (méthanol ou éthanol) pendant 48 h à  $-20^{\circ}\text{C}$ . L'extrait est récupéré après centrifugation 15 min à 4500 rpm.

Des dosages des phénols totaux (Skerget et al., 2005) et des *Ortho*-di-phénols (Blekas et, 2002) ont été réalisés sur les différents extraits. Une analyse de la variance (ANOVA) des résultats obtenus est réalisée par le logiciel Microsoft® Office Excel 2003.

### 3. Résultats et Discussion

Dans ce travail, nous nous intéressons aux composés phénoliques des différents organes d'olivier et en particulier aux *Ortho*-di-phénols. Ces derniers sont les plus actifs antioxydants parmi les composés phénoliques d'olive.

Les teneurs en phénols totaux varient de 12,5 à 59 mg/g Matière Sèche (Tableau 1) selon l'organe concerné (feuille, pulpe, noyau ou rameau), selon le degré de maturité (3 niveaux) et selon le solvant d'extraction utilisé (méthanol ou éthanol).

En prenant comme référence les teneurs en phénols totaux des extraits de pulpe d'olive (de 31 à 59 mg/g Matière Sèche), les autres organes d'olivier contiennent des teneurs non négligeables (de 12,5 à 51 mg/g Matière Sèche) quelque soit le solvant d'extraction utilisé.

D'après le Tableau 1, l'éthanol possède des rendements d'extraction des phénols totaux plus importantes que le méthanol (une différence significative), et ceci indépendamment des organes étudiés et de leur degré de maturité.

La teneur en phénols totaux est influencée par le degré de maturité des feuilles d'olivier - comme cela a été signalé par Fabbri et al. (comm. pers., 2008), et de celui de pulpe d'olive - résultats en concordance avec les conclusions de Briante et al. (2002) et de Malik & Bradford, (2006). Cependant, la concentration en phénols totaux dans les extraits à partir des noyaux d'olive est significativement indépendante de leur degré de maturité.

**Tableau 1.** Teneurs en phénols totaux dans des extraits phénoliques à partir des différents organes d'olivier de différents degrés de maturité, en utilisant le méthanol ou l'éthanol comme solvant d'extraction.

| Echantillons            | [phénols] (mg/g) MS |                    | p-value         |
|-------------------------|---------------------|--------------------|-----------------|
|                         | Extrait méthanol 2% | Extrait éthanol 2% |                 |
| Feuille Age 1           | 28,04 $\pm$ 1,80    | 29,34 $\pm$ 3,31   | <b>0,5817</b>   |
| Feuille Age 2           | 29,57 $\pm$ 0,92    | 33,84 $\pm$ 0,61   | <b>0,0026 *</b> |
| Feuille Age 3           | 37,17 $\pm$ 1,28    | 51,56 $\pm$ 2,94   | <b>0,0014 *</b> |
| <b>p-value</b>          | <b>0,0004 *</b>     | <b>0,0000 *</b>    |                 |
| Pulpe olive verte 1     | 38,98 $\pm$ 2,11    | 59,05 $\pm$ 5,86   | <b>0,0050 *</b> |
| Pulpe olive tournante 2 | 31,65 $\pm$ 2,00    | 50,35 $\pm$ 0,35   | <b>0,0000 *</b> |
| Pulpe olive rouge 3     | 32,24 $\pm$ 1,77    | 50,60 $\pm$ 2,38   | <b>0,0004 *</b> |
| <b>p-value</b>          | <b>0,0068 *</b>     | <b>0,0442</b>      |                 |
| Noyau olive verte1      | 14,13 $\pm$ 4,20    | 17,79 $\pm$ 0,35   | <b>0,2072</b>   |
| Noyau olive tournante2  | 16,51 $\pm$ 0,50    | 19,80 $\pm$ 0,91   | <b>0,0054 *</b> |
| Noyau olive rouge 3     | 12,57 $\pm$ 1,08    | 19,30 $\pm$ 0,93   | <b>0,0012 *</b> |
| <b>p-value</b>          | <b>0,2363</b>       | <b>0,0456</b>      |                 |
| Rameau (d< 2 mm)        | 24,77 $\pm$ 2,96    | 24,27 $\pm$ 0,90   | <b>0,7930</b>   |

L'indice (\*) indique une différence significative entre les échantillons à un niveau 99% (p-value < 0.01).

L'abondance des *Ortho*-di-phénols par rapport aux phénols totaux extraits a été analysée dans les différents extraits phénoliques à partir des différents organes d'olivier de différents degrés de maturité. Elle varie de 20% à 80%. Les extraits méthanoïques à partir des feuilles sont plus riches en *Ortho*-di-phénols (60% à 70%) que les extraits éthanoïques (36 % à 45%). Contrairement aux feuilles, l'extraction par éthanol à partir de pulpes et noyaux d'olive de différent degré de maturité permet

d'obtenir des extraits riches en *Ortho*-di-phénols (avec un pourcentage d'abondance de 50% à 80%), par comparaison aux extraits par méthanol (22% à 30%). Les résultats sur les pulpes d'olive sont semblables à ceux sur les noyaux. Quant au rameau, l'abondance des *Ortho*-di-phénols par rapport aux phénols totaux est indépendante du solvant d'extraction utilisé.

Les résultats de ce présent travail expliquent l'intérêt aux composés phénoliques étendu à tous les produits de l'olivier. Les composés phénoliques sont présents dans tous les organes étudiés d'olivier avec des teneurs non négligeables par comparaisons à celui des fruits.

Au cours de cette dernière décennie, énormément de travaux de caractérisation et valorisation des composés phénoliques sont réalisés sur les olives et leurs dérivés (pâte d'olive, huile d'olive, margine, margion, grignon) (Blekas et al., 2002 ; Briante et al. 2002 ; De Marco et al., 2007 ; Malik et Bradford, 2006 ; Romero et al., 2004 ; Winkelhausen et al. 2005) mais aussi sur les feuilles d'olivier (Fabbri et al., comm. pers., 2008 ; Japon-Lujan et al., 2006 ; Skerget et al., 2005). Beaucoup de brevets sont apparus proposant des applications et des procédés de valorisation des composés phénoliques des feuilles d'olivier, des olives et leurs dérivés.

Cependant, très peu de travaux s'articlent autour de composés phénoliques du rameau d'olivier (Japon-Lujan et al., 2006) et des noyaux d'olives (Ryan et al. 2002), malgré la richesse de ces tissus en composés phénoliques et malgré leurs abondances comme matière première. Les rameaux sont des produits de la taille d'olivier tandis que les noyaux d'oliviers sont des sous produits de l'industrie de transformation des olives (olives dénoyautés et fermentés, pâtes d'olive).

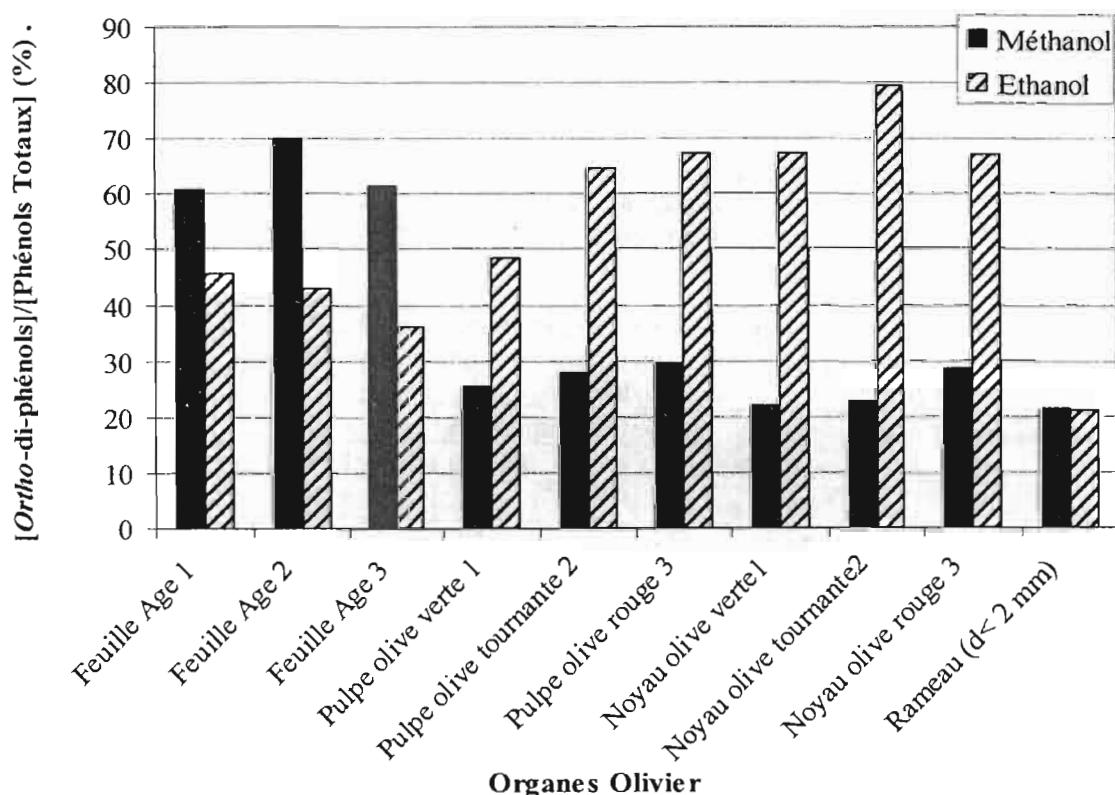


**Figure 1:** Proportions des *Ortho*-di-phénols par rapport aux phénols totaux dans des extraits phénoliques à partir des différents organes d'olivier de différents degrés de maturité, en utilisant le méthanol ou l'éthanol comme solvant d'extraction.

#### 4. Conclusions

Les teneurs en composés phénoliques dans les feuilles, noyaux d'olive et rameaux sont non négligeables par rapport à celui des pulpes d'olive (prises comme référence). Les différents organes d'olivier sont riches en *Ortho*-di-phénols, les plus actifs antioxydants parmi les composés phénoliques d'olives. D'après ces résultats, les différents organes d'olivier (feuilles, pulpe d'olive, noyaux d'olive et rameaux) pourraient être considérés comme sources potentielles intéressantes de composés phénoliques.

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## Utilisation des marqueurs moléculaires SSR pour l'identification variétale

Zaher Hayat, Belkassem Boulouha, Mohamed Baaziz, Spipada

### Résumé

La culture d'olivier (*Olea europaea*) au Maroc, représente 58% de la surface occupée par l'arboriculture nationale. Malgré l'importance de ce secteur, l'oléiculture marocaine ne répond pas encore aux attentes en matière de production et de qualité. L'amélioration de notre production oléicole ne peut être réalisée que par un travail de sélection clonale pour exploiter la diversité génétique existante au sein de notre verger et par l'amélioration génétique qui consiste à la création d'une variabilité génétique qui permettra la réalisation de progrès dans les performances et les potentialités de notre patrimoine oléicole. Toutefois, la sélection à l'intérieur de la picholine marocaine a généré des génotypes différents par un ou deux caractères morphologiques discriminants, cependant difficilement identifiables par les agriculteurs et les pépiniéristes pendant la phase juvénile, ce qui pose le problème de la confirmation de l'authenticité par les services de contrôle. D'où, la nécessité d'utiliser les marqueurs moléculaires pour l'identification du matériel sélectionné et résoudre le problème de son authenticité. D'un ensemble de marqueurs microsatellites (SSR) constitué de 30 paires d'amorces décrites antérieurement, dix ont été employées pour différencier 19 cultivars d'olivier (7 variétés méditerranéennes, 4 variétés autochtones et 7 clones sélectionnés à partir de la picholine marocaine). Quatre marqueurs SSR ont suffi pour différencier entre les clones sélectionnés par l'INRA Maroc (Haouzia, Menara, M26, S7, S8, M14 et M16) et la Picholine marocaine. Les résultats obtenus lors de ce travail constituent un point de départ vers des recherches plus spécifiques pour l'identification variétale chez l'olivier sur base d'empreintes génétiques.

**Mots clés:** *Olea europaea*, sélection clonale, identification variétale, microsatellites, empreintes génétiques.

### Abstract

In this work we report on the use of thirty previously developed olive microsatellite markers for the identification and differentiation of a set of nineteen olive varieties (7 Mediterranean varieties, 4 local varieties and 7 selected clones from the Picholine marocaine). 50% of analysed microsatellite markers revealed a high level of polymorphism. Fifty-one alleles were detected at nine loci analysed with an average number of 5.6 alleles per locus in the nineteen cultivars examined. A minimum number of four microsatellite markers was chosen for the rapid and unambiguous varietal identification of the selected clones (Haouzia, Menara, M26, S7, S8, M14 and M16) and the Picholine marocaine.

The described results come to support the results obtained by other researchers concerning the utility of the microsatellites for the identification of the species and the cultivars.

The data obtained can be used for the varietal survey and construction of a database of all olive varieties grown in Morocco providing also additional genetic information on the agronomic and quality characteristics of the olive varieties.

**Key words:** (*Olea europaea* L.), clonal selection, varietal identification, microsatellites.

## Reducing olive (*Olea europaea* L.) shotberries by cross pollination in years with unfavorable climate during anthesis

Georgios C. Koubouris<sup>1,2</sup>, Ioannis T. Metzidakis<sup>1</sup>, Miltiadis D. Vasilakakis<sup>2</sup>

### Abstract

In olive, seed development is important for fruit growth, pattern of flower and fruit abscission, and level of flowering during the following year. Olive shotberries are small, commonly seedless fruit expressing quantitative parthenocarpy and they either abscise before harvest or are of no commercial value. Consequently, extended incidence of shotberries has negative impact on fruit and oil yield and can seriously affect economic performance of olive orchards. Aim of this paper was to determine impact of self-, cross- and free-pollination on shotberry production of olive cultivars 'Koroneiki' and 'Mastoidis' in relation to climatic factors. Field observations and experiments were carried out, over a 3-year period (2005-2007), in the Institute of Olive Trees and Subtropical Plants, N.A.G.R.E.F., Chania – Greece. The percent of shotberries/flowers was recorded after: 1) self pollination, 2) cross-pollination 3) free multi-cultivar pollination. Significant differences were recorded between treatments, cultivars and years. Lowest values were observed in free-pollination and highest percentages were recorded in self-pollination for both cultivars especially in years with low temperature incidents during flowering. Higher overall incidence of shotberries observed in 2007 could be related to climatic factors such as low temperature during anthesis. Fresh weight of shotberries was significantly lower than that of normal fruit. Significant variation observed in shotberry fresh weight in the present study indicates different levels of stimulus expression even in flowers of the same inflorescence though more research is necessary in that field. Shotberry size was also markedly lower. Our results indicate that parthenocarpic fruit have competitive disadvantage in accumulation of assimilates. 'Koroneiki' and 'Mastoidis' were reciprocally the most effective pollinator varieties in reducing shotberries. In practice, planting trees of a compatible pollinator variety in selected positions of an olive orchard is expected to contribute to normal fruit development and diminish shotberries, especially in years with unfavorable climate.

**Key words:** Olive shotberries, pollination, 'Koroneiki' and 'Mastoidis', climatic factors.

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## Effects of medium physical status and darkening on *in vitro* micropropagation of olive cultivars from Liguria (Italy)

Tania Bracci, Chiara Michelazzo, Luca Sebastiani

### Abstract

In order to improve olive *in vitro* micropropagation of autochthonous Ligurian olive cultivars, the medium culture physical status and the darkening effects were evaluated. Olive shoots were collected from previously established *in vitro* plants of Ligurian cultivars (Liccione, Mortina, Pignola). The experiments were carried out on liquid and solidified OMM medium culture. The darkening effect was obtained coating the bottom side (up to the medium upper level) of the culture vessel with black adhesive tape. Result showed that the physical status of medium significantly affected the number of nodes, which was higher in solid than in liquid culture. On the contrary fresh weight Relative Growth Rate of shoots was not influenced by this parameter but was positively affected by the darkening of medium, being highest on the darkened medium.

**Keywords:** *Olea europaea* L., Ligurian germplasm, micropropagation, Relative Growth Rate, coefficient of multiplication.

### Résumé

Les effets de l'état physique et de l'obscurcissement du milieu de culture ont été évalués afin d'améliorer la prolifération *in vitro* de l'olivier. Les microboutures d'olivier provenaient de plantes des trois cultivars (Liccione, Mortina, Pignola) de la région Liguria précédemment établies *in vitro*. Les essais ont été effectués sur un milieu de culture OMM liquide et solidifié. L'effet de l'obscurcissement a été obtenu couvrant avec une bande adhésive noire l'extérieur du vase de culture jusqu'à recouvrir le milieu. Les résultats ont montré un effet significatif de l'état physique du milieu de culture sur le nombre de nœuds qui a été plus grand sur le milieu solide que sur le liquide. Au contraire ce traitement n'a pas eu une incidence sur le *Relative Growth Rate* du poids frais des explants qui a été plutôt influencé par l'obscurcissement du milieu de culture. En effet ce paramètre a été plus haut sur le milieu noir.

**Mots clés:** *Olea europaea* L., germoplasme de la Liguria, micropropagation, *Relative Growth Rate*, coefficient de multiplication.

## Utilisation de marqueurs microsatellites pour la caractérisation de variétés d'olives tunisiennes.

M. Fendri, I. Trujillo, A. Trigui, M.I Rodríguez-García, J.D. Alché

### Résumé

Dans cette étude, un ensemble de 8 marqueurs microsatellites (SSRs) a été utilisé pour l'analyse de 84 individus appartenant à la banque de germoplasme de "Boughrara"- Sfax. De plus une observation morphologique des endocarpes des fruits a été réalisée afin de confirmer toutes les conclusions obtenues par l'analyse moléculaire. Le contenu en information polymorphique des marqueurs utilisés démontre leur utilité dans l'identification et la classification des variétés d'olives tunisiennes. Les moyennes d'hétérozigoties et le nombre total d'allèles détectés attestent de la grande variabilité présente dans la collection en question. Cependant, de nombreuses duplications ont été détectées dans cette collection. L'analyse factorielle a permis d'indiquer les tendances d'agroupement entre les génotypes identifiés suivant leurs origines géographiques et l'usage de leurs fruits.

**Mots clés:** Cultivars, Endocarpes, Identification, Microsatellite, Olive.

## Use of microsatellite markers for the characterization of Tunisian olive cultivars

### Abstract

In this work, 84 accessions belonging to "Boughrara"- Sfax germoplasm bank have been screened using a set of 8 selected microsatellite markers (SSRs). Moreover, morphological observation of fruit endocarps has been also performed in order to confirm all conclusions made by the molecular screening. Averages of the polymorphic information content demonstrated that the majority of the utilised markers are very useful for identification and classification of Tunisian olive cultivars. A high variability within the collection was shown by the elevated averages of heterozygosity indexes and the total number of alleles. However, several duplications were detected within the collection. Factorial analysis displayed the cluster tendencies among the identified genotypes using geographical origin and fruit uses as the criteria.

**Key words:** Cultivars, Endocarps, Identification, Microsatellite, Olive.

### 1. Introduction

L'olivier a été l'espèce cultivée par excellence dans le bassin méditerranéen depuis les temps les plus anciens jusqu'à nos jours. La contribution de la Tunisie dans le développement de l'oléiculture dans cet espace a été très importante. Aujourd'hui, il existe dans ce pays tout un patrimoine autour de cette espèce. L'étude du réservoir génétique constitue l'un des créneaux essentiels dans l'actualité ayant pour objectif de caractériser, conserver et améliorer les variétés d'oliviers autochtones, fruit d'un long processus d'adaptation environnementale. Les méthodes traditionnelles utilisées dans la classification des variétés se basent sur la caractérisation morphologique, agronomique et technologique. Des approches de ce type ont permis de cataloguer un nombre important de variétés tunisiennes et de donner une idée sur le niveau de diversité génétique dans cette espèce (Trigui et al., 2002). Cependant, ces méthodes restent insuffisantes pour aboutir à une caractérisation complète notamment dans les collections nationales et les banques de germoplasme. L'utilisation d'outils moléculaires se présente alors comme une solution fiable pour aboutir à une meilleure classification.

Plusieurs méthodes moléculaires ont été utilisées dans l'étude de l'olivier à savoir les isoenzymes (Ouazzani et al., 1996) et les marqueurs basés sur l'analyse de l'ADN. L'utilisation de ces derniers a débuté avec les RFLPs (Besnard et al., 2002) mais suite à l'apparition de la technique de la PCR, d'autres types de marqueurs ont été utilisés notamment les RAPDs (Belaj et al., 2001), les AFLPs (Grati kamoun et al., 2006) et les SSRs (Belaj et al., 2004; Rekik et al., 2008). Les microsatellites (SSRs) se considèrent encore comme l'une des techniques les plus performantes pour l'étude de la diversité génétique et la discrimination variétale dans l'olivier.

Dans ce travail, une analyse moléculaire par une série de marqueurs microsatellites utilisés avec succès dans diverses collections du monde notamment celles de la Banque Mondiale de Germoplasme de l'Olivier de Cordoue a été réalisée sur 84 accessions d'oliviers tunisiens. Cette analyse a permis de mettre en évidence le niveau de diversité génétique dans ce matériel végétal et aussi de donner une information complémentaire aux études agronomiques, morphologiques et technologiques réalisés sur les mêmes individus. Finalement, l'étude a permis aussi de caractériser les différentes tendances d'agroupement entre les génotypes identifiés.

## 2. Matériel et méthodes

L'étude a été réalisée sur 84 accessions appartenant à la collection du Conservatoire National de l'Olivier de « Boughrara » (Sfax) qui est le fruit d'un programme national de prospection. La majorité des accessions proviennent à l'origine d'oliveraies autochtones du sud tunisien. L'ADN a été obtenue à partir des feuilles des individus en question en utilisant un protocole d'extraction basé sur la méthode du CTAB (De la Rosa et al., 2002). Dans cette analyse 8 marqueurs microsatellites ont été utilisés (Sefc et al., 2000 ; Carriero et al., 2002 ; Cipriani et al., 2002).

Le protocole de PCR-SSRs utilisé dans ce travail a été décrit par (Belaj et al., 2004). La détection des allèles a été réalisée dans un séquenceur automatique ABI PRISM 3100 GENETIC ANALYSER (Applied Biosystems). Plusieurs outils bioinformatiques ont été utilisés pour visualiser les allèles, étudier les paramètres de variabilité, classer les individus et caractériser les relations génétiques (GENOTYPER 3.7, CERVUS 3.0, DARWIN 5.0.155)

## 3. Résultats et discussion

L'ensemble des 8 marqueurs a démontré une grande utilité dans la caractérisation des variétés d'oliviers dans la collection étudiée. Effectivement, ils ont présenté un grand niveau de polymorphisme dans ce matériel végétal comme le démontre l'exemple de la Figure 1. La moyenne du contenu en information polymorphique est égale à 0,64. Un nombre total de 64 allèles a été obtenu lors de l'analyse. Les valeurs observées d'hétérozygotie démontrent que 79% des individus identifiés sont des hétérozygotes. Etant des indicateurs du niveau de diversité génétique, ces données démontrent bien la grande variabilité présente dans le germoplasme tunisien représenté en partie par la collection de « Boughrara » (Sfax). Ces résultats apportent une confirmation non seulement pour les études morphologiques et agronomiques réalisées sur un certain nombre d'accessions de cette même collection, mais aussi pour plusieurs travaux antérieurs sur la diversité de l'oliveraie tunisienne d'une manière générale (Trigui et al., 2002 ; Taamai et al., 2006).

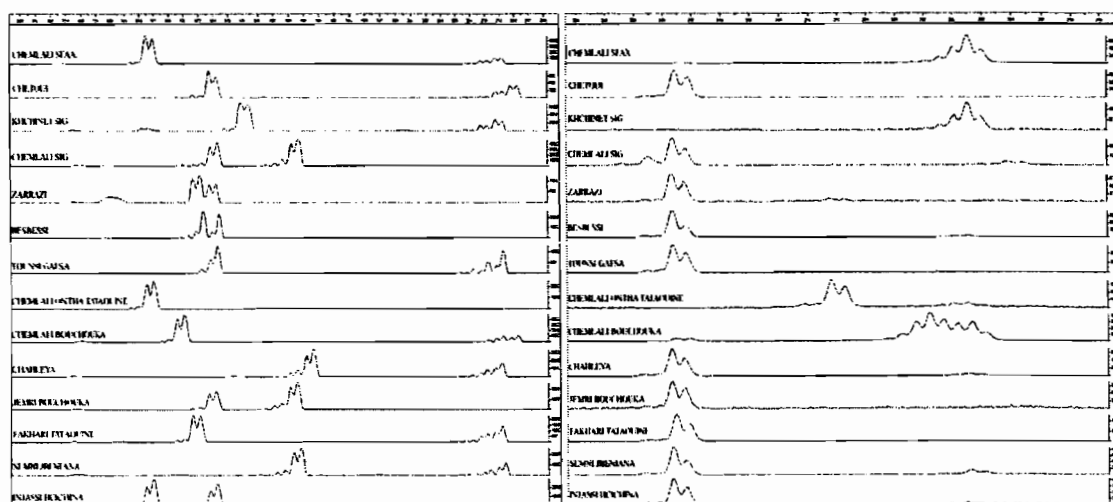


**Figure 1:** Exemples de détection d'allèles au niveau du marqueur le plus polymorphique UDO99-43 (à gauche) et du moins polymorphique ssrOeUA-DCA15 (à droite).



Entre les allèles détectés, il existe des allèles dominants dont la fréquence a atteint jusqu'à environ 62% dans certains marqueurs (comme l'allèle 261 du marqueur *ssrOeUA-DCA15*). Cependant, il existe aussi des allèles uniques présentés par un seul individu (comme l'allèle 177 du marqueur *UDO99-043*). Ces derniers permettent une identification rapide des géotypes correspondants lorsqu'on dispose d'une large base de données d'allèles détectés. Cette étude a démontré la présence de plusieurs cas de duplications dans la collection, un nombre important d'entre elles correspondent à la variété 'Chemlali Sfax'. Cela permet de donner un élément supplémentaire pour une meilleure gestion de la collection en question.

Une analyse factorielle de correspondance ayant pour objectif de caractériser les possibles groupements entre les différents géotypes identifiés a permis de mettre en évidence une tendance de séparation des géotypes traditionnellement destinés à l'élaboration d'olive de table par rapport aux cultivars utilisés pour l'extraction de l'huile ou à usage mixte.

### Remerciement

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## Mesure de la germination *in vitro* du pollen d'olivier en utilisant la cytométrie en flux

Rodríguez-García MI, Rejón JD, Alché JD, Castro AJ

### Résumé

Dans ce travail, nous décrivons un nouveau protocole pour évaluer le taux de germination *in vitro* du pollen d'olivier en utilisant la cytométrie en flux. Après la culture *in vitro* dans un milieu adéquat, les populations des pollens germiné et non germiné (10,000-15,000 par échantillon) ont été facilement séparés et comptés sur la base de leur taille dans 10-15 minutes. Nous étudions aussi l'effet de plusieurs paramètres tel que l'origine génétique (i.e. variété), ainsi que la température et le temps de stockage sur la germinabilité du pollen. Cette nouvelle méthode est plus rapide et fiable du point de vue statistique que la méthode classique de comptage dans le microscope optique. Le protocole peut être utile pour les améliorateurs afin d'évaluer la capacité de germination après le stockage du pollen utilisé pour la pollinisation dans les programmes d'amélioration végétale.

**Mots clés:** olivier, pollen, germinabilité, stockage, cytométrie en flux.

## Measurement of olive pollen *in vitro* germination rates using flow cytometry

### Abstract

In this work, we describe a new method for measuring pollen germination rates *in vitro* using flow cytometry. After germination in a suitable culture medium, populations of germinated and non-germinated pollen grains (about 10,000 per sample) were easily discriminated by size and counted in 10-15 minutes only. We also analyzed the effects of several parameters like the genetic origin (i.e. cultivar), and both the temperature and time of storage on olive pollen germinability. This new method proved to be faster and statistically more robust and reliable than traditional counting-based methods. The protocol could be useful for breeders to evaluate how extended storage might affect the germinability of the pollen batches, which are used for pollination in breeding programs.

**Key words:** olive, pollen, germinability, storage, flow cytometry.

### 1. Introduction

Dans le cadre des programmes d'amélioration de l'olivier, la viabilité et la capacité de germination du pollen des nouvelles variétés sélectionnées sont déterminées afin d'évaluer la performance du pollen (Ferri et al. 2008). En plus, ces deux paramètres sont indispensables pour le processus de sélection des meilleures variétés pollinisatrices.

La germination *in vitro* est la méthode classique pour déterminer la germinabilité du pollen. Plusieurs facteurs comme la température, le temps de stockage et l'humidité relative peuvent modifier la germinabilité du pollen de l'olivier (Pinney et Polito 1990 ; Ferri et al. 2008). Après la germination *in vitro*, un comptage des grains de pollen germés et non germés a été réalisé sous un microscope optique. Toutes les données obtenues ont été soumises à une analyse statistique.

Dans ce travail, nous décrivons une nouvelle méthode pour évaluer le taux de germination *in vitro* du pollen de l'olivier en utilisant la cytométrie en flux.

## 2. Matériel et Méthodes

### 2.1. Récolte du pollen

Le pollen de l'olivier (*Olea europaea* L. cv. Picual) a été obtenu pendant le printemps de l'année 2009. Les grains ont été récoltés par l'agitation des inflorescences à l'intérieur de sacs en papier.

### 2.2. Germination in vitro du pollen

Après la récolte, le pollen a été réhydraté pendant 60 min dans une boîte de Petri à 30°C avec une humidité relative de 80-85%. Ensuite, les échantillons (0.02 g) ont été transférés à un milieu de germination (M'rani-Alaoui 2000) et incubés à 25°C pendant 6 h à l'obscurité. Après la germination, le pollen a été fixé à l'aide d'une solution d'acide acétique et d'éthanol (3:1). Le comptage a été réalisé sur mille grains de pollen (200 grains x 5 répétitions indépendantes) sous un microscope optique. Le taux de germination du pollen a été calculé de la manière suivante: (nombre des grains du pollen germés/nombre total des grains du pollen) x100.

### 2.3. Cytométrie en flux

Le pollen fixé a été centrifugé et resuspendu dans 300 µl de PBS. Ensuite ils ont été analysés dans un cytomètre FACSCalibur (BD Biosciences, USA) en utilisant les paramètres FSC (le forward scatter ou lumière absorbée renseigne sur la taille de la particule) et SSC (le side scatter ou lumière diffusée renseigne sur la morphologie et la structure de la particule). Les données obtenues ont été analysées avec le logiciel CELLQUEST PRO (BD Biosciences). Le cytomètre a été calibré à l'aide du pollen hydraté comme marqueur de taille. Les grains de pollen possédant un tube pollinique  $\geq 3$  fois le diamètre du grain ont été considérés comme des grains de pollen germés (Pinney et Polito 1990). En total, cinquante mille grains de pollen (10.000 grains x 5 répétitions indépendantes) ont été analysés.

### 2.4. Analyses statistiques

Les données obtenues avec les deux méthodes ont été analysées par le test U de Mann-Whitney ( $p=0.05$ ).

## 3. Résultats et Discussion

La Figure 1 illustre le pollen de l'olivier réhydraté après sa culture dans le milieu de germination pendant 6 h à température ambiante. Les populations de pollen germé et non germé (10,000 grains/répétition) ont été facilement séparées et analysées dans le cytomètre en flux en se basant sur leur taille et leur forme en quelques minutes. Le comptage visuel sous le microscope démontre que le pollen de l'olivier (cv. Picual) a atteint un taux de germination de 17% (Figure 2). Un pourcentage inférieur (5,5%) a été obtenu après les analyses dans le cytomètre en flux. Ces différences se sont montrées significatives lors du test U de Mann-Whitney ( $p=0.05$ ). Le rapport entre les valeurs de germinabilité obtenus par les deux méthodes (visuel/cytométrie) indique que le taux de germination obtenu par la méthode classique (comptage visuel) est surestimé, soit 3 fois le taux réel. Certains avantages présentés par cette nouvelle méthode pourraient expliquer les différences détectées, notamment : 1) la taille de l'échantillon ( $N$ = nombre de grains du pollen) est plus grande, 2) la totalité de l'échantillon est analysée, et 3) l'influence du degré de subjectivité lors du comptage est nulle.

En résumé, cette nouvelle méthode s'est avérée non seulement beaucoup plus rapide mais aussi plus fiable du point de vue statistique par rapport à la méthode classique du comptage visuel. Le protocole peut être utile pour les améliorateurs afin d'évaluer la capacité de germination du pollen après son stockage et garantir son efficacité dans la pollinisation lors des programmes d'amélioration végétale. Ce protocole peut aussi contribuer à une sélection plus fiable des meilleures variétés pollinisatrices.

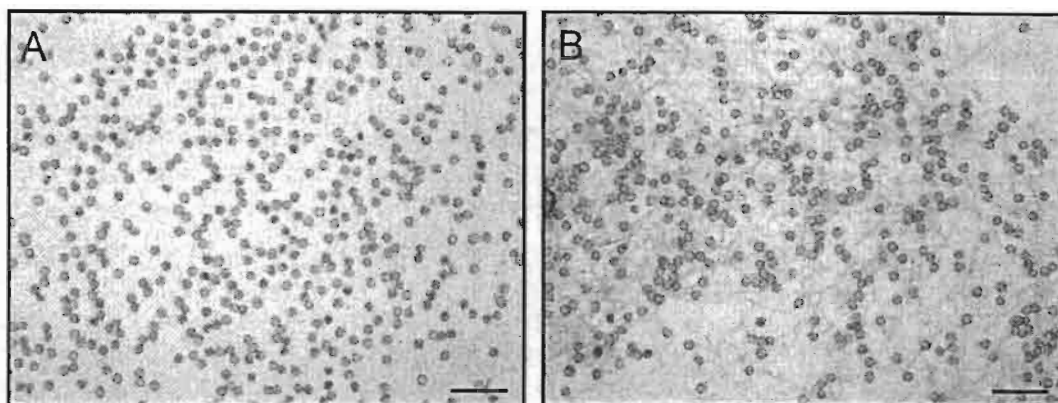


**Figure 1:** (A) Pollen de l'olivier après la réhydratation. (B) Pollen de l'olivier après avoir germé pendant 6 h dans un milieu de culture. Les images ont été obtenues sous un microscope Axioplan (Carl Zeiss, Allemagne) avec un appareil photo ProgRes C3 et à l'aide du logiciel ProgRes CapturePro (Jenoptik Laser, Allemagne). Échelle= 100 µm.

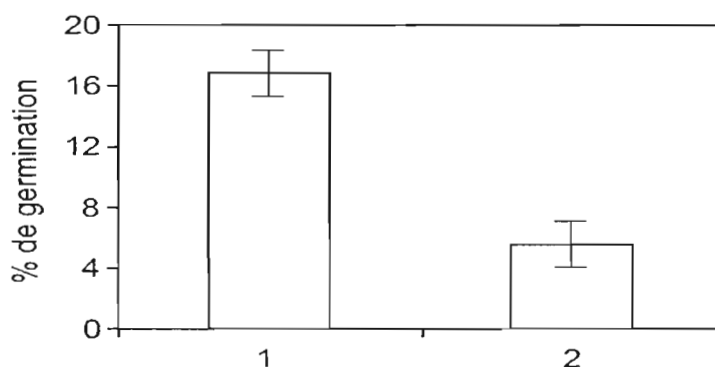


**Figure 2:** Taux de germination du pollen de l'olivier calculée après le comptage visuel (1) et par la cytométrie en flux (2).

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## Characterisation and Identification of Tunisian Olive Tree Varieties by Microsatellite and SNP Markers

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### Abstract

Genetic diversity within the species *Olea europaea* L., across the Mediterranean basin is enormous. This poses problems; different varieties may bear the same name (homonymy) while the same variety may be known by different names from one region to another Olive (synonymy). Molecular markers are now the preferred method to overcome these difficulties major varietal identification. Among molecular markers available, the SSR loci are codominant markers and seem more suitable than others for genotyping plant material. Microsatellites are among the most powerful markers to reveal polymorphism. Their high informativity makes the markers of choice in studies of genetic diversity. This paper presents the results of molecular characterization and identification of 20 Tunisian olive varieties using ten SSR markers. Genotyping of PCR products was performed using the Bioanalyzer.

All PCR products homozygotes and heterozygotes were sequenced to determine the number of repeats and the sizes of alleles for each marker. The number of alleles for the ten SSR markers varies from three to six and their average levels of heterozygosity ranged from 30% to 95% DCA18 for GAPU71B. The data obtained were coded 1 / 0 depending on the presence or absence of bands so they can be exploited in statistical programs to estimate the genetic similarity between varieties of olive (coefficient of similarity Jacquard) and establish a hierarchical classification of the varieties studied. We have shown the efficiency of SSR markers for the identification and discrimination of twenty varieties studied and even between individuals belonging to the same variety. We have also shown that there is a partial correlation between the traits given by the SSR markers and morphological traits and chemical. To confirm the results found by the SSR markers, we also studied the intervarietal diversity using the newly discovered SNP markers. However, due to the absence of genomic sequences of species of olive their discovery of SNPs and their use remained limited. Given the paucity of genomic sequences of olives available in the Gene bank, we chose to sequence two genes lupeol synthase (OEW) and cycloartenol synthase (OEX) in which their cDNAs are available in this bank. Nine new SNP markers have been discovered by comparing the sequences of two genes involved in biosynthesis of triterpene alcohols from the 24 varieties studied. These SNPs are located in the introns of genes sequenced. SNPs are then used to measure degrees of similarities among the 24 Tunisian olive cultivars. We found that there is a partial homology between the dendrograms based on SNP markers and SSR markers. This result is expected because the SNPs are biallelic contrast to SSR markers. A combination of SNP and SSR markers has been proposed that can be used for identification of cultivars in the state juvenile or traceability of the oil.

**Additional index words:** cultivar genotyping, SSR markers, SNP markers, *Olea europaea*, genetic diversity.

## Etude du polymorphisme moléculaire de l'olivier en Tunisie en utilisant les marqueurs SSR et SNP

### Résumé

La diversité génétique au sein de l'espèce *Olea europaea* L., dans l'ensemble du bassin méditerranéen est importante ce qui pourra engendrer des problèmes de dénomination taxonomique (homonymie et synonymie). Les marqueurs moléculaires représentent aujourd'hui la méthode de choix pour surmonter ces difficultés majeures d'identification variétale. Parmi les marqueurs moléculaires disponibles, les SSR sont des locus de marqueurs codominants et semblent être les plus adaptés pour le génotypage du matériel végétal. Le présent travail consiste à la caractérisation moléculaire et l'identification de 20 variétés d'olivier tunisiennes en utilisant dix marqueurs SSR. Le génotypage des produits PCR a été réalisée en utilisant le *BioAnalyzer*. Tous les produits PCR homozygotes et hétérozygotes ont été séquencés afin de déterminer le nombre de répétitions et les tailles des allèles pour chaque marqueur. Le nombre d'allèles pour les dix marqueurs

SSR varie de trois à six et la moyenne de leurs taux d'hétérozygotie varie de 30% pour DCA18 à 95% pour GAPU71B. Les données obtenues ont été codées en 1/0 selon la présence ou l'absence de bandes afin qu'elles puissent être exploitées dans des programmes statistiques pour estimer la similarité génétique entre les variétés d'olivier, (coefficient de similarité de Jacquard) et établir une classification hiérarchique des variétés étudiées. Nous avons montré ainsi l'efficacité des marqueurs SSR pour l'identification et la discrimination des vingt variétés étudiées et même entre les individus appartenant à la même variété. Nous avons montré aussi qu'il y a une corrélation partielle entre les caractères génétiques donnés par les marqueurs SSR et les caractères pomologiques et chimiques.

Pour confirmer les résultats trouvés par les marqueurs SSR, nous avons aussi étudié la diversité inter variétale en utilisant les marqueurs SNP. Pour s'y faire, nous avons choisi de séquencer deux gènes impliqués dans la biosynthèse des alcools triterpéniques Lupeol synthase (OEW) et Cycloartenol synthase (OEX) dont leurs cDNA sont disponibles dans le Gene Bank. Neuf nouveaux marqueurs SNP ont été découverts en comparant les séquences de deux gènes chez les 20 variétés étudiées.

Ces marqueurs SNP sont localisés dans les introns des gènes séquencés. Les marqueurs SNP sont ensuite utilisés pour mesurer les degrés de similarité entre les 20 variétés d'olivier étudiées. Nous avons constaté qu'il y a une homologie partielle entre les dendrogrammes basés sur les marqueurs SNP et les marqueurs SSR. Ce résultat est attendu puisque les marqueurs SNP sont bialléliques contrairement aux marqueurs SSR. Une combinaison de marqueurs SNP et SSR pourrait être proposé pour l'identification des variétés d'olivier à l'état juvénile et la traçabilité des huiles d'olive.

**Mots clés:** *Olea europaea*, diversité, marqueur moléculaire, SSR, SNP, génotypage.

## 1. Introduction

In Tunisia, olive (*Olea europaea* L.) is cultivated throughout the country. Olive farms cover more than one-third of arable land producing 6.0% of the world olive production and contributing for 45.0% of food export receipts, 4.5% of total exports and 10.0% of the total agricultural production value. The Tunisian olive grove lands, although dominated by two major varieties: 'Chemleli' in the South and the Center and 'Chetoui' in the North, is rich in cultivars. Many of these cultivars have been characterized by pomological and chemical traits (Mehri et al., 1997; Grati Kamoun and Khlif, 2001, Grati Kamoun, 2007), with isozyme markers (Grati Kamoun et al., 2002), and more recently with AFLP markers (Grati Kamoun et al., 2006; Taamalli et al., 2006) and SSR markers (Taamalli et al., 2006 ; 2007). However, there is still a need for a better genetic diversity assessment and varietal identification using high throughput marker technologies such as SSR and SNP markers. In this study we report the discovery of nine new SNP markers within two genes of terpenoids synthase (sterol precursors) to studies a genetic diversity among Tunisian olive varieties and to compare their performance with SSR markers.

## 2. Materials and Methods

### Plant materials

Twenty-four accessions of *Olea europaea* originating from different growing areas in Tunisia were used this study.

### DNA Extraction from Leaves

Genomic DNA was extracted from fresh leaves of the cultivars by using a CTAB method Described by authors [10].

### Primer desing for SNP markers

PCR primers were designed using Primer3 program [24] and the sequence of partial or complete cDNA of OEW and OEX genes available from GenBank at NCBI website (<http://www.ncbi.nlm.nih.gov>) under accession numbers NC6456433 and NC6456464, respectively. The sequences were amplified by polymerase chain reaction (PCR) and sequenced.

### SNP marker discovery strategies

The most direct approach to the discovery of DNA polymorphisms is direct sequencing of PCR products from a number of diverse individuals. Polymorphisms between the sequences were identified by sequence alignment using ClustalW [26]. Furthermore, the trace outputs from the sequencer were evaluated by eye to identify possible areas of heterozygous sequence. To minimize false positives due to sequencing artefacts, potential SNPs were resequenced.

### Microsatellite markers

Ten published microsatellites markers were used in this study. Four markers (GAPU59, GAPU71A, GAPU71B, GAPU103A) from the primer set designed by Carriero et al., (2002), four markers (UDO03, UDO12, UDO28, UDO39) from Cipriani et al., (2002) and two markers (DCA9, DCA18) from Sefc et al., (2000) were selected.

PCR amplification and genotyping. SSR amplification was carried out as described by Muzzalupo et al., (2006a) using primers given in the original publications.

PCR products were analysed with a 2100 Bioanalyzer (Agilent Technologies, Waldbronn, Germany) by using DNA 500 LabChip® kits (Burns et al., 2003; Muzzalupo et al., 2007b), providing an estimation of the length of any amplified product. Amplified bands were cut out of the gel, purified using the QIAquick Gel Extraction kit (Qiagen Spa, Milan, Italy) and sequenced.

### Genetic diversity and data analyses for SSR and SNP markers

The SNP and SSR genotypes were then recoded in a 1 / 0 data matrix where 1 indicates the presence of a given allele and 0 its absence. Jaccard's similarity coefficient was calculated to measure the genetic variability among the cultivars [15]. Based on the genetic similarity matrix, the cultivars were clustered by the unweighted pair group method with arithmetic averaging (UPGMA) using the program NTSYS-pc version 2.1 [23].

## 3. Results and Discussion

### Targeted sampling for SNP discovery

The aim of this work was to discover new Single Nucleotide Polymorphisms (SNPs) within the lupeol synthase (OEW) and Cycloartenol synthase (OEX) genes in olive cultivars and assess their potential in olive cultivar discrimination. These two genes were chosen because they are key contributors in the oleanolic acid synthesis and their cDNA sequences have been previously identified in olive [25]. The total length of high quality sequences analysed obtained for the two genes was 1375 bp.

Comparison of this sequences to the cDNA original sequences from which the primers were designed by BLAST2seq [27] showed that only 96 bp were homologous to cDNA (correspond to exons) while the remaining 1279 bp correspond to introns. SNP discovery was validated by the identification of genotype of the 24 cultivars using direct sequencing of PCR products and manual comparative analysis of sequences of the different cultivars.

Nine SNPs were identified within the intronic sequences and their distributions five of them were transversions and four were transitions (Table 1). This high level of polymorphism showed a great variability in olive germplasm suggesting that further SNP identification can be effectively performed using a smaller number of olive cultivars.

**Table 1:** Position and types of the nine SNPs discovered.

| Gene | SNP position* | Type |
|------|---------------|------|
| OEW  | 28            | C/T  |
|      | 31            | C/G  |
|      | 36            | C/T  |
|      | 159           | C/T  |
|      | 250           | C/G  |
|      | 289           | G/T  |
| OEX  | 1             | G/T  |
|      | 33            | C/T  |
|      | 155           | C/T  |



**Table 2:** Genotypic, Allelic frequencies and power of discrimination (PD) of the nine SNPs discovered.

| Locus    | Accession numbers | Size of amplicon in base pairs | Na  | Ho    | He    | PD    | r     |
|----------|-------------------|--------------------------------|-----|-------|-------|-------|-------|
| GAPU59   | EU376393          | 214 (208-218)                  | 4,0 | 0,450 | 0,583 | 0,725 | 0,084 |
| GAPU71A  | EU376392          | 212 (210-228)                  | 4,0 | 0,550 | 0,665 | 0,770 | 0,069 |
| GAPU71B  | EU376384          | 124 (121-144)                  | 4,0 | 0,950 | 0,665 | 0,630 | –     |
| GAPU103A | EU376386          | 150 (136-184)                  | 5,0 | 0,700 | 0,771 | 0,720 | 0,039 |
| UDO03    | EU376385          | 135 (135-202)                  | 4,0 | 0,600 | 0,719 | 0,825 | 0,069 |
| UDO12    | EU376389          | 166 (166-193)                  | 4,0 | 0,850 | 0,616 | 0,705 | –     |
| UDO28    | EU376387          | 154 (143-210)                  | 5,0 | 0,800 | 0,801 | 0,815 | 0,000 |
| UDO39    | EU376388          | 164 (108-232)                  | 6,0 | 0,750 | 0,724 | 0,755 | --    |
| DCA09    | EU376390          | 172 (172-206)                  | 4,0 | 0,550 | 0,637 | 0,750 | 0,053 |
| DCA18    | EU376391          | 177 (174-190)                  | 3,0 | 0,300 | 0,562 | 0,675 | 0,168 |

### SNP markers characteristics

Table 2 shows the genotypic and allelic frequencies of the nine SNPs and their power of discrimination (PD) which varies from 0.43 for the marker oew36 to 0.63 for the marker oew31 with an average value of 0.53. Similar values of power of discrimination were found by [21]. The combined power of discrimination for the nine SNPs was 0.9956274976 which means that the probability of finding two cultivars with the same combination of genotypes for the nine markers is about 5 in one thousand. This relatively weak value is expected since the SNP markers are biallelic. It is interesting to note the high heterozygote frequency for markers oew36 and oex33.

### SSR markers characteristics

Ten microsatellite markers were used to assess the genetic diversity of the 16 Tunisian varieties. We improved the specificity of this analysis by sequencing of all amplicons so that the exact number of repeat units was established. A total of 43 alleles were found for the ten loci with an average of 4 alleles per locus, ranging from 3 for DCA18 to 6 for UDO39 (Table 2). The observed heterozygosity ranged from 0.30 to 0.95 (average 0.625). The power of discrimination (PD) ranged from 0.63 (GAPU71B) to 0.82 (UDO03) with an average value of 0.71. This is higher than that found by Cipriani et al., (2002) in 12 Italian cultivars (0.44) and by Muzzalupo et al., (2006b) on 39 Italian cultivars (0.38). Markers UDO12, UDO28 and UDO39 have high levels of heterozygosity and a PD value consistent with that found in other studies (Cipriani et al., 2002; Muzzalupo et al., 2006b). Among the GAPU markers, GAPU71B has a very high heterozygosity while the two DCA markers exhibit low heterozygosity (Table 3). This is in contrasts with results reported by Sefc et al., (2000), probably because includes a lot of different olive cultivars, mostly of Iberian origin.

**Table 3** Repeat motif and sequence size of the SSR amplification products observed among the 20 Tunisian olive accessions.

| Genes | SNP    | Theoretical heterozygosity (%) | Observed heterozygosity (%) | Power discrimination (PD) | Genotypic frequencies (%) |          |          | Allele frequencies (%) |          |
|-------|--------|--------------------------------|-----------------------------|---------------------------|---------------------------|----------|----------|------------------------|----------|
| OEW   | oew28  | 22.41                          | 29.16                       | 0.59                      | 54.16T/T                  | 16.66C/C | 29.16C/T | 68,75 :T               | 31,25 :C |
|       | oew31  | 49.91                          | 45.83                       | 0.63                      | 37.5G/G                   | 12.5C/C  | 45.83C/G | 60,41 :G               | 39,58 :C |
|       | oew36  | 49.91                          | 70.83                       | 0.43                      | 4.16T/T                   | 25 C/C   | 70.83C/T | 60,41 :C               | 39,58 :T |
|       | oew159 | 46.37                          | 33.33                       | 0.61                      | 50 T/T                    | 16.66C/C | 33.33C/T | 66 :T                  | 34 :C    |
|       | oew250 | 36.86                          | 37.5                        | 0.51                      | 58.33G/G                  | 4.16 C/C | 37.5G/C  | 77,08 :G               | 22,91 :C |
|       | oew289 | 22.41                          | 37.5                        | 0.59                      | 12.5T/T                   | 50G/G    | 37.5G/T  | 68,75 :G               | 31,25 :T |
| OEX   | oex1   | 47.74                          | 45.83                       | 0.60                      | 41.66T/T                  | 12.5G/G  | 45.83T/G | 64,58 :T               | 35,41 :G |
|       | oex33  | 43.11                          | 58.33                       | 0.48                      | 41.66T/T                  | 0C/C     | 58.33C/T | 70,83 :T               | 29,16 :C |
|       | oex155 | 49.91                          | 29.16                       | 0.64                      | 25T/T                     | 45.83C/C | 29.16C/T | 60,41 :C               | 39,58 :T |



For each locus, the GenBank (<http://www.ncbi.nlm.nih.gov/Genbank>) accession numbers for the total nucleotide sequences, the size range in base pairs, the number of alleles ( $N_a$ ), the observed heterozygosity ( $H_o$ ), the expected heterozygosity ( $H_e$ ), the power of discrimination ( $PD$ ) and the probability of null alleles ( $r$ ) are.

### Consensus dendrogram based for SSR and SNP markers

GS matrices with SSR and SNP markers were used to draw a consensus dendrogram for these two marker types. Two groups were obtained by cutting the dendrogram at a similarity equal to 0,44 (Fig.1 ). Group 1 comprises the varieties Chemleli Sfax, Zalmati, Chemleli ontha, Fougi and Chemleli Zarzis. This group is common to all dendrograms based on SSR markers, SNP and SNP + SSR (Rekik et al .2008). The proximity of Lguim and Beldi in group II was also consistent in all studied dendrograms also for both varieties Toffehi and Chemcheli at the group II consensus dendrogram.

This group includes the varieties of group II and III of the SSR dendrogram. We noted that this dendrogram allows separation of all the varieties studied and shows that combining the two types of markers can provide a better characterization of the cultivars.

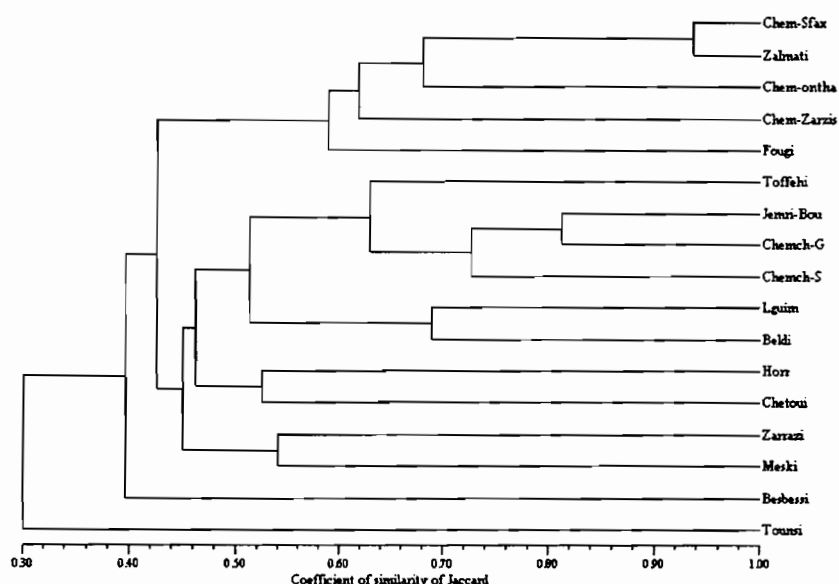


**Figure 1:** Dendrogram of 16 olive cultivars generated by unweighted pair group method using an arithmetic average cluster analysis using Jaccard similarity coefficients from simple sequence repeat markers and single nucleotide polymorphism markers

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## Etude de l'héritabilité des caractères pomologiques, végétatifs et reproductifs des hybrides d'olivier (*Olea europaea* L.) issus de croisements dirigés entre Meski X Picholine.

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### Résumé

Le présent travail a pour objectif l'étude de l'héritabilité des caractères pomologiques et des potentialités végétatives et reproductives des hybrides d'olivier issus de croisements dirigés entre *Meski* et *Picholine*. Les résultats obtenus après 3 ans d'observation ont montré que tous les hybrides ont hérité le caractère de l'autoincompatibilité pollinique de la variété porte graine *Meski*. Par ailleurs, certains hybrides ont présenté des caractères pomologiques identiques à la variété pollinisatrice *Picholine* tel que la forme allongée des fruits et des noyaux et la forme elliptique des feuilles. Cependant, d'autres hybrides ont présenté des caractères plus performants que leurs parents tels qu'un taux élevé de fleurs parfaites et une amélioration des caractéristiques des grains de pollen (viabilité et germination). Ceci pourrait être dû à un brassage chromosomique entre les deux parents.

**Mots clés:** Héritabilité, hybrides, caractères pomologiques, végétatifs et reproductifs.

## Heritability study of pomological, vegetative and reproductive characters of olive tree hybrids (*Olea europaea* L.) issued from *Meski* X *Picholine*.

### Abstract

In this approach, we study the heritability of pomological, vegetatives and reproductives characters of olive tree hybrids issued from *Meski* and *Picholine* crossing. The results obtained after 3 years of observation showed that all hybrids had inherited the self incompatibility character from their female genitor *Meski*. However, some hybrids had presented pomological characters identical to their male genitor such as elongated shape of fruits and stones and elliptical shape of leaves. Nevertheless, other hybrids had showed better characters than their genitors such as a high rate of perfect flowers and an improvement of pollen's characters (viability and germination). This is may be the results of a chromosomal brewing between parents.

**Key words:** Heritability, hybrids, pomological, vegetatives and reproductives characters.

### 1. Introduction

Dans le cadre de l'amélioration du patrimoine génétique de l'olivier, un programme d'hybridation croisé a été initié en Tunisie depuis 1993. Des croisements dirigés ont été effectués entre les variétés *Meski* et *Chétoui* avec des pollinisateurs locaux et étrangers. Le programme d'amélioration génétique vise essentiellement l'augmentation du potentiel de production et l'amélioration de la qualité du produit fini. Cependant, au cours de l'évaluation de la descendance, certains caractères agronomiques prometteurs ont été constatés. De ce fait, nous avons jugé utile d'étudier l'héritabilité des caractères pomologiques et des potentialités reproductives d'une population présentant le nombre le plus élevé d'individus (46).

### 2. Matériel et méthodes

Les hybrides ayant fait l'objet de l'étude de l'héritabilité de caractères sont issus du croisement *Meski* et *Picholine* (*Meski* étant la variété porte graine). Ils sont âgés de 9 ans, plantés selon un écartement de 6X3m et ils sont conduits en irrigué.

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La caractérisation pomologique a été effectuée en se basant sur la méthodologie de caractérisation primaire de l'olivier éditée par le Conseil Oléicole International dans le cadre du projet RESGEN / C01 (2003).

L'étude des potentialités reproductives a porté sur le taux d'avortement ovarien, la viabilité des grains de pollen après coloration au carmin acétique ainsi que la germination des grains de pollen sur un milieu gélosé composé de 10% de saccharose, 100ppm d'acide borique et 0,5% d'agar (Mehri et Kamoun-Mehri, 1995)

### 3. Résultats et discussions

Les résultats relatifs à la caractérisation pomologique des feuilles ont révélé que les hybrides ont présenté des différences hautement significatives par rapport aux parents. En effet, les hybrides 21J, 14J, 6J, 13I, 16I, 22I, 14H, 17H, 21H, 18H et 12H ont présenté des feuilles de forme elliptique identique à celle du parent femelle *Meski*, alors que les hybrides 15H, 12J, 12I, 19I et 23I ont présenté des feuilles de forme elliptique-lancéolée telle que leur parent male *Picholine*. Cependant, l'hybride 18I a présenté des feuilles de forme lancéolée.

Les caractères quantitatifs relatifs aux fruits et aux endocarpes (Tableau 1 et 2) ont montré des différences hautement significatives entre les hybrides et les parents. En effet, le poids moyen des fruits le plus élevé a été enregistré chez l'hybride 19I (7,22g) rappelant celui de *Meski* (7,24g). Par ailleurs, les hybrides 21J, 16J, 13I, 14H, 17H, 21H, 18H et 12H ont présenté des fruits de poids élevé tel que leur parent male *Picholine*.

Les hybrides 22I, 23I et 18H ont présenté des fruits de forme allongée, alors que le reste des hybrides ont présenté des fruits de forme ovoïde telle que leurs parents.

D'autre part, les hybrides 15H, 16J, 14J, 6J, 16I, 22I, 23I, 21H et 18H sont caractérisés par des endocarpes de forme allongée identique à celle du parent male *Picholine*, alors que le reste des hybrides ont présenté des endocarpes de forme elliptique telle que leur parent femelle *Meski*. Telle variabilité d'expression de caractères pomologiques a été signalée par Bartolini et al., (2006) chez une descendance des hybrides d'olivier issus du croisement entre *Konservolia* et *Leccino*.

L'étude des potentialités reproductives, effectuée durant 3 années d'observation, nous a permis de déceler des hybrides ayant des caractères plus performants que leur parents. En effet, les hybrides 18I, 12I, 10J, 16J, 21J et 8K ont présenté un faible taux d'avortement ovarien (<20%) en comparaison avec la moyenne du taux d'avortement ovarien des parents (28,74%). (Tableau 2)

D'autre part, les hybrides 13H, 16H, 18I, 15I, 6J, 9J, 23J et 9K ont présenté des taux de viabilité et de germination des grains de pollen meilleurs que ceux de leurs parents respectivement 88,86% et 26,66%. (Tableau 2).

Les essais d'autopollinisation effectués en plein champs durant 3 campagnes, ont montré que tous les hybrides issus du croisement *Meski* X *Picholine* sont fortement autoincompatibles, d'après la classification signalée par Moutier (2001) sur l'olivier. Le caractère de l'autoincompatibilité pollinique semble être hérité de la variété porte graine *Meski*.

**Tableau 1:** Caractérisation primaire des fruits et des endocarpes des hybrides et de leurs parents.

| Hybrides         | Poids Fruits  | Forme Fruits | Poids Endocarpes | Forme Endocarpes |
|------------------|---------------|--------------|------------------|------------------|
| 15H              | 3,54 ±0,62 d  | Ovoïde       | 0,36 ±0,06 bcd   | Allongée         |
| 21J              | 4,28 ±1,14 f  | Ovoïde       | 0,74 ±0,1 k      | Elliptique       |
| 16J              | 4,16 ±0,57 ef | Ovoïde       | 0,43 ±0,1 ef     | Allongée         |
| 14J              | 1,41 ±2,06 ab | Ovoïde       | 0,28 ±0,04 ab    | Allongée         |
| 12J              | 1,06 ±1,14 a  | Ovoïde       | 0,29 ±0,06 ab    | Elliptique       |
| 6J               | 1,60 ±0,27 bc | Ovoïde       | 0,23 ±0,04 a     | Allongée         |
| 12I              | 3,76 ±0,81 de | Ovoïde       | 0,49 ±0,09 fg    | Elliptique       |
| 13I              | 4,60 ±1,03 fg | Ovoïde       | 0,6 ±0,08 hi     | Elliptique       |
| 16I              | 1,99 ±0,37 c  | Ovoïde       | 0,39 ±0,07 cde   | Allongée         |
| 18I              | 3,36 ±0,64 d  | Ovoïde       | 0,46 ±0,11 ef    | Elliptique       |
| 19I              | 7,22 ±1,77 k  | Ovoïde       | 0,56 ±0,15 gh    | Elliptique       |
| 22I              | 3,34 ±0,71 d  | Allongée     | 0,64 ±0,11 ij    | Allongée         |
| 23I              | 1,63 ±0,31 bc | Allongée     | 0,32 ±0,06 bc    | Allongée         |
| 14H              | 4,22 ±0,8 ef  | Ovoïde       | 0,55 ±0,15 gh    | Elliptique       |
| 17H              | 5,78 ±1,39 ij | Ovoïde       | 0,9 ±0,19 k      | Elliptique       |
| 21H              | 4,80 ±0,95 gh | Ovoïde       | 0,4 ±0,06 de     | Allongée         |
| 18H              | 5,13 ±1,29 h  | Allongée     | 0,59 ±0,15 hi    | Allongée         |
| 12H              | 5,58 ±0,95 hi | Ovoïde       | 0,82 ±0,09 k     | Elliptique       |
| <i>Meski</i>     | 7,24 ±0,79 k  | Ovoïde       | 0,68 ±0,15 kl    | Elliptique       |
| <i>Picholine</i> | 6,00 ±0,91k   | Ovoïde       | 0,55 ±0,06 gh    | Allongée         |

Les moyennes suivies des mêmes lettres ne présentent pas de différences significatives (Test de Duncan  $\alpha = 0,05$ )

**Tableau 2:** Taux d'avortement ovarien et valeurs intrinsèques des grains de pollen.

| Hybrides         | % Avortement ovarien | % Viabilité<br>Grains de pollen | % Germination<br>Grains de pollen |
|------------------|----------------------|---------------------------------|-----------------------------------|
| 13H              | 25,64 ±7,78 cdef     | 91,21 ±1,79 def                 | 39,04 ±3,13 d                     |
| 16H              | 22,37 ±4,48bcde      | 94,4 ±1,94 f                    | 34,12 ±3,59 d                     |
| 22H              | 32,53 ±8,44 fg       | 84,64 ±1,97 bcd                 | 31,35 ±2,32 bc                    |
| 18I              | 18,29 ±7,54 abc      | 92,72 ±4,36 ef                  | 30,19 ±1,44 abc                   |
| 15I              | 32,5 ±7,5 fg         | 91,57 ±1,44 def                 | 39,56 ±1,92 d                     |
| 12I              | 11,56 ±6,4 a         | 81,98 ±2,39 abc                 | 30,5 ±1,62 abc                    |
| 8I               | 20,21 ±9,57 abcd     | 88,61 ±8,59 cdef                | 34,69 ±6,76 cd                    |
| 6J               | 31,6 ±10,67 fg       | 89,78 ±2,17 cdef                | 30,88 ±1,83 abc                   |
| 10J              | 18,25 ±6,62 abc      | 88,65 ±5,98 cdef                | 51,58 ±1,65 e                     |
| 16J              | 19,18 ±5,58 abcd     | 82,42 ±2,19 abc                 | 38,95 ±4,25 d                     |
| 14J              | 39,31 ±7,42 g        | 82,17 ±4,12 abc                 | 52,51 ±2,4 e                      |
| 21J              | 12,87 ±7,42 a        | 86,37 ±5,93 bcde                | 38,43 ±4,67 d                     |
| 11K              | 33,82 ±8,69 fg       | 76,27 ±4,29 a                   | 25,07 ±1,92 a                     |
| 10K              | 32,45 ±7,09 fg       | 84,9 ±3,67 bcde                 | 25,19 ±2,26 a                     |
| 9K               | 30,86 ±9,8 efg       | 92,24 ±1,85 def                 | 26,72 ±2,13 ab                    |
| 8K               | 16,01 ±5,62 ab       | 79,96 ±6,53 ab                  | 29,63 ±3,22 abc                   |
| <i>Meski</i>     | 30,06 ±5,8 ef        | 82,15 ±2,65 abc                 | 28,18 ±2,19 ab                    |
| <i>Picholine</i> | 27,42 ±20,78 def     | 95,58 ±1,94 f                   | 25,14 ±2,76 a                     |

Les moyennes suivies des mêmes lettres ne présentent pas de différences significatives (Test de Duncan  $\alpha = 0,05$ )

#### 4. Conclusion

L'étude de l'héritabilité de caractères des hybrides issus du croisement *Meski* X *Picholine* montre une variabilité intervariétale inouïe témoignant la richesse génétique des hybrides. En effet, nous avons pu distinguer des hybrides présentant des caractères parentaux et d'autres présentant des nouveaux caractères. L'expression de ces nouveaux caractères est probablement due à un brassage chromosomique entre les parents.

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## **Etude de comportement des variétés d'olivier en collection Mondiale de Tassaoute (Marrakech, Maroc)**

Sikaoui Lhassane, Boulouha Belkassem

### **Résumé**

Depuis 2003, la Collection Mondiale d'olivier de Tassaoute est en cours d'installation. A ce jour, 489 variétés de 12 pays oléicole sont plantées. En plus de son rôle de la conservation des ressources génétiques de l'olivier, cette collection permet d'étudier le comportement des variétés du pourtour méditerranéen dans les conditions du sud de la méditerranée avec la conduite en irrigué. Les premiers résultats concernent la phénologie, l'entrée en production, la production cumulée 2007-2008 et la pomologie des fruits. Ces paramètres sont étudiés chez 285 cultivars installés en 2003 et 2004. La production en 2008 est supérieure à 12 Kg par arbre chez 17 variétés. Les paramètres de croissance végétative (vigueur, rythmicité de croissance) et de fructification (floraison, pollinisation, nouaison fructification) de ces génotypes sont étudiés.

**Mots clés:** Collection, Mondiale, Olivier, Marrakech, comportement.

## SSR markers for characterization and identification of Tunisian olive cultivars (*Olea europaea* L.)

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### Abstract

A large number of varieties of *Olea europaea* L. are extended throughout the country, which gives rise to difficulties in characterization, management and preservation of germoplasms. Five Simple-Sequence-Repeats (SSRs) primer pairs were used to characterize 10 olive accessions. The five microsatellite loci used in this study were polymorphic and generated a total of 56 polymorphic alleles with an average of 11 alleles by locus. For all genotypes, the highest number of polymorphic alleles was obtained by the locus UDO043 and GAPIU 103.

The Polymorphism Index Content (PIC) ranged from 0.836 for the locus *ssrOeUADCA-04* and 0.919 for the locus GAPIU-103. The analysis of the dendrogram and the Principal Coordinates Analysis (PCoA) produced by SSR markers grouped studied cultivars into four distinct clusters by cutting the dendrogram at GS value of 0.1. Accessions of Marsaline, Fouji and Fokhari are grouped in one clear cluster. Zarrazi and Meski varieties are grouped in separated clear class. The last group was formed by Picholine and Chemcheli varieties.

**Key words:** SSR, Genotyping, Genetic diversity, *Olea europaea*.

### Résumé

Un grand nombre de variétés appartenant à l'espèce *Olea europaea* L. sont cultivées dans tout le territoire Tunisien. Cette diversification variétale donne lieu à des difficultés dans la caractérisation, la gestion et la préservation du patrimoine national. Cinq paires d'amorces de microsatellites : Simple Sequence Repeats (SSRs) ont été utilisées pour caractériser 10 accessions d'oliviers. Les cinq loci microsatellites utilisés dans cette étude sont polymorphes et ont généré un total de 56 allèles polymorphes avec une moyenne de 11 allèles par locus. Pour tous les génotypes, le plus grand nombre d'allèles polymorphes a été obtenue par le locus UDO043 et le locus GAPIU 103.

L'analyse du dendrogramme montre que les cultivars étudiés sont groupés en quatre groupes distincts. Un groupe formé par les cultivars Marsaline, Fouji et Fokhari. Les cultivars Zarrazi et Meski sont groupés dans deux groupes distincts et le dernier groupe est formé par les variétés Picholine et Chemcheli.

### 1. Introduction

La culture d'olivier est très ancienne, elle s'est développée en plusieurs pays méditerranéens bien avant l'époque romaine. L'olivier et ses produits (huile d'olive et olive) sont considérés comme une ressource alimentaire essentielle. L'arbre et son fruit sont au cœur de groupes humains qui en dépendaient étroitement. Tant de techniques, de pratiques paysannes et domestiques et tant de traditions y sont associées, qu'il est permis de parler d'une vraie civilisation d'olivier.

En Tunisie, l'origine des noms de variétés est directement liée à la toponymie, à des caractères génétiques propres de la variété considérée voire à des faits historiques à un nom de lieu ou d'une localité, à la forme et à la couleur du fruit et à l'architecture de l'arbre (Trigui, 1996). L'ancienneté de la culture de l'olivier et l'importance alimentaire de son fruit ont favorisé l'optimisation des méthodes de multiplication (greffage, bouturage, souchets...) et ont favorisé une grande diversité variétale locale par la population pour assurer leurs besoins en huile, olive et fourrage (Msallem et Hellali, 2000).



Avec l'accroissement de l'intérêt porté à l'olivier (huile et olive), des programmes de recherches ont été instaurés en Tunisie pour améliorer la culture d'olivier par l'inventaire des variétés encore présentes (morphologique, organoleptique et génétique) (Trigui et Msallem, 2002). Le recours à de nouvelles biotechnologies, comme l'utilisation des marqueurs moléculaires est obligatoire pour résoudre des problèmes taxonomiques comme l'homonymie et la synonymie et pour assurer le maintien du patrimoine variétal existant (Pollastri, 2008).

Au début, l'identification et l'inventaire des variétés cultivées en Tunisie est basée sur des critères phénologiques et morphologiques ayant traits aux fruits, feuilles et fleurs (Trigui, 1996). Cependant, ces marqueurs morphologiques sont sensibles aux facteurs environnementaux. Certains auteurs ont appliqué des marqueurs biochimiques comme la technique des iso-enzymes pour identifier et étudier la variabilité génétique de l'olivier (Ouazzani et al. 1995). Les mêmes auteurs ont trouvé des difficultés au niveau de la détermination des marqueurs biochimiques spécifiques ainsi qu'au niveau de l'étude de leurs structures génétiques par la dernière méthode.

En dépit des méthodes utilisées, le recours à d'autres méthodes co-dominantes d'analyse génétique comme la méthode moléculaire SSR (Simple Sequence Repeats ou Microsatellite) est devenue nécessaire (Sefc et al. 2000).

Le but de ce présent travail est d'étudier la diversité génétique inter-variétale de l'olivier Tunisien et d'évaluer l'efficacité de la technique microsatellite pour la caractérisation moléculaire des variétés indigènes d'olivier.

## 2. Matériel et méthodes

### 2.1. Matériel végétal

Les feuilles de jeunes pousses de chaque accession ont été collectées au printemps de différentes collections variétales de la Tunisie, seulement pour la variété Zarrazi, les feuilles de cette variété sont collectées dans la région de Zarzis (voir Tableau 1).

**Tableau 1:** Individus étudiés et leurs origines.

| Géotype    | Utilisation             | Collection  | Origine géographique |
|------------|-------------------------|-------------|----------------------|
| Zarrazi    | Huile et olive de table | -           | Zarzis               |
| Picholine1 | Huile et olive de table | Ksar Ghriss | Sidi BouZid          |
| Picholine2 | Huile et olive de table | Ksar Ghriss | Sidi BouZid          |
| Marsaline1 | Huile et olive de table | Ksar Ghriss | Sidi BouZid          |
| Marsaline2 | Huile et olive de table | Ksar Ghriss | Sidi BouZid          |
| Chemcheli  | Huile et olive de table | Boughrara   | Sfax                 |
| Fouji      | Huile et olive de table | Boughrara   | Sfax                 |
| Fakhari    | Huile et olive de table | Boughrara   | Sfax                 |
| Meski1     | Olive de table          | Boughrara   | Sfax                 |
| Meski2     | Olive de table          | Bouighrara  | Sfax                 |

### 2.2. Extraction de l'ADN

L'ADN génomique est extrait à partir de feuilles, en utilisant le bromure d'ammonium hexadecyltriméthyle (CTAB), selon la méthode décrite par Doyle and Doyle (1990), suivie par une extraction (phénol/chloroforme). La concentration de l'ADN a été déterminée par dosage spectrophotométrique. Finalement, l'ADN a été repris en suspension dans une solution de tampon TE (pH 8) et stocké à -20°C.

### 2.3. Analyse moléculaire

Les cinq amorces microsatellites fluorescentes ont été utilisées dans d'autres travaux sur la caractérisation des variétés d'olivier cultivés et sauvages, sont considérées comme des marqueurs moléculaires fiables et reproductibles (Cipriani et al. 2002) (Tableau 2).

L'amplification de l'ADN est effectuée dans un volume total de 10 µl contenant 1x tampon, 0.75 mM MgCl<sub>2</sub>, 2.5 mM de chaque dNTP, 10 mM d'amorce, 0,5 Unité de Taq DNA polymérase (Gotaq,

Promega) et 50 ng/ $\mu$ l d'extrait contenant de l'ADN. Les conditions de la PCR consistent en une dénaturation initiale (3 minutes à 93°C), 35 cycles d'amplification (1mn de dénaturation à 93 °C, 1 mn d'hybridation à une température optimale allant de 50 °C à 57°C, 90 secondes d'extension à 72°C) et une extension finale (10 minutes à 72°C).

0.5  $\mu$ l du produit PCR est introduit à un volume de 12  $\mu$ l de formamide déionisée et 0,5  $\mu$ l de marqueurs de poids (Gene Scan 500 LIZ). Chaque échantillon est chargé sur le séquenceur automatique d'ADN ABI-310 (électrophorèse à capillaire). Les pics ainsi révélés sont visualisés par les logiciels GeneMapper v4.0 (PE Applied Biosystems).

**Tableau 2:** Séquence des amorces, température d'hybridation (°C), taille générée (pb), nombre d'allèle générée de cinq amorces utilisées dans cette présente étude.

| Nom de l'amorce | Répétition du motif | Taille générée (pb) | Nombre d'allèle |
|-----------------|---------------------|---------------------|-----------------|
| UDO099-043      | (GT)12              | 156-223             | 15              |
| GAPU-103        | (TC)26              | 127-199             | 15              |
| ssrOeUA DCA-04  | (GA)16              | 127-184             | 10              |
| ssrOeUA DCA-03  | (GA)19              | 122-251             | 11              |
| EMO-90          | (CA)10              | 123-200             | 14              |

## 2.4. Analyses des données

Pour chaque individu étudié, les pics sont codés de la manière suivante : 1 : pour la présence du pic et 0 pour son absence. Le dendrogramme est construit par la méthode UPGMA (Unweighted Pair-Group with Arithmetic Averages) fondée sur l'indice de similarité de Jaccard (Sneath et Sokal, 1973) en utilisant le programme d'analyse et de groupement NTSYS.

## 3. Résultats et discussion

Les résultats obtenus par la technique moléculaire microsatellite montrent une certaine homogénéité entre les variétés Tunisiennes étudiées. Cette homogénéité génétique est montrée par les résultats générés par le dendrogramme (Fig. 1). Les cinq amorces microsatellites utilisées ont généré un total de 56 allèles polymorphes avec une moyenne de 11 pics polymorphes par amorce (Tableau 2). Pour tous les génotypes, le nombre le plus élevé de pics polymorphes a été obtenu par les locus UDO043 et GAPU 103. Les résultats obtenus par ce marqueur moléculaire ont montré une importante homogénéité génétique inter-variétale. Les mêmes résultats ont été aussi trouvés par Rekik et al. (2008) qui ont étudié la variabilité génétique de l'olivier Tunisien. Ces auteurs expliquent cette homogénéité génétique entre les cultivars par l'origine commune de l'olivier et de sa culture ancienne en Tunisie, conformément à ce qui a été décrit par Grati-Kamoun et al. (2006) en utilisant la technique AFLPs comme marqueur moléculaire.

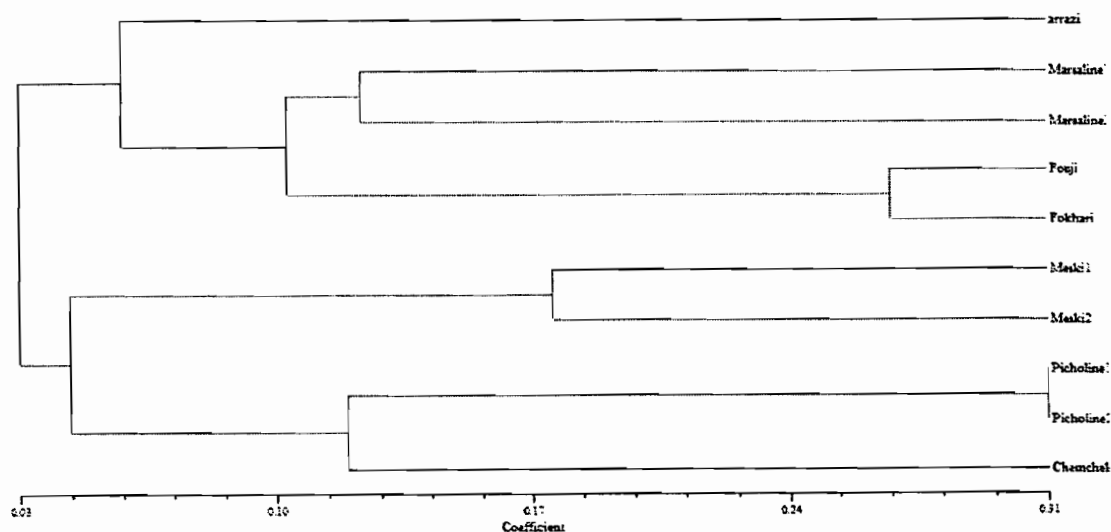


**Figure 1:** Dendrogramme généré par la méthode SSR basé sur la distance génétique de Jaccard.

Il est important de noter que la ressemblance génétique entre les variétés étudiées peut être expliquée par une sélection clonale très sévère appliquée par les agriculteurs pour répondre à leurs besoins quotidiens (aliment et fourrage). Ces agriculteurs utilisent des variétés de qualité supérieure (vigueur, adaptation édaphique...), ce qui a permis l'existence des variétés polyclonales facilement propagées par les méthodes de multiplication asexuée (Mehri et al. 2003). L'existence des variétés dans le même groupe peut être expliquée par une distribution géographique locale restreinte, d'une certaine adaptation édapho-climatique de ces cultivars et par l'allogamie (pollinisation avec les mêmes variétés) de l'olivier. Des travaux antérieurs de (Hannachi et al. 2008) ont montré une uniformité morphologique des variétés Tunisiennes au niveau des traits morphologiques. Donc, l'élargissement du spectre des variétés étudiées et des amorces testées peut générer d'autres pics polymorphes et par conséquent peut changer le groupement des cultivars étudiés.

#### 4. Conclusion

Dans nos conditions d'expérimentation, la technique utilisée au sein de ce travail nous a permis de produire des empreintes génétiques permettant d'identifier chaque variété étudiée. L'utilisation d'une autre méthode basée sur le même principe de l'amplification de l'ADN par PCR comme les SNP (Simple Nucleotide polymorphism) et le séquençage pourra générer plus de bandes polymorphes et donc pourrait mieux discriminer les variétés étudiées.

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## Expression studies of AOX during adventitious root induction in *Olea europaea* L.

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### Abstract

Recently, identification of four alternative oxidase (AOX) gene sequences from olive (*Olea europaea* L.) has been reported from our group. Three genes could be classified as members of the sub-family AOX1 and one as belonging to the sub-family of AOX2. AOX has been proposed as functional marker candidate for easy-to-root olive genotypes. Validation of AOX as an appropriate marker candidate needs to highlight a close relationship between AOX expression and AOX activity to root induction and/or initiation. Here we present AOX gene expression data during IBA treatments of olive shoot cuttings and the result of studies on the effect of AOX inhibitors and stimulators. Furthermore, variable 3'-UTRs are presented as a source for differential gene regulation in the OeAOX2 gene.

**Key words:** *Olea europaea* L., adventitious rooting, AOX gene expression, 3'-UTRs, functional marker

### Études d'expression de l'AOX pendant l'induction de l'enracinement adventive en *Olea europaea* L.

### Résumé

L'identification de quatre séquences du gène de l'alternative oxidase (AOX) d'olivier (*Olea europaea* L.) a été rapportée récemment de notre groupe. Trois gènes ont pu être classifiés comme membres de la sous-famille AOX1 et un autre comme appartenant à la sous-famille AOX2. L'AOX a été proposé comme marqueur fonctionnel pour l'identification des génotypes d'olivier à enracinement facile par bouturage. De toute façon, la validation d'AOX en tant que candidat à marqueur doit se baser sur un étroit rapport entre son l'expression et son activité, concernant l'induction et/ou le déclenchement du enracinement adventive. Ici nous présentons des données d'expression du gène d'AOX pendant des traitements de boutures d'olivier avec d'IBA et aussi le résultat des études sur l'effet des inhibiteurs et des stimulateurs d'AOX. Egalement, les variables 3' - UTRs sont aussi présentés comme source pour le règlement génétique différentiel dans le gène d'OeAOX2.

**Mots clés:** *Olea europaea* L., enracinement adventive, expression d'AOX, 3'-UTRs, marqueur fonctionnelle.

Alternative oxidase (AOX) was recently proposed as a functional marker for efficient cell reprogramming under stress (Arnholdt-Schmitt et al. 2006a). Adventitious root induction and initiation is stress-related and is being explored in olive as one example of cell-reprogramming. This approach is thus combining fundamental research related to molecular biology and cell biology and applied studies of commercial interest for cost-efficient olive tree propagation (Arnholdt-Schmitt et al. 2006b and c, see also the strategic paper on research strategies for molecular plant breeding Arnholdt-Schmitt 2005). It is thought to support the developed hypothesis in view of a functional marker candidate for easy-to-root olive genotypes to assist olive tree breeding. Validation of AOX as an appropriate source for polymorphic sequences as marker candidates that can discriminate olive trees needs to highlight a close relationship between AOX expression and AOX activity to root induction and/or initiation.

AOX is an inner mitochondrial membrane protein that functions as terminal oxidase in the alternative (cyanide-resistant) pathway of respiration where it generates water from ubiquinol (Umbach et al. 2002). AOX serves to relieve oxidative stress originating from environmental stresses by limiting mitochondrial reactive oxygen species (ROS) formation and preventing specific components of the

respiration chain from over-reduction (Popov et al. 1997, Purvis 1997) and canalizing ROS signals (Amirsadeghi et al. 2007). We hypothesize that root induction is a plant cell reaction linked to oxidative stress and that activity of stress-induced AOX is importantly involved in adventitious rooting.

Recently, Santos Macedo et al. (2006a, b, 2008a, b, and 2009) have reported the isolation of four AOX genes from olive and highlighted the existence of polymorphic sequences within the OeAOX2 gene from diverse origins. Additionally, the group of Arnholdt-Schmitt has shown from across species studies that polymorphic plant AOX sequences have the potential to mark individual plants and varieties (Cardoso et al. 2009, Costa et al. 2009, Ferreira et al. 2009). These observations on natural AOX gene diversity and diversity in breeding lines together with supporting opinion and review papers from experts in the field of AOX research (see AOX Special Issue, 2009) encourage proceeding with this novel functional marker approach related to molecular breeding on rooting-efficient olive trees.

Experimental studies with the inhibitor of AOX protein activity, SHAM, and the two AOX stimulators, H<sub>2</sub>O<sub>2</sub> and pyruvate, are giving first indications about the involvement of AOX in adventitious rooting (Santos Macedo et al. 2009). During a rooting assay with the easy-rooting cultivar 'Cobrançosa', semi-hardwood cuttings treated with SHAM (with or without IBA) revealed a significant decrease in root induction (maximal of 28%) to values observed for the negative control (without IBA treatment).

Contrarily, treatments with H<sub>2</sub>O<sub>2</sub> and pyruvate revealed an increase in root induction. These physiological studies on the involvement of AOX in rooting were complemented by OeAOX2 expression assays performed by RT-PCR. Expression was studied in tissue rings removed from semi-hardwood shoot cuttings of 'Cobrançosa' at t0, 12h and 14 days after IBA treatment at the site of root primordial appearance during rooting assays (see Santos Macedo et al. 2009). A high expression of OeAOX2 was observed at the stage of shoot cutting from olive trees, followed by a decrease in OeAOX2 expression and an increase at 14 days. Two weeks after auxin treatment marks the time when first signs of root induction can be observed at histological level (Peixe et al. 2007).

Genes can be regulated at various levels. Polymorphisms in important *cis* or *trans* positions to the gene and within-gene sequences at critical positions for regulation have the potential to interfere with gene function. Thus, these positions are all candidates for functional markers that can mark different stress-related behavior of organisms if related to a gene involved in plant-environment interaction. 3'-untranslated region (3'-UTR) in genes are known to include critical sites for gene regulation, including also miRNA target sites, and can be a source for sequence signals that induce size variations at genome or transcript level (Poliodoros et al. 2009). The isolation of a partial OeAOX2 gene sequence at gDNA level from three olive cultivars (characterized by differences of rooting behavior) allowed the identification of several polymorphisms. Insertion/deletions (InDels) and single nucleotide polymorphisms (SNPs) were identified at different gene regions – exons, intron and 3'-UTR – within and between cultivars. From the three gene regions the 3'-UTR was the most important source for polymorphisms showing 5.7% of variability. cDNA from micro shoots of 'Galega vulgar' revealed transcript length variation for the 3'-UTR of OeAOX2 ranging between 76 and 301 bp.

The identified polymorphisms and 3'UTR length variation can be explored in future studies for effects on gene expression and stability as well as posttranscriptional and posttranslational control and a potential linkage to olive rooting phenotypes in view of marker-assisted plant selection.

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## Etude du bouturage semi-ligneux de l'olivier cultivar «Ousleti »

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### Résumé

Le bouturage semi ligneux a donné entière satisfaction pour certains cultivars d'olivier mais il n'en est pas le cas pour la variété « Oueslati » où des problèmes persistent. L'objectif de ce travail est de contribuer à l'amélioration de la rhizogénèse chez le cultivar « Ouslati ». Pour ce faire, des boutures ont été prélevées de différentes régions de la Tunisie et traitées par différentes doses d'auxine. Ces essais ont été complétés par une analyse chimique et une étude anatomique des boutures. Les résultats ont montré que des fortes doses d'auxine ont permis d'augmenter la rhizogénèse. Les résultats ont montré aussi l'existence de faibles taux des réserves nutritives, et d'un anneau sclérenchymateux continu peuvent inhiber l'enracinement.

**Mots clés:** Auxine, bouturage semi-ligneux, olivier, Ousleti, rhizogénèse.

### Study of the semi-hardwood cuttings of the olive tree cultivar "Ousleti"

### Abstract

The semi-hardwood cuttings gave satisfaction for some cultivar of olive tree but it is not the case for the cultivar "Oueslati" where problems persist. The objective of this work is to contribute to the improvement of the rhizogenese for cultivar "Ouslati. This study was realized on cuttings collected from different regions of Tunisia and treated by auxine application. These essays were completed by a chemical analysis and an anatomical study of cuttings. The results showed that the high level of auxine allowed to increase the rhizogénèse. The results showed also that reduction of the nutritional reserves and the presence of a discontinuous zone of sclerenchyme which inhibits the implanting.

**Key words:** Auxine, semi-hardwood cuttings, olive, ousleti, rhizogenèse.

### 1. Introduction

L'olivier est une espèce pérenne, à feuillage persistant, qui s'est propagée aisément grâce à sa rusticité et sa résistance aux adversités. Sa facilité à se reproduire et son aptitude remarquable à végéter au sein d'un environnement pédoclimatique presque toujours capricieux, ont permis son expansion dans des régions difficiles (Msallem et Charfi 1997). Par ailleurs, l'olivier montre une diversité des techniques de multiplication qui peuvent avoir lieu soit par la voie de la multiplication traditionnelle soit de façon plus complexe par la voie des techniques de bouturage semi-ligneux et de la micropropagation. Le bouturage semi-ligneux, en particulier, est un mode de multiplication végétatif non destructif qui permet la production de milliers de plants en un temps relativement court. C'est entre autres pour cette raison que ce type de bouturage est actuellement le plus utilisé pour la propagation de l'olivier. L'objectif de ce travail est de contribuer à l'amélioration de la rhizogénèse chez la variété « Ouslati ». Pour ce faire différentes dates de prélèvement des boutures, différentes doses d'auxines et la scarification ont été essayés. Ces essais ont été complétés par une étude anatomique et une analyse chimique de certains éléments minéraux.

### 2. Matériel et méthodes

#### 2.1. Préparation des boutures

Les essais ont été réalisés sur des boutures de la variété « Ouslati » prélevées des régions Ouslatia, jebel Ouslet, et El-Alaa, à trois dates différentes: Janvier (P1), Février (P2) et Mars (P3). Des boutures ayant une longueur comprise entre 12 et 15 cm, et portant 4 feuilles à leurs parties apicales ont été préparées. Pour se faire les bases des boutures ont été trempées dans une solution d'éthanol 50% Contenant la dose d'AIB désirée : D1: 2000 ppm ; D2 : 3000 ppm ; D3: boutures scarifiées et



trempees dans 3000 ppm, comparees à un témoin = 0 AIB. Ensuite, les boutures ont été maintenues à l'air ambiant durant 20 min, et placé dans un bac de multiplication rempli de perlite humide sous serre vitrée dont le chauffage basal est assuré avec une résistance électrique (19°C-20°C). Les boutures ont été placées et conservées pendant 60 jours à une humidité relative variant de 80% à 90%.

## 2.2. Etude anatomique des boutures

Des coupes transversales ont été réalisés sur la base des boutures témoins avant leur mise en place, et sur des boutures après un séjour de deux mois dans le bac à multiplication. Une fois coupés, les segments de la base des boutures ont été placés dans de l'alcool à 70%.

## 2.3. Détermination des teneurs en éléments minéraux et organiques

A chaque prélèvement, les feuilles et le bois des boutures ont été séparées et lavés minutieusement avec de l'eau distillée puis égouttés et mis à 60°C. Après le séchage, le matériel végétal a été broyé et conservé. Le dosage des éléments N, P, K, Ca et Na dans les feuilles et le bois des boutures a été réalisé.

La matière azotée (en pourcentage de la matière sèche) a été exprimée comme suit :

$$N (\%) = \frac{(V-V_0) \times 1.4}{10}$$

Où : V = volume H<sub>2</sub>SO<sub>4</sub> (HCl) ajouté à l'échantillon ; V<sub>0</sub> = volume H<sub>2</sub>SO<sub>4</sub> (HCl) ajouté au témoin

La teneur en phosphore (P) exprimée en (%) de la matière sèche a été calculée :

$$P\% = N \times 50 \times V / P \times 1/10^4$$

Où : N: concentration du phosphore en mg/l dans la solution photométrée ; V: volume de la solution à minéraliser (100 ml) ; P: poids de la prise de l'échantillon en g.

La teneur en potassium ou en sodium en % de la matière sèche a été exprimée comme suit:

$$\text{Teneur (\%)} = I \times D \times 10^{-2}$$

Où : I : lecture en µg/ml ; D : dilution de la solution à photomètre.

La teneur du calcium (Ca %) a été calculée comme suit :

$$Ca \% = \frac{V \times 0,2}{P}$$

Avec : V : volume de EDTA versé ; P : poids initial de l'échantillon (avant calcination).

## 2.4. L'analyse statistique des données

Le dispositif expérimental utilisé est le bloc aléatoire complet sachant que les boutures préparées ont été mises en 4 lots à raison de 50 boutures par lot. L'ensemble des mesures a fait l'objet d'une analyse de variance à deux facteurs au seuil du risque 5%. Cette analyse de la variance est complétée par une comparaison multiple des moyennes par le test Newman-Keuls, au seuil de 5% avec le logiciel Statitcf (version 5).



### 3. Résultats

#### 3.1. Effet de la date de prélèvement sur la rhizogénèse

Les résultats obtenus ont montré que le pourcentage d'enracinement de la variété «Ouslati», varie de 0 à 4% (Figure 1). Pour les dates (Janvier) et (Mars) l'application de 3000 ppm a donné le meilleur pourcentage d'enracinement avec respectivement 2 et 4%.

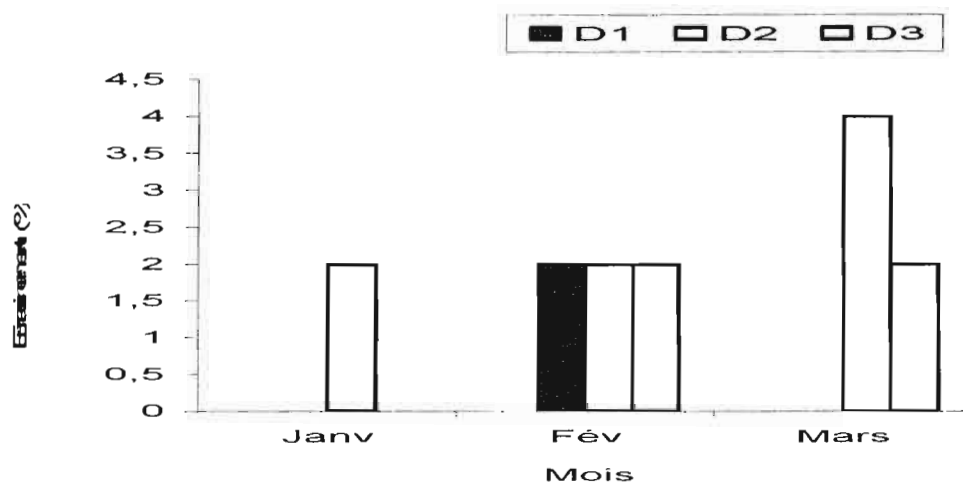


**Figure 1:** Effet de la date de prélèvement et de la dose d'AIB sur le taux d'enracinement des boutures (D1 : 2000 ppm, D2 : 3000 ppm, D3 : 3000 ppm + scarification).

Pour la deuxième date (Février) l'application de 3000 ppm avec ou sans scarification et l'application de 2000 ppm ont donné le même pourcentage d'enracinement (2%). Concernant les témoins le pourcentage d'enracinement est nul. Ainsi les meilleures dates de prélèvement sont Janvier et Mars avec une moyenne de 1,5% de boutures enracinées, suivie de celle du Février avec une moyenne de 0,5%. L'analyse statistique au seuil de 5% n'a pas montré de différence significative entre les traitements et entre les dates.

#### 3.2. Etude anatomique des boutures

La coupe transversale réalisée à la base des boutures témoin a montré l'existence d'un anneau sclérenchymateux continu (Figure 2) ce qui explique la difficulté de l'enracinement des boutures de la variété « Ouslati ».

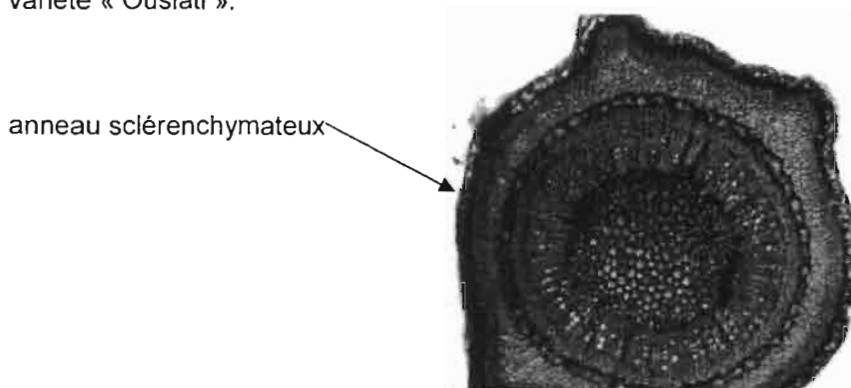


**Figure 2:** Coupe transversale à la base d'une bouture témoin montrant la présence d'un anneau sclérenchymateux continu.

#### 3.3. Variation de la teneur des éléments minéraux dans les feuilles et le bois des boutures

Les résultats de cet essai ont montré que la teneur en azote totale dans les feuilles de la partie basale est plus élevée que celui dans les parties apicales et médianes. Il est à noter aussi que quelque soit la position, les feuilles sont plus riches en azote que le bois. Les résultats ont montré aussi que dans les

feuilles et le bois des différentes parties des brindilles, la teneur en phosphore (P) est faible en comparaison avec l'azote. La teneur en phosphore est plus élevée dans les feuilles que dans le bois et particulièrement à la partie apicale avec un taux de 0,06%. Alors que dans les parties médianes et basales les teneurs sont similaires soit dans les feuilles ou dans le bois avec un taux de 0,05% dans les feuilles et 0,04% dans le bois. La teneur en P dans le bois est identique dans les différentes parties de la brindille, alors que dans les feuilles elle diminue en passant de la partie apicale aux parties médiane et basale. Les teneurs en sodium et en Potassium dans les différentes parties d'une brindille (apicale, médiane et basale) sont plus élevées dans le bois pour les parties apicale et basale.

Alors que pour la partie médiane ces teneurs sont plus élevées dans les feuilles. Concernant le Potassium le taux le plus élevé est enregistré dans la partie apicale avec 0,085% dans les feuilles et 1,05% dans le bois. Ce pendant pour le Sodium le taux le plus élevé est enregistré dans la partie basale avec 0,04% dans les feuilles et 0,06% dans le bois. Les résultats ont montré aussi que la teneur en Calcium dans les feuilles est plus élevée que la teneur dans le bois pour les parties apicale, médiane et basale. En comparant ces différentes parties, on remarque que la partie basale est plus riche en calcium que les autres parties soit dans les feuilles ou dans le bois, avec respectivement 2,1% et 1,02%.

#### 4. Discussions

L'effet de la date de prélèvement sur le pourcentage d'enracinement pour la variété « Ouslati » a été constaté pour les trois dates (janvier, février et mars). Les dates qui ont donné le meilleur résultat sont celles de février et mars avec un pourcentage total de 1,5%, le plus bas pourcentage d'enracinement a été obtenu en janvier avec 0,5%. Charfi (1990) a trouvé que février est le meilleur mois de prélèvement, au cours duquel 48% de réussite ont été atteintes pour la variété « Meski ». Alors que Ben Hadj Salah (1991) a constaté qu'en janvier le meilleur résultat d'enracinement est atteint avec 22,7% pour les variétés « Meski », « Arbequina » et « Besbessi ». Les différences constatées peuvent être due, soit à l'effet variétal, soit à la variabilité des conditions climatiques annuelles du pays, soit à l'effet des deux facteurs à la fois.

Les essais réalisés par Charfi (1990) ont montré que 3000 ppm est la meilleure concentration d'AIB qui a permis l'obtention du pourcentage d'enracinement le plus élevé pour les trois variétés : « Chetoui », « Meski » et « Picholine » ; cette même dose a enregistré le meilleur taux d'enracinement pour la variété « Ouslati ». En effet pour cette variété la concentration 3000 ppm d'AIB a pu donner le meilleur pourcentage d'enracinement en janvier et mars avec respectivement 2 et 4%. Pour le mois de février, l'application de 3000 ppm avec ou sans scarification et l'application de 2000 ppm ont donné le même pourcentage d'enracinement 2%. Le faible taux de la rhizogénèse pourrait être du également à l'hétérogénéité de la température de fond des bacs. En effet cette température est plus élevée au milieu qu'aux extrémités. L'essai a montré que les boutures placées au milieu sont enracinées alors que celles aux extrémités ne le sont pas. Pour la variété étudiée, on a remarqué aussi que la scarification n'a pas donné d'effet sur l'enracinement ; ce phénomène a été noté par Ben Hadj Salah (1991) qui pensait que l'effet défavorable de la scarification ne peut être expliqué que par l'absorption super optimale de la solution hormonale à travers la blessure causée par la scarification. D'autre part, la présence d'un anneau sclérenchymateux continu pourrait être l'origine du faible taux d'enracinement.

L'analyse chimique des réserves nutritives des feuilles et du bois des boutures de la variété « Ouslati », a montré une diminution de divers éléments minéraux de février à mars en se rapprochant de la floraison. Ces variations sont remarquables pour l'azote, le potassium, le calcium et le phosphore. Cette diminution pourrait être expliquée par la mobilisation de ces éléments en réserves vers les organes nouvellement formés (pousses végétatives et fleurs). Les réserves enregistrées de ces éléments sont plus élevées dans les feuilles que dans le bois sauf pour le phosphore dont le taux est plus élevé dans le bois que dans les feuilles. Celano et al. (1999) ont montré que la concentration des différents organes végétatifs en NPK augmente durant la période végétative. L'apparition des nouvelles pousses et des inflorescences pourraient être le résultat de la réduction des réserves nutritives et par la suite elle peut entraîner une diminution du taux d'enracinement. Enfin, et à fin d'améliorer le pourcentage d'enracinement des boutures de la variété « Ouslati », il serait judicieux de penser à : choisir la meilleure date de prélèvement et des pieds mères vigoureux, de même origine et ayant reçus les soins culturaux nécessaires (Irrigation, taille, fertilisation, etc...), et augmenter la dose d'AIB en appliquant une concentration comprise entre 3000 ppm et 5000 ppm.

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## Étude de la diversité génétique de deux variétés d'huile d'olives tunisiennes

Ben Ayed Rayda Naziha Grati-Kamoun · Fabienne Moreau · Ahmed Rebaï

### Résumé

La potentialité des microsatellites dans la traçabilité des huiles tunisiennes a été évaluée par comparaison des profils génétiques de six marqueurs SSR de l'ADN extrait de l'huile et des feuilles de deux principaux cultivars d'olive: *Chemlali* et *Chetoui*. Différents protocoles d'extraction d'ADN à partir d'huile ont été testés et nous avons constaté que le protocole le plus reproductible en termes de succès d'amplifications microsatellites était le Kit QIAamp DNA Stool (Qiagen). Après l'amplification et l'analyse génotypique de six SSR, nous avons démontré que le profil de l'ADN purifié à partir d'huile monovariétal ne correspond pas exactement au profil de l'ADN isolé à partir des feuilles du même cultivar. En fait, nous avons identifié au niveau d'ADN d'huile la présence des allèles provenant des pollinisateurs présents dans le génome de l'embryon de la graine. Ces allèles existent sous forme de pics mineurs et peuvent être aisément distingués des allèles de l'arbre, de sorte qu'ils n'interfèrent pas avec la traçabilité de l'huile d'olive.

**Mots clés:** huile d'olive, les microsatellites, l'huile, la pollinisation, la traçabilité

### Comparative study of microsatellite profiles of DNA from oil and leaves of two Tunisian olive cultivars

#### Abstract

The potential of microsatellites in traceability of Tunisian oils was assessed by comparison of the genetic profile for six SSR markers of DNA extracted from oil and leaves of the two Tunisian major olive cultivars: *Chemlali* and *Chetoui*. Different protocols of DNA extraction from oil were tested and compared and we found that the most reproducible protocol in terms of successful microsatellites amplification was the Qiagen QIAamp DNA Stool extraction Kit, which we slightly modified. After amplification and automatic genotyping of six SSRs, it has been shown that the pattern of the DNA purified from monovarietal oil did not correspond exactly to the profile of the same cultivar leaves DNA. In fact, we found evidence for the presence in the oil of alleles originating from the pollinators, which are present in the genome of the seed embryo. These alleles appear as minor peaks and could be easily distinguished from the mother tree's alleles, so they do not interfere with oil traceability.

**Key words:** Olive, Microsatellites, Oil, Pollinator, Traceability.

#### 1. Introduction

L'olivier (*Olea europaea* L.), est une espèce diploïde ( $2n = 46$ ), de pollinisation croisée et parfois auto-incompatible (Besnard *et al.*, 2000 ; Diaz *et al.*, 2006).

L'huile d'olive qui est l'objet d'un commerce international, est concurrencé par d'autres huiles c'est pour cela elle doit correspondre à des normes de composition et des labels d'origine et de qualité. Par conséquent, il est crucial d'élaborer des procédures de traçabilité qui permettent une identification rapide et facile des cultivars et des huiles. En Tunisie, l'oléiculture est axée sur deux cultivars dominants qui représentent 80% de l'huile d'olive exportée. Toutefois, les appellations des cultivars de l'huile d'olives ont été souvent mêlées avec des dénominations locales ou commerciales en se basant sur les caractères biochimiques et morphologiques. Néanmoins, ces traits ne sont pas suffisants pour vérifier l'authenticité d'huile d'olive (Christopoulou *et al.*, 2004). Aussi la caractérisation chimique des acides gras, les stérols, les phénols et les métabolites secondaires ne permet pas d'identifier les cultivars à cause de l'effet de l'environnement sur le phénotype et la composition chimique (Busconi *et al.*, 2003). En outre, les analyses chimiques nécessitent de nombreux tests coûteux (Woolfe *et al.*, 2004). Les marqueurs moléculaires ont fait leur preuve comme des outils efficace pour la description de la diversité génétique parmi ceux les marqueurs SSR (microsatellites ou Simple sequence Repeat). De plus, ces marqueurs ont été utilisés pour résoudre les problèmes de traçabilité et la provenance de l'huile d'olive (Testolin *et al.*, 2005).

L'extraction de l'ADN à partir d'huile d'olive est une tâche très difficile en raison de sa faible quantité et de sa forte dégradation par les nucléases présentes dans l'huile d'olive (Testolin *et al.*, 2005 ; Muzzalupo *et al.*, 2002 ; De la Torre *et al.*, 2004). Afin de réaliser l'amplification de l'ADN dégradé, il est nécessaire d'utiliser les marqueurs moléculaires les plus fiables comme les microsatellites (SSR) en raison de leurs nombreux avantages : multialléliques, codominants, très polymorphes et largement répartis le long des génomes des plantes (Jobling *et al.*, 2004).

Dans la présente étude, afin d'évaluer les potentialités des marqueurs SSR dans la traçabilité des huiles d'olives, nous avons étudié et comparé les profils génétiques de six marqueurs SSR des échantillons d'ADN à partir d'huile d'olive et des feuilles des deux principales variétés en Tunisie: *Chemlali* et *Chetoui*.

## 2. Matériel et Méthodes

### 2.1. Matériel végétal

Le matériel végétal utilisé dans cette étude est constitué de deux variétés : le cultivar *Chemlali* et le cultivar *Chetoui*. Deux arbres par variété ont été étudiés.

### 2.2. Extraction de l'ADN

- De jeunes feuilles fraîchement cueillies sont utilisées pour extraire l'ADN en utilisant le protocole CTAB décrite par Rekik *et al.*, (2008).

- L'ADN a été extrait à partir des échantillons d'huile d'olive en utilisant différents procédés d'extraction et des kits (tableau 1). Les performances de ces protocoles ont été évaluées par le succès de l'amplification PCR pour toutes les amorces SSR.

La quantité d'ADN a été mesurée par l'incorporation du colorant H33258 détectée par le spectrofluoromètre (Tecan GENIOS Plus).

### 2.3. Marqueurs microsatellites

Six marqueurs microsatellites ont été utilisés dans cette étude ; deux marqueurs (DCA1, DCA3) de Sefc *et al.* (2000), trois marqueurs (GAPU59, GAPU71A, GAPU71B) de Carriero *et al.* (2002) et l'un des marqueurs (UDO12) de Cipriani *et al.* (2002) qui ont été sélectionnés pour leur polymorphisme élevé dans de nombreux cultivars d'olive (Muzzalupo *et al.*, 2009) et aussi pour les variétés tunisiennes (Rekik *et al.*, 2008).

### 2.4. PCR et séquençage

Les PCR ont été effectuées dans un volume finale de milieu réactionnel est de 15 µl : 10 ng d'ADN génomique de l'huile d'olive et 20 ng d'ADN génomique des jeunes feuilles, 2mM MgCl<sub>2</sub>, 0,05 mM de chaque dNTP, 0.1 µM d'amorce direct (l'amorce direct a été marqué par un colorant fluorescent FAM), 0.4µM d'amorce inverse, 0,5 unité de Go Taq Flexi (Promega), 1× tampon (10×). Le programme de thermocycleur (Applied Biosystems, 96 puits) est le suivant : un cycle à 95 ° C pendant 5 min, suivi de 35 cycles (à 95°C pendant 30s, T<sub>m</sub> °C pendant 45s et à 72°C pendant 45s), puis un cycle à 72°C pendant 10min. avec T<sub>m</sub>= 50°C pour les couples d'amorces des marqueurs SSRs DCA1, DCA3, GAPU59 et GAPU71B et T<sub>m</sub>= 57°C pour les microsatellites GAPU71A et UDO12.

Cinq microlitres de produit des PCR ont été mélangés avec 0.3µl de (marqueur 420pb) et 14,7 µl H<sub>2</sub>O déminéralisée, centrifugés à 2000 tr / min pendant 1 min, dénaturés à 94 ° C pendant 3 min, refroidis dans la glace et analysés avec un séquenceur capillaire. (Genetic of Applied 3130xl Biosystems).

L'analyse des données

Les allèles détectés pour chaque microsatellite ont été enregistrés dans une matrice de données de présence (1) et l'absence (0) des bandes (chaque allèle représentant une bande). Comme la plupart des cultivars d'olive sont auto-incompatibles, donc l'ADN extrait à partir d'huile contient des allèles de l'arbre ainsi que des allèles de l'embryon (des allèles du pollinisateur). Nous avons essayé de retracer l'origine des allèles de pollinisateurs en parcourant les génotypes des autres variétés tunisiennes étudiées pour les mêmes marqueurs à partir des études précédentes par Taamalli *et al* (2006) et Rekik *et al.* (2008).

### 3. Résultats et Discussion

#### 3.1. Extraction de l'ADN

Différentes méthodes d'extraction d'ADN à partir des échantillons d'huile d'olive ont été comparés (tableau 1). Les amplifications PCR étaient incompatibles avec toutes les méthodes testées. Néanmoins, les résultats les plus reproductibles sont obtenus lorsque l'ADN a été récupéré à partir d'huile d'olive à l'aide d'un Kit QIAamp DNA stool Kit (Qiagen) en utilisant le protocole (détection de pathogène)(Testolin *et al.*, 2005).

**Tableau 1** : les protocoles utilisés pour l'extraction d'ADN à partir d'huile

| Les procédures d'extraction d'ADN | Amplification des microsatellites | Références                                                  |
|-----------------------------------|-----------------------------------|-------------------------------------------------------------|
| Protocol using added water        | <b>Non</b>                        |                                                             |
| Wizard kit                        | <b>oui (mais inconsistent)</b>    | <a href="http://www.promega.com">http://www.promega.com</a> |
| CTAB                              | <b>non</b>                        | (Busconi <i>et al.</i> , 2003)                              |
| Qiagen QIAamp DNA stool           | <b>oui</b>                        | (Testolin and Lain, 2005)                                   |

La quantité d'ADN extrait de l'huile d'olive vierge a été quantifiée par un spectrophotomètre et les quantités trouvées sont très faibles allant de 5 à 10 ng / microlitres.

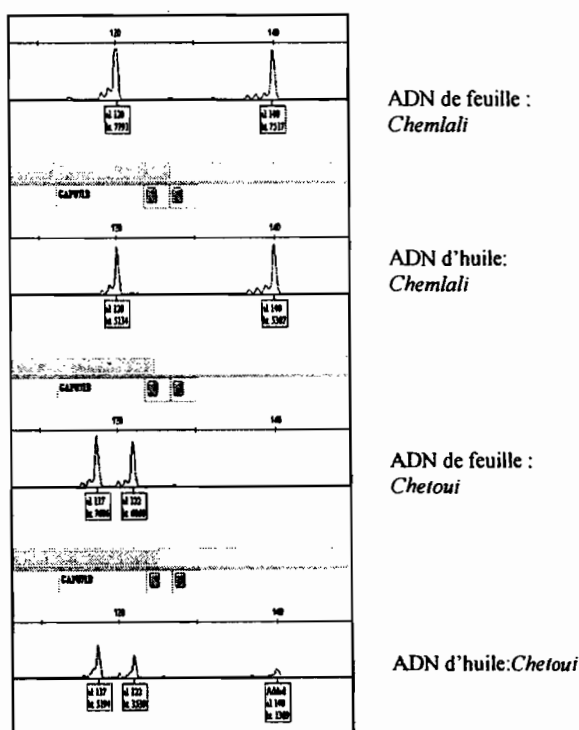
#### 3.2. Identification des cultivars

Aucune différence n'a été observée entre les deux cultivars *Chetoui* et *Chemlali* pour le marqueur DCA3, mais des profils différents ont été trouvés pour les autres marqueurs. DCA3 a aussi le plus petit nombre d'allèles montrant son faible potentiel de traçabilité. Au contraire, le marqueur GAPU71A a pu, seul, différencier ces deux variétés d'olives car ils n'ont aucun allèle en commun. Ce résultat est cohérent avec les conclusions de Rekik *et al.* (2008) qui ont trouvé que le marqueur GAPU71A a l'hétérozygotie la plus élevée parmi un ensemble de vingt variétés tunisiennes.

#### 3.3. Les génotypes d'Huile d'olives

En comparant les profils obtenus à partir de l'ADN extrait des feuilles, les génotypes des échantillons d'huile ont montré des différences notables pour certains marqueurs SSR ; le profil contient des pics de faible intensité qui existent exclusivement dans les échantillons d'huile alors que les pics de haute intensité sont partagés par les échantillons des feuilles (Figure 1). Ces allèles proviennent très probablement de la contribution paternelle (pollinisateur) et sont présentes dans l'embryon. Il est intéressant de noter que, pour certains marqueurs, les échantillons d'huile ont exactement le génotype de la mère qui indique soit l'auto-pollinisation soit la pollinisation par un autre arbre de la même variété. Il est également intéressant de noter que les allèles des variétés *Chemlali* (présent dans le centre et dans le sud du pays) ont été détectées dans les échantillons d'huile de *Chetoui* (cultivé dans le nord) pour le marqueur GAPU71B et l'inverse a été observé pour le marqueur UDO12.

**Figure 1** : Électrophorèse capillaire du marqueur microsatellite GAPU71B.



Cela montre que ces deux cultivars peuvent être polliniser l'un de l'autre et que le pollen d'olive peut voyager sur de longues distances. Ceci a déjà été souligné par Ribeiro *et al.*, (2005) qui a trouvé des preuves que le pollen d'olive peut atteindre jusqu'à 200 km.

La composition génétique diversifiée des embryons n'invalide pas l'analyse moléculaire des cultivars utilisés pour les producteurs d'huile d'olive, car les profils d'ADN à partir d'huile montrent des pics très faibles correspondant aux allèles pollinisateurs par rapport aux principaux pics correspondant aux allèles de l'arbre mère. Il est donc possible, non seulement de retracer l'origine de l'huile commerciale obtenue par broyage des fruits entiers mais aussi d'identifier, dans certaines circonstances, le pollinisateur. Par conséquent, l'interprétation des profils d'ADN récupéré à partir d'huile en utilisant les marqueurs microsatellites sont suffisantes pour vérifier l'authenticité d'huile d'olive et aussi pour répondre aux questions de provenance. Nos résultats sont contradictoires avec ceux de Doveri *et al.*, (2006) qui a suggéré que la présence d'ADN paternel des embryons dans l'huile rendrait la traçabilité de l'huile d'olive très difficile en pratique.

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## Studying the Genetic Determinism of Olive Tree Architecture in a F1 Progeny Olivière x Arbequina

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### Abstract

Tree architecture is of major importance for the agronomic performance of fruit tree orchards. It influences tree adaptability to cultivation systems, and determines yield and fruit quality. The study of the genetic determinism of architectural traits has been initiated in apple tree and has opened new perspectives for breeding programs. In the present study, we aim at investigating the existence of genetic determinism in the olive tree architecture. A F1 hybrid population was created at Montpellier (France) between two genotypes with contrasted architecture, Olivière and Arbequina. A phenotyping methodology of architectural traits was applied and led us to analyze traits related to growth, branching and fruiting behavior, that were measured during the 3 first years of tree growth after plantation. Analyses show the influence of tree ontogeny and morphogenetic gradients on the tree architectural development that must be taken into account during quantitative genetic analyses. The phenotypic variability observed within the population was decomposed into genotypic and residual (environmental) effects in order to identify the most heritable and discriminating phenotypic architectural characters depending on tree age and branching orders. In parallel, an initial genetic map based on the same hybrid population was constructed with 44 SSR loci and 494 AFLP (Khadari *et al.*, in preparation). This will allow us to initiate QTLs mapping related to architectural traits in the next future, as we will continue further phenotyping campaigns for the most relevant traits.

**Keywords:** Olive tree architecture, Phenotyping Methodology, Quantitative Genetics, Growth, Branching, Flowering, Fruiting.

### Résumé

L'architecture de l'arbre a une incidence majeure sur les performances agronomiques des arbres fruitiers en verger. Elle influence la capacité d'adaptation des arbres aux systèmes de culture et conditionne leur productivité et la qualité des fruits. L'objectif de la présente étude, est d'explorer le déterminisme génétique des caractères architecturaux chez l'olivier. Une population hybride F1 a été créée entre deux génotypes à architectures contrastées, Olivière et Arbequina. Une méthodologie de phénotypage a été appliquée, nous permettant d'analyser les caractères liés à la croissance, la ramification et la fructification, et qui ont été mesurés au cours des 3 premières années de croissance des arbres. Les analyses montrent l'influence de l'ontogenèse de l'arbre et des gradients morphogénétiques au cours du développement des arbres, qui doivent être pris en compte dans les analyses de génétique quantitative. La variabilité phénotypique a été décomposée en effets génotypiques et résiduel afin d'identifier les caractères les plus héréditaires.

### 1. Introduction

Genetic studies are needed to design strategies for including traits related to tree architecture such as regular bearing in breeding programs. In order to investigate the genetic determinism of architectural traits in the olive tree, a F1 progeny was created from a cross between two contrasted cultivars giving high architectural variability. During the 3 first years of growth, a phenotyping methodology was applied, inspired from previous studies apple tree, based on a detailed decomposition of tree architecture into topological and geometrical variables (Segura *et al.*, 2006). This strategy highlighted the existence of morphogenetic gradients that must be taken into account in the estimation of genetic effects on architectural traits.

Finally, this estimation was carried out on both growth and branching traits and led us to identify the most heritable traits.

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## 2. Materials and methods

### 2.1. Plant material

The progeny under study derived from an 'Olivière' X 'Arbequina' cross and counts 162 genotypes. The parents have been chosen for their contrasted architecture (Moutier et al. 2004; 2008). 'Olivière' (female parent) is a vigorous French cultivar with a fast growth rate, a big fruit and a rapid entrance in production. The 'Arbequina' male parent is a Spanish cultivar with low vigor and small fruit. In 2005, the 162 hybrids have been randomly planted after cutting (6m x 2m; N/S orientation in plot) as 6 repetitions per hybrid at INRA- Montpellier experimental station. For the architectural study, trees were not pruned and trunks were cut back to 20cm. Standard irrigation and treatments were carried out.

### 2.2. Architectural description

A topological and geometrical description was performed on a total of 324 trees (2 repetitions/genotype) for the three first years of growth. All growth units (GUs), whatever the branching order<sup>1</sup>, were observed in 2005 while a subsample corresponding to the main path of GUs in the tree and along the longest lateral branch was observed in 2006 and 2007. GUs were classified into three types according to their length: long (length  $\geq 20$  cm); medium (5 cm  $\leq$  length  $< 20$  cm) and short (length  $< 5$  cm). Bottom and top diameters, basal angle and cord were measured on the longest lateral branch (Figure 1). Tree topology was formalized according to the coding methodology defined by Godin et al. (1999). Thus, a multiscale tree graph (MTG) was used to calculate architectural traits at 3 different scales: branch, GU and internodes (Table 1). Data extraction from MTGs was performed using VPlants software (<http://openalea.gforge.inria.fr>).

### 2.3. Statistical Analysis

A one-way ANOVA (with a TYPE III sum of square for unbalanced design) was applied to estimate the genotypic effect on phenotypic variance for the architectural quantitative characters. Broad sense heritabilities ( $H^2$ ) of growth and branching characters were calculated from the variance additive model decomposing phenotypic values into genotypic and environmental effect. Broad sense heritability corresponds to the ratio between the genotypic and phenotypic variances (Gallais, 1989) and can be approximate as a function of Fisher statistic for the genotypic factor (Knapp et al., 1985):

$$H^2 = \sigma^2_G / \sigma^2_P = \sigma^2_G / (\sigma^2_G + \sigma^2_\epsilon); H = 1 - 1/F$$

A confidence interval based on the Fisher theoretical distribution is estimated for the heritability significance appraisal. Generally, a variable is considered heritable when the correspondent low limit for the confidence interval is greater than zero and when the heritability value is greater than 0.2 (Gallais, 1989). All statistical analyses were performed using R software<sup>2</sup>

## 3. Results

### 3.1. Architectural analysis

Every year after plantation, the olive trees developed axes at several branching orders (Figure 2). These axes did not show any morphological differentiation since they were all orthotropic with an opposite decussate phyllotaxy and a rhythmic growth, even though no or few morphological markers were visible (data not shown).

### 3.2. Primary Growth

The number of GUs developed per tree was the greatest in the second year after plantation in 2006 (Figure 2). Moreover, an increase in the average length, positively correlated to the internodes number, was found in the second year when compared to first year of growth (Figure 3). This primary growth launching was observed mainly at orders 0 and 1 and was followed by a decrease at order 2 in both first and second year of growth. A stable phase was reached from order 3 and in the third year of growth at all observed orders (2 to 5; Figure 3).

<sup>1</sup> Branching orders were incremented from axes developed after cutting, which were considered as order 0 (Figure 1).

### 3.3. Branching

Whatever the year and branching order, the three types of GUs (long, medium and short) were observed as laterals (Figure 3). However, lateral GU types changed depending on the age of their parent GU: the older the parent GU the higher the proportion of long lateral GUs (Figure 4). The mean number of lateral GUs per GU decreased with years and branching orders, in a similar way to that observed for GU length and number of nodes (data not shown).

### 3.4. Broad Sense Heritability

Due to significant effects of branching orders on all variables (data not shown), only particular combinations of orders were considered for estimating the genotypic effects: all measured orders were considered for the first year after plantation, order 2 and 3 for the second year of growth and orders 2 to 5 for the third year of growth. Topological variables showed intermediate to high heritability values (Table 2). The highest values were found in 2005 for the number of internodes per GU (Nb\_IN) and the short GUs (Nb\_S) in 2006, with  $H^2$  value greater than 0.5 and a lower limit of the confidence interval of 0.38. The geometrical variables showed relatively low heritability values except for the mean

## 4. Discussion and Conclusion

The analysis of growth and branching during the three first years of ('Olivière' X 'Arbequina') progeny highlighted the existence of morphogenetic gradients related to tree ontogeny. A growth increase was observed in the second year of growth, showing an establishment phase after tree plantation that was followed by a more stable phase at high orders and in the third year of growth. These results are consistent with previous quantitative studies on fruit and forest trees (Costes et al., 1992, 2003; Barthélémy et al., 1997; Fournier et al., 1998; Seleznyova et al., 2002). High trait variability was also found depending on the branching order that can be interpreted as resulting from frequent sympodial branching in the olive tree. Both tree age and branching orders were thus accounted for in our quantitative genetic study, as previously proposed by Segura et al. (2008). This study will allow the next phenotyping campaigns to focus on the most heritable and less correlated variables. In addition, combined with a first genetic map built for the same progeny (Khadari et al., submitted), this study will allow us to initiate QTLs mapping for architectural traits.

**Table 1:** List of quantitative variables related to tree topology and geometry with detailed formula for calculated variables.

| Growth Units Variables               | Abbreviations | Formula             | GU2005 | GU2006 | GU2007 |
|--------------------------------------|---------------|---------------------|--------|--------|--------|
| <b>Topology</b>                      |               |                     |        |        |        |
| <i>Growth</i>                        |               |                     |        |        |        |
| Number of Internodes / GU            | Nb_IN         |                     | X      | X      | X      |
| <i>Branching</i>                     |               |                     |        |        |        |
| Number of axillary shoots / GU       | Nb_AS         |                     | X      | X      | X      |
| Number of long ax. GUs               | Nb_L          |                     | X      | X      | X      |
| Number of medium ax. GUs             | Nb_B          |                     | X      | X      | X      |
| Number of short ax. GUs              | Nb_S          |                     | X      | X      | X      |
| <b>Geometry</b>                      |               |                     |        |        |        |
| GU length (mm)                       | L             |                     | X      | X      | X      |
| Mean Internode Length /GU(mm)        | Mean IN_L     | $L / Nb\_IN$        | X      | X      | X      |
| Length of the longest internode (mm) | IN_Max        |                     | X      | X      | X      |
| Basal GU diameter (mm)               | Bdiam         |                     | X      | X      | X      |
| GU top diameter (mm)                 | Tdiam         |                     | X      | X      | X      |
| Longest branch cord (mm)             | cord          |                     | X      | X      | X      |
| Longest branch basal angle(°)        | B_angle *     |                     | X      | X      | X      |
| <b>Tree variables</b>                |               |                     |        |        |        |
|                                      | Abbreviation  | Formula             | 2005   | 2006   | 2007   |
| Height (m)                           | H             |                     |        | X      |        |
| Projection (m)                       | Proj          |                     |        | X      | X      |
| Basis area (m <sup>2</sup> )         | B_area        | $\pi(Proj^2)$       |        | X      | X      |
| Tree volume (m <sup>3</sup> )        | V             | $1/3 * B\_area * H$ |        | X      | X      |
| Trunk basal diameter (mm)            | Tr_Bdiam      |                     | X      | X      | X      |

<sup>2</sup>R Development Core Team (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org>.

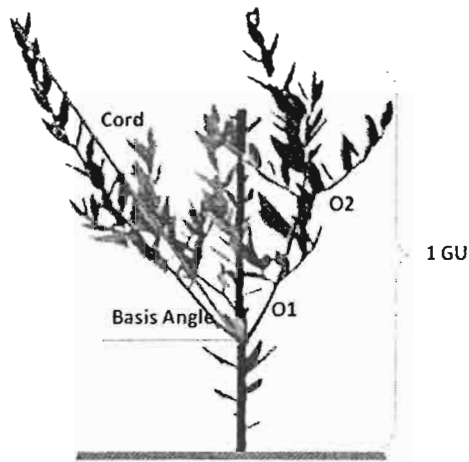


**Figure 1:** Schematic representation of a 1-year old Olive tree with a single growth unit (GU) along the trunk (Order 0).

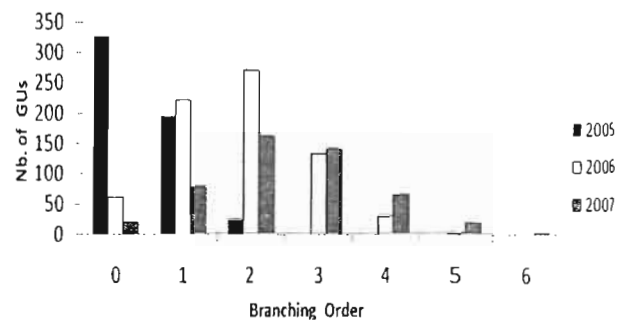


**Figure 2:** Number of growth units developed per tree as a function of years and branching orders, calculated on 3-years old Olive trees of an 'Olivière' x 'Arbequina' progeny.

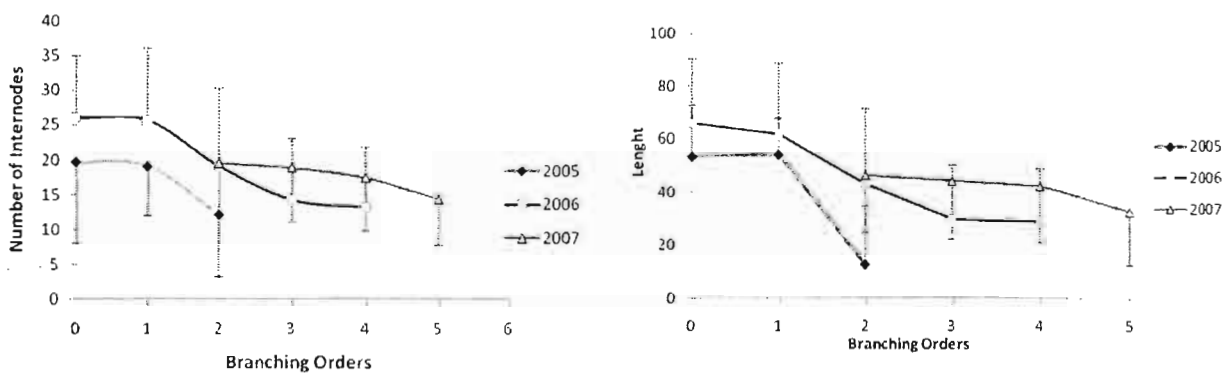


**Figure 3:** Mean values and confidence intervals of the number of nodes and length per growth units (GU) as a function of years and branching orders, calculated on 3-years old trees of an 'Olivière' x 'Arbequina' progeny.

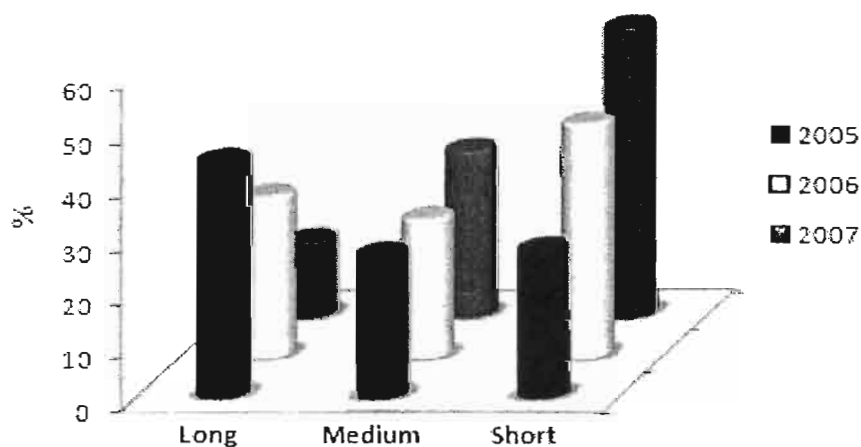


**Figure 4:** Percentage of lateral growth unit (GU) types (long, medium or short) depending on parent growth units age, calculated on 3-years old olive trees of an 'Olivière' x 'Arbequina' progeny.

**Table 2:** Individual broad sense heritability values  $H^2$  with confidence interval (CI) and Pr (>F) significance (S), for topological and geometrical variables calculated on 3-years old olive trees from an 'Olivière' x 'Arbequina' progeny.

|                       |             |               |     |             |               |     |             |               |     |
|-----------------------|-------------|---------------|-----|-------------|---------------|-----|-------------|---------------|-----|
| Nb_IN /GU             | 0,491       | (0,33 ; 0,60) | *** | 0,342       | (0,12 ; 0,50) | **  | 0,305       | (0,08 ; 0,47) | *** |
| Nb_AS /GU             | 0,470       | (0,30 ; 0,58) | *** | 0,414       | (0,22 ; 0,56) | *** | 0,326       | (0,11 ; 0,49) | **  |
| Nb_L                  | 0,422       | (0,24 ; 0,55) | *** |             |               | NS  |             |               | NS  |
| Nb_M                  | 0,390       | (0,20; 0,52)  | *** | 0,215       | (0 ; 0,41)    | *   | 0,308       | (0,08 ; 0,47) | **  |
| Nb_S                  | 0,332       | (0,13; 0,48)  | *** | 0,513       | (0,35 ; 0,63) | *** |             |               | NS  |
| <b>Geometry</b>       |             |               |     |             |               |     |             |               |     |
| GU Length             | 0,322       | (0,09; 0,49)  | **  | 0,288       | (0,05 ; 0,46) | **  | 0,312       | (0,09; 0,48)  | **  |
| Cord                  | 0,338       | (0,11; 0,50)  | **  | 0,246       | (0; 0,44)     | *   | 0,249       | (0; 0,43)     | **  |
| IN_L Mean             |             |               | NS  | 0,539       | (0,38 ; 0,65) | *** | 0,519       | (0,36; 0,63)  | *** |
| IN_Max                | 0,322       | (0,01; 0,55)  | *   |             |               | NS  | 0,278       | (0; 0,49)     | *   |
| Bdiam_GU05            | 0,251       | (0; 0,45)     | *   | 0,434       | (0,23 ; 0,58) | *** | 0,274       | (0,01; 0,46)  | *   |
| Bdiam_GU06            | -           | -             | -   |             |               | NS  | 0,330       | (0,1; 0,49)   | **  |
| Bdiam_GU07            | -           | -             | -   | -           | -             | -   |             |               | NS  |
| Tdiam                 |             |               | NS  |             |               | NS  |             |               | NS  |
| B_angleGU06           | -           | -             | -   |             |               | NS  |             |               | NS  |
| B_angleGU07           |             |               | NS  |             |               | NS  | 0,342       | (0,12; 0,50)  | **  |
| <b>Tree Variables</b> |             |               |     |             |               |     |             |               |     |
|                       | <b>2005</b> |               |     | <b>2006</b> |               |     | <b>2007</b> |               |     |
|                       | $H^2$       | CI (95%)      | S   | $H^2$       | CI (95%)      | S   | $H^2$       | CI (95%)      | S   |
| Height                | -           | -             | -   |             |               | NS  | 0,232       | (0; 0,44)     | *   |
| Projection            | -           | -             | -   | 0,341       | (0,1; 0,52)   | **  | 0,373       | (0,14; 0,55)  | **  |
| Basis area            | -           | -             | -   | 0,409       | (0,19; 0,57)  | *** | 0,388       | (0,16; 0,56)  | **  |
| Tree volume           | -           | -             | -   | 0,421       | (0,19; 0,59)  | *** | 0,352       | (0,11; 0,53)  | **  |
| Tr_Bdiam              | 0,322       | (0,07; 0,50)  | **  | 0,296       | (0,04; 0,48)  | *   | 0,277       | (0,01; 0,47)  | *   |
| Δ Tr_Bdiam            | -           | -             | -   | 0,320       | (0,07; 0,50)  | **  | 0,375       | (0,15; 0,54)  | **  |

(S) : Pr(>F) signif. codes: 0 '\*\*\*\*' 0.001 '\*\*\*' 0.01 '\*\*' 0.05 '\*' NS, 1 ; (-) : data not observed.

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## **Identification of greek olive cultivars and traceability of their products using molecular marker techniques**

G. Mitsopoulos, A. Linos and M. Hagidimitriou

### **Abstract**

The every day evolved market demand for olive tree product quality improvement and assurance, as well for ascertaining their origin. The purpose of this study was the identification of eight olive cultivars and the traceability of their products using molecular markers that have been derived from two methods, RAPDs and SSR. Leaves and drups were collected from eight cultivars of 4 years old olive trees. Out of 6 RAPD primers, a total of 60 polymorphic parts of DNA were generated. Out of 3 SSR primer pairs, a total of 12 alleles were generated. The molecular size of the polymorphic segments varied from 150 to 1200 bp for the RAPDs and SSR. The RAPD primers gave products of similar patterns between leaf and drups from the same variety. The SSR primers gave products of identical alleles patterns between leaves and drups from the same variety.

**Key words:** Olive, molecular markers, RAPD, SSR.

## Characterization and Identification of the Main Algerian Olive Cultivars by Molecular Markers

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### Abstract

The present study is aimed to identify main and local cultivars from the north-eastern Algeria using SSRs and SNPs markers. Eight microsatellite and nine SNPs were tested on 18 varieties; among them, 4 cultivars (two Algerian and two Tunisian) from the World Germplasm Bank of Cordoba (IFAPA), were also analysed. All microsatellites were polymorphic, showing 10.71 alleles per locus on average and mean observed heterozygosity value of 0.83. Although less polymorphic than SSR, SNP markers were useful as well for characterization and identification purposes. The results indicates a high degree of polymorphism and variability in a small sample of Algerian varieties. SSR and SNP markers are recommended for olive fingerprinting in order to generate a database for olive cultivars identification.

**Key words:** SSR, SNP, genetic variability, *Olea europaea*

## Caracaterisation et identification des variétés principales algérienne à l'aide des marqueurs moléculaires.

### Résumé

Cette étude avait pour objectif l'identification des principaux et locaux variétés du Nord-Est de l'Algérie à l'aide de 8 locus SSR et 9 SNP. Ces marqueurs ont été testées sur 18 variétés, parmi eux, 4 (deux algériens et deux tunisiens) provenant du Banque de Germoplasme de Cordoba (IFAPA). Tous les microsatellites étaient polymorphes, montrant 10,71 allèles par locus et une valeur moyenne d'hétérosygosité observée de 0,83. Bien que moins polymorphe que SSR, les SNP sont aussi utiles pour la caractérisation et l'identification. Les résultats indiquent un haut degré de polymorphisme et de la variabilité dans un petit échantillon des variétés algériennes. Les marqueurs SSR de SNP sont recommandés pour le fingerprinting afin de générer une base de données pour l'identification variétale.

**Mots clés:** SSR, SNP, variabilité génétique, *Olea europaea*

### 1. Introduction

Algeria is a country in the north Africa where the olive crop has been mainly managed by traditional farming systems (Cavaillès H., 1938; Loussert and Brousse, 1978). Nowadays, olive cultivars occupies 33% of national arboriculture area and 2% of the arable land, being foreseen an extension of the olive growing area (Data from the Ministry of Agriculture, 2009). Most of them are very local and are situated in the most northern regions of the country. The richness of genetic diversity of Algerian olive cultivars and the general criteria of their naming make their characterization and identification necessary. Hauville (1953) evidenced the presence of 150 olive cultivars in Argelia. In contrast, in a recent survey reported only 39 (ITAFV 2006), might indicate certain loss of genetic diversity. The use of molecular markers could help to have a better knowledge of the olive genetic resources of Algeria as well as to prevent their loss. To our knowledge, studies on genetic diversity and identification of Algerian olive cultivars by means of DNA markers have not been carried out so far. Only very few cultivars from this country have been included in previous studies at Mediterranean level (Besnard et al., 2001; Belaj et al., 2002; Idrissi and Ouazzani, 2003). In the present study, SSR and SNP markers are used to characterize and identify olive cultivars from the north-eastern Algeria, on of the most important regions in terms of olive cultivation and where most representative cultivars of the country are found.

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## 2. Materials and methods

Molecular analysis was performed on 18 olive cultivars. Fourteen of them were collected from north-east Algeria. The genetic profiles of the Algerian cultivars were compared with a set of Tunisian (1), Moroccan (1) and Algerian (2) cultivars provided from the the World Olive Germplasm Bank of Córdoba (IFAPA "Alameda del Obispo). Ten microsatellite markers and seven SNP markers were tested on the set of cultivars. Microsatellite used (DCA3, DCA5, DCA9, DCA11, DCA18 GAPU59, GAPU71 B, GAPU103 A, EMO03 and UDO99-043) were selected among those reported in the literature (Sefc et al. (2000), Carriero et al. (2002), De la Rosa et al (2002), Cipriani et al.(2002). PCR were carried out with the composition and conditions described in De la Rosa et al. (2002). PCR products were separated using an automatic capillary sequencer (ABI 3130 Genetic Analyzer Applied Biosystems/HITACHI). The software Genescan version 3.7 and Genotyper 3.7 from Applied Biosystems were used for sample analysis and peak sizes determination. All the seven SNPs (Cycl 1, Cycl 2, Lup, Ant 1, LS5, Cbp and LS2) were genotyped as described by the authors (Reale et al. (2006). PIC,  $H_e$  and  $H_o$  were estimated using the CERVUS software, version 3.0.3.

## 3. Results

The 10 SSRs used in this study showed a high polymorphism and discriminating power in both Algerian and the full sample that include cultivars from Algeria, Tunisia and Morocco. The total number of alleles amplified was 76. The number of alleles per locus was 7.60 (minimum 5 for GAPU71B, maximum of 10 for Udo99-043, DCA9 and DCA18). The expected heterozygosity was mean value of 0.81 (maximum 0.729 and minimum 0.901). The observed heterozygosity varied from 0.636 to 1 with a mean value of 0.84. The PIC values ranged from 0.860 (DCA18) to 0.663 (GAPU59) with an average of 0.7477 and classified all loci as informative markers ( $PIC > 0.5$ ). The 7 SNPs used in this study showed a high polymorphism and discriminating power. The expected heterozygosity ranged between 0.533 at locus Cycl-1 and 0 at LS2 (mean value of 0.3166). The observed heterozygosity ones varied from 0 to 0.750 (mean value of 0.3551). The PIC values ranged from 0.375 (Cycl-1) to 0 (LS2). The combination of the allelic patterns obtained with the 10 SSRs was able to identify all accessions analyzed. It was possible to achieve the same result using only three loci (DCA18, DCA5 and DCA9).

The 7 SNP markers, showed low discrimination power. This can be explained by their biallelic nature which calls for the extension of the analysis to a higher number of markers for genetic variability studies. Anyway, these markers are a very useful tool in addition to microsatellites, considering also the different mechanism originating mutations. The average similarity value among genotypes (similarity matrix not shown) was 0.47 with most similarity measures among genotypes ranging between 0.36 and 0.90. The cophenetic correlation coefficient between the dendrogram and the original distance matrix was not very high but significant ( $r=0.75$ ). From this dendrogram, most of the cultivars could be classified into three main groups (Figure 2). In the first group, 5 cultivars were from Algerian and only one was from Tunisia ('Chemlali'). Similarity values ranged between 0.53 and 0.72 in this group. The second group was composed of three sub-clusters. The first sub-cluster included four cultivars 'Chemlal', 'El-Knarfi', 'Derdouri' and 'Chetoui'. The cultivars 'Rouihni' and 'Sigoise' from Algeria and the cultivar 'Picholine Marocaine' from Morocco are grouped in the second sub-cluster. The third sub-cluster included the cultivars 'Lokchiri' and 'Chemlal de Kabylia' from Algeria. The remaining three cultivars 'Azeradj', 'Laaninbi' and 'Bliti' from Algeria, were not included in any group.

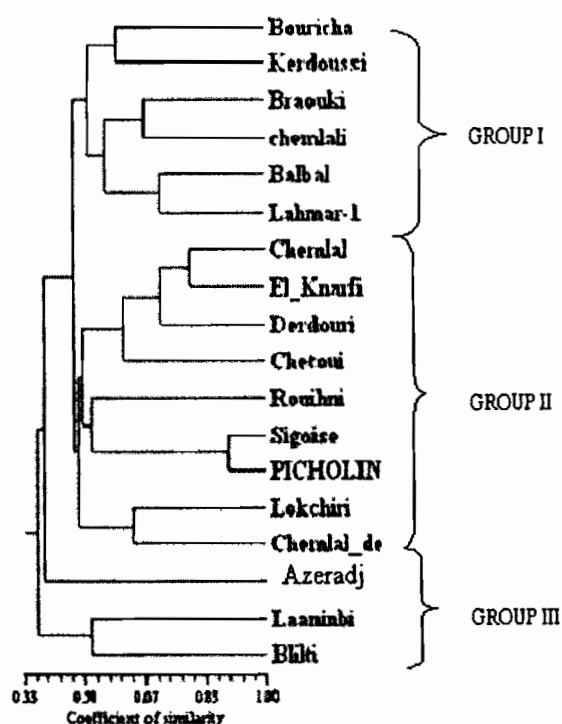


**Figure 2:** Dendrogram of the Algerian olive cultivars using UPGMA clustering method Discussion.



The results obtained in this work confirmed the high efficiency of SSR and SNP markers for genotyping olive cultivars and highlighted the genetic richness available in the olive germplasm of olive areas as Algeria. Regarding the local germplasm of Algeria, a high level of genetic variability was shown. The values of heterozygosity and number of alleles found each was comparable with those reported in several studies, performed by SSR markers, on olive germplasm of other northern Africa countries (Taamalli et al., 2008; Rekik et al., 2008). The clustering of the cultivars originating from the same or nearby region suggests a common genetic base and an autochthonous origin for these cultivars. This result agrees with the hypothesis of autochthonous origin of most of the olive cultivars as well as their limited diffusion from the centers of origin (Besnard et al. 2001; Idrissi and Ouazzani, 2003). The clustering of 'Chemlali', the olive from Tunisia, with the Algerian cultivars 'Bouricha', 'Kerdoussi', 'Braouki', 'Balbal' and 'Lahmar-1' together with some molecular similarities may be indicative the common origin. Our results support the utility of SSR and SNP markers in identification and diversity studies in olive. The identification of cultivars is one of the major applications of the molecular markers in olive. Therefore, the potential of the markers to yield different genotypes for as many cultivars as possible is of great interest, and selection of the most information markers reduces the number of loci to be investigated for reliable cultivar distinction. SSR and SNP based fingerprinting of olive cultivars may generate a molecular database that will facilitate management of cultivar collections, trade control of plant material, and selection of genotypes for breeding programs. For such a purpose, further studies with larger olive samples are needed in the future.

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## **Thème 2**

**Bonnes pratiques de conduite de l'olivier: acquis et innovations**

S1-P1

## Etude du comportement du psylle de l'olivier *Euphyllura olivina* Costa (Hemiptera: psyllidae) sur quelques variétés d'oliviers en Tunisie

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### Résumé

En Tunisie, le psylle de l'olivier est un ravageur d'une grande importance économique. Il s'attaque aux jeunes pousses et aux grappes florales et cause des pertes importantes au niveau de la production, Ce qui a nécessité plusieurs études sur les caractéristiques biologiques et environnementales afin d'établir une stratégie de lutte intégrée. Toutes les recherches ont été réalisées exclusivement sur la variété Chemlali.

Le présent travail vise l'étude du comportement de ce ravageur *Euphyllura olivina* sur d'autres variétés d'olivier plantées dans les mêmes conditions culturales (conduite intensive).

Le suivi a été réalisé dans la station de Taous dans la région de Sfax dans une parcelle irriguée (7 x 7m). Les variétés étudiées pendant 3 années (2007 – 2009) sont: les variétés à huile : Chemlali, Koroneiki et Zalmati et les variétés de table : Picholine, Meski et Manzanille.

Les résultats obtenus ont montré une différence significative au niveau de l'attractivité des variétés pour la réception du psylle et la ponte des œufs. Ainsi, Chemlali et Koroneiki sont les variétés les plus réceptives, suivies de Zalmati et Manzanille, et finalement Picholine et Meski. Par ailleurs, on a enregistré deux pics de ponte au début et à la fin de la saison printanière.

Pour le développement larvaire, le nombre des larves enregistrées pour les deux variétés Chemlali et Koroneiki est plus important en comparaison avec les autres variétés.

### Behavioural study of *Euphyllura olivina* Costa (Hemiptera: Psyllidae) on different olive tree cultivars

#### Abstract

Among harmful pests of olive tree in Tunisia, the Psylla *Euphyllura olivina* causes several damages on young shoots and clusters. Current studies of the biological and environmental characteristics of the insect were suggested in order to establish a strategy for the integrated control. However, all research studies have been deal only with the cultivar Chemlali.

Therefore, the present work aims to evaluate the behaviour of Psylla towards different cultivars of olive trees under similar growth conditions (intensive conduction). The survey was undertaken in the experimental station of Taous in Sfax region under irrigated parcel of density 7x7. The cultivars studied during three years (2007-2009) were: Chemlali, Koroneiki and Zalmati (cultivars for olive oil) and Picholine, Meski and Manzanille (cultivars for table olive). The results show a significant attractiveness relationship between the cultivar and the activity of lying by the insect. Indeed, the cultivars Chemlali and Koroneiki appear to be the more receptive followed by Manzanille and Zalmati than finally, the cultivars Picholine and Meski appear to be with less receptivity. Furthermore, it was noted the existence of two peaks of eggs laying in the beginning and the end of Spring. Concerning the larval growth, it was registered that the two cultivars: Chemlali and Koroneiki have emerged the important number of larvae.

**Key words:** Psylla *Euphyllura olivina*, olive tree, cultivars, activity of lying, larval growth, receptivity.

## 1. Introduction

Le psylle de l'olivier, est un ravageur primaire en Tunisie, dont les larves se nourrissent de la sève des grappes florales et des jeunes pousses (Jardak *et al.*, 2004). Le danger de ce ravageur est de plus en plus important surtout dans les zones côtières où l'humidité relative est élevée et la température est clémente (Chermiti, 1989). Ces conditions favorisent la pullulation du ravageur et engendrent des dégâts énormes surtout si la densité du ravageur dépasse le seuil économique (chute de 30% des fruits noués pour une infestation de 60%) (Ksantini, 2003).

Plusieurs travaux ont permis d'élucider certains aspects relatifs à la morphologie de l'insecte, sa bio-écologie, sa répartition géographique, ses dégâts et les méthodes de lutte (Arambourg, 1964, Jardak *et al.*, 1985, Chermiti, 1992, Ksantini *et al.*, 2002 et Jarraya, 2003). Ceci a permis une réduction de l'effectif (97.9%) des oliviers traités; en passant d'une moyenne de 2 831 027 pieds de 1982 à 1986, jusqu'à seulement 60 014 en 2002 et 2006 (Ksantini, com. personnelle). De même, l'ensemble de ces travaux ont concerné essentiellement la variété Chemlali.

Par ailleurs, la nouvelle planification vise l'augmentation des superficies en intensif pour d'autres variétés (Msallem *et al.*, 2004). Alors que ce nouveau mode de conduite présente des conditions très favorables au développement du psylle.

C'est pourquoi, nous avons entrepris ce travail pour étudier le comportement du psylle de l'olivier sur quelques variétés à huile et d'autres à table cultivées dans les mêmes conditions édaphoclimatiques et culturales.

## 2. Matériels et méthodes

### 2.1. Biotope d'étude

Il s'agit de la station expérimentale "Taous" de l'Institut de l'Olivier, qui se trouve dans une région semi-aride (Sfax, Tunisie). Elle contient plusieurs cultures comme l'olivier, l'amandier et le pistachier. En plus, elle est équipée d'une station météorologique. La parcelle d'étude est composée d'oliviers conduits en mode intensif (7 X 7 m), plantés en avril 2001 et irrigués par un système goutte à goutte.

### 2.2. Les variétés étudiées

Les variétés à huile sont : Chemlali, Zalmati et Koroneiki. Tandis que, les variétés destinées à la production des olives de table sont : Meski, Picholine et Manzanille.

### 2.3. Les techniques employées

#### 2.3.1. Echantillonnage des rameaux

Pour chaque variété, on prélève des échantillons de 5 arbres choisis vigoureux. L'échantillonnage consiste en un prélèvement au hasard, de 5 rameaux par arbre ; des quatre côtés et un du milieu, tel qu'on examine 25 rameaux par variété pour chaque prélèvement. Les échantillons sont pris hebdomadairement.

#### 2.3.2. Observations effectuées au laboratoire

Les rameaux sont mesurés afin de déterminer leurs longueurs. L'estimation de la population du psylle de l'olivier a été effectuée par détermination du nombre de chaque stade de l'insecte : l'œuf et les différents stades larvaires ( $L_1$ ,  $L_2$ ,  $L_3$ ,  $L_4$ ,  $L_5$  et  $L_5$  parasitées).

Ces stades sont relevés en précisant leurs états (vivants, morts et parasités).

Les données, obtenues du comptage des stades pré-imaginaux du psylle sur les différentes variétés, sont ramenées au mètre linéaire pour faciliter la comparaison des résultats entre les différentes variétés.

### 2.3.3. Analyse Statistique

Les analyses statistiques ont été réalisées à l'aide du logiciel SPSS sous interface Windows (version 11.0) à  $P < 0.05$  (Test Duncan) en utilisant les données de trois années successives à savoir la densité des œufs et celle des larves par mètre linéaire et ce pour six variétés étudiées.

## 3. Résultats et discussions

### 3.1. Comparaison de la ponte entre les différentes variétés

Le suivi des résultats de la ponte, effectué sur les différentes variétés durant les périodes printanières des trois années d'étude, a révélé une nette différence entre la préférence du ravageur pour les différentes variétés. En effet, il a été montré que la variété Chemlali est la plus réceptive à la ponte du psylle contrairement à la variété Meski (Figure1).

En se basant sur la différence entre le nombre des œufs pondus sur les différentes variétés durant les années d'étude, nous avons observé une grande augmentation des pontes durant les printemps des années 2008 et 2009 par rapport à 2007.

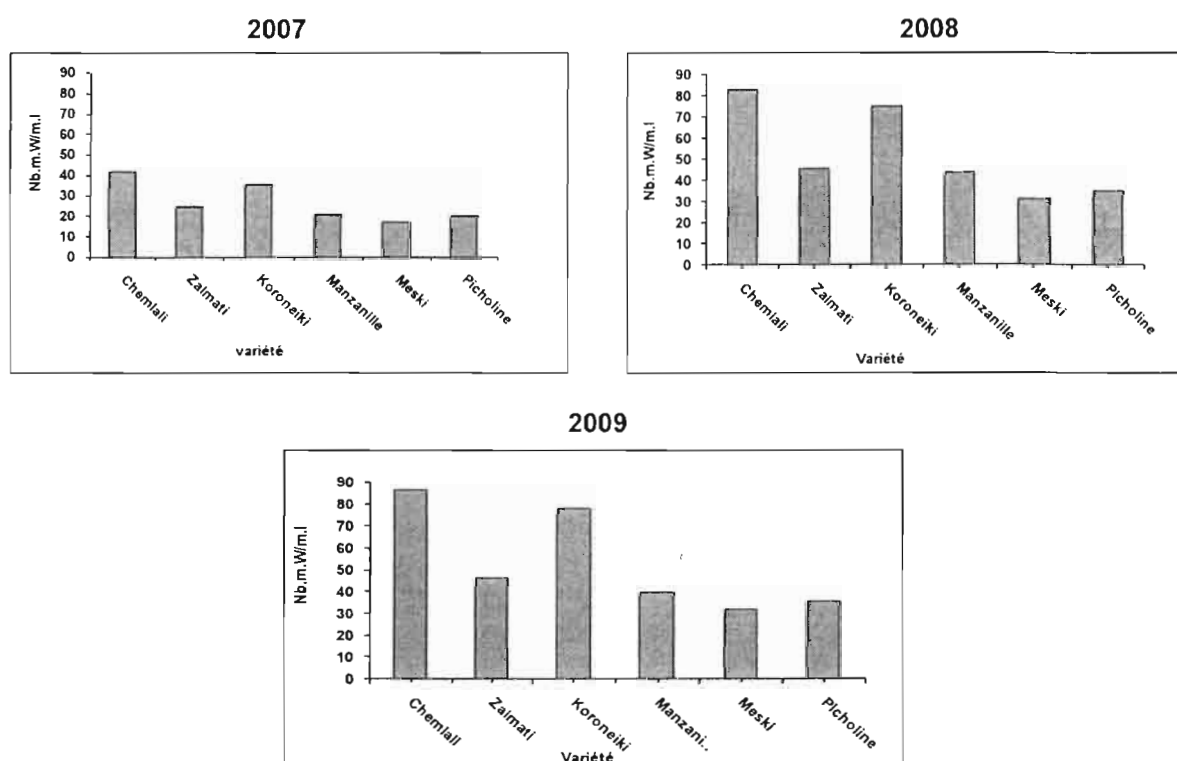


**Figure 1:** Nombre moyen des œufs déposés par mètre linéaire sur quelques variétés durant les saisons printanières de 2007 à 2009 à la station expérimentale Taous.

Par ailleurs, l'analyse statistique appliquée aux moyennes des œufs déposés par mètre linéaire, durant les années d'étude, a montré une légère différence entre les quatre variétés Zalmati, Manzanille, Picholine et Meski, mais des différences hautement significatives avec ces variétés et les autres deux variétés Chemlali et Koroneiki (tableau I).

**Tableau I:** Nombre moyen des œufs déposés par mètre linéaire pour six variétés étudiées.

| Variété    | Chemlali  | Koroneiki | Zalmati   | Manzanille | Picholine | Meski     |
|------------|-----------|-----------|-----------|------------|-----------|-----------|
| Nb.m.W/M.l | 69.9840 a | 62.1000 a | 38.6240 b | 34.9000 b  | 28.8980 b | 26.6460 b |

\* Les chiffres suivis de lettres différents sont significativement différents au seuil de 5%.  
Nb.m.W/M.l: Nombre moyen des œufs déposés par mètre linéaire.

### 3.2. Comparaison de la densité larvaire entre les différentes variétés

Le comptage du nombre des larves sur les variétés étudiées montre une supériorité des variétés à huile sur les variétés de table. Ceci pourrait être dû à la faible ponte sur les variétés à table en comparaison aux variétés à huiles durant les trois années d'étude. En plus, on a enregistré une diminution des effectifs des larves durant la campagne 2007 en comparaison à 2008 et 2009 (Fig. 2).

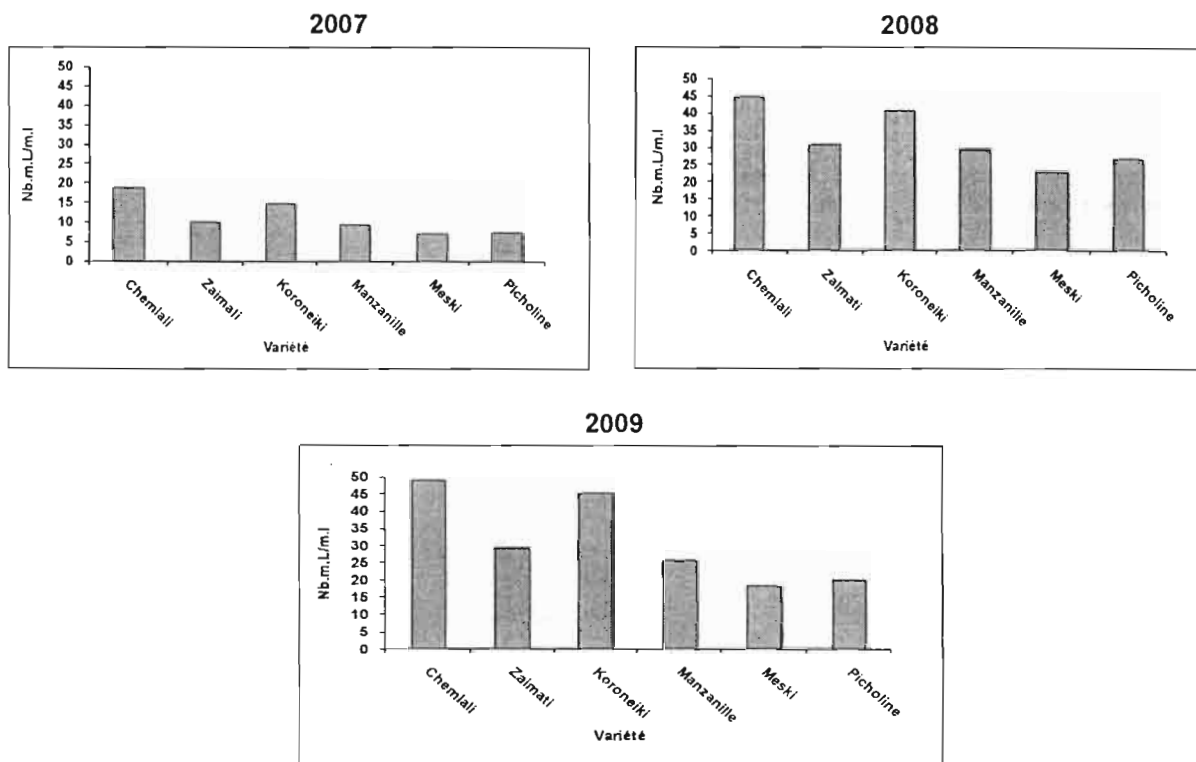


**Figure 2:** Nombre moyen des larves par mètre linaire sur quelques variétés durant les saisons printanières de 2007 à 2009 à la station expérimentale Taous.

De même, on note la présence d'une différence significative au sein du même groupe de variétés et ceci est aussi valable pour les variétés à huiles et celles de table pour les trois années. En effet, l'effectif des larves sur la variété Manzanille est plus élevé par rapport à la Picholine et Meski (variétés de Table). En plus, on note une réduction du nombre des larves sur la variété Zalmati par rapport aux autres variétés à huile (tableau II).

**Tableau II:** Nombre moyen des larves par mètre linaire pour six variétés étudiées.

| Variété    | Chemlali | Koroneiki | Zalmati | Manzanille | Picholine | Meski   |
|------------|----------|-----------|---------|------------|-----------|---------|
| Nb.m.L/M.l | 36.64 a  | 32.84 a   | 23.32 b | 21.65 b    | 17.75 c   | 15.79 c |

\* Les chiffres suivis de lettres différents sont significativement différents au seuil de 5%.

Nb.m.L/M.l: Nombre moyen des larves par mètre linaire.

On peut, ainsi, conclure que les deux variétés Chemlali et Koroneiki sont les plus réceptives pour la ponte des œufs et les plus favorables pour le développement larvaire en comparaison aux autres variétés et spécialement la variété Meski.

### 3.3. Suivre de la dynamique des populations du psylle sur les différentes variétés

L'analyse de la structure des populations des individus immatures du Psylle, durant les années d'étude sur les différentes variétés, a permis d'expliquer certaines caractéristiques biologiques de l'insecte :

On a observé deux pics de ponte avec la présence d'un léger décalage (seulement une semaine) entre les variétés à huile et celles de table. D'une façon générale, durant 2008 et 2009, on a

enregistré le début de la ponte vers mi février avec un premier pic à peu près durant mi mars et le deuxième pic vers la fin du mois d'avril. Alors qu'en 2007, le début de la ponte a eu lieu au début de février et l'apparition du premier pic vers la fin du même mois et le deuxième vers la fin du mois d'avril.

1. L'absence d'une nette différence de l'apparition des larves entre les différentes variétés. En effet, l'apparition des larves a commencé vers la fin du mois de février et la différence réside uniquement au niveau des densités. Ces dernières ont été assez élevées chez les variétés Chemlali et Koroneiki, moyenne chez les variétés Zalmati et Manzanille et basses chez Picholine et Meski.

2. Le faible effectif des larves âgées par rapport au nombre d'œufs déposés s'explique par un taux élevé de mortalité chez des larves jeunes et la faible éclosion des œufs. Ceci est, essentiellement, dû aux températures élevées et à l'impact des ennemis naturels surtout les prédateurs et spécialement *Anthocoris nemoralis*. Ces résultats correspondent, d'une part, à ceux de Zouiten *et al.* (2001) et Jardak *et al.* (2004); Où, ils ont évoqué que la hausse anormale des températures au printemps induit à un blocage du développement des stades larvaires et à une quiescence ovarienne chez l'adulte.

D'autre part, d'après les travaux de l'AFIDOL (2009) et de M'hamed et Chamseddine (1999), ce ravageur est, en effet, soumis à l'action d'insectes prédateurs qui limitent fortement les populations du psylle. Ces auxiliaires sont principalement de la famille des « punaises » et sont présents dans les vergers. Les larves de chrysopes, de syrphes ou celles des araignées peuvent aussi avoir une influence sur les colonies du psylle.

3. Le développement larvaire est interrompu après le deuxième pic de ponte tel qu'on n'a pas détecté la présence des larves âgées. Cependant, on a enregistré une première génération et une deuxième génération incomplète sur les diverses variétés étudiées. Ces résultats se concordent avec celles trouvées par Arambourg et Chermiti (1986), Tajnari (1992) et Civantos (2000) qui ont observé deux générations durant la période printanière.

En guise de conclusion et à la lumière des résultats obtenus, il est impératif d'approfondir d'avantage l'étude de l'impact des variétés sur le développement des populations du psylle de l'olivier et ceci en étudiant la composition de la sève dans les différentes parties de la plante et son rôle dans le choix de l'insecte pour une variété donnée.

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## Caractéristiques des feuilles des vitro-oliviers au cours de l'acclimatation des vitroplants

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### Résumé

Le suivi de l'acclimatation des vitro-plants de 4 variétés d'olivier (Chemlali de Sfax, Chemchali Gafsa, Zarrazi et Oueslati) a permis de mettre en exergue des changements au niveau des feuilles de ces plantules qui se traduisent par la réduction du nombre et le changement de la forme des stomates, quant aux trichomes, ils sont beaucoup plus nombreux et plus dentés après le transfert ex vitro surtout au niveau de la face inférieure des feuilles. La détermination des valeurs de la photosynthèse nette et ses paramètres au cours de l'acclimatation ont permis de conclure qu'au delà de 3 mois de transfert, les vitro-oliviers de toutes les variétés sont considérés comme bien acclimatés étant donné qu'ils ont acquis leur capacité autotrophique surtout après que la photosynthèse nette a enregistré des valeurs assez importantes en même temps que la transpiration et la conductance stomatique régressent sensiblement.

**Mots clés:** Acclimatation, vitro-plants, olivier, trichomes, stomate, photosynthèse.

### Abstract

The follow up of olive tree (Chemlali Sfax, Gafsa Chemchali, Zarrazi and Oueslati cultivars) in vitro plantlets acclimatization permitted to record changes in the leaves resulting in reduced of stomata number, and increased number of trichomes especially on the underside of leaves. The determination of net photosynthesis values and its parameters during acclimation led to the conclusion that beyond 3 months of transfer, vitro-trees of all varieties are considered as well acclimated as the net photosynthesis recorded high values. At the same time transpiration and stomata conductance significantly regressed. As for the content of chlorophyll, it registered an increase during the acclimation.

**Key words:** Acclimation, trichomes, stomata, photosynthesis, olive tree, in vitro.

### 1. Introduction

En Tunisie, des expérimentations sur la micropropagation de l'olivier ont démarré il y a quelques années et les phases de multiplication et d'enracinement ont été réussies pour certaines variétés tunisiennes (Chaari *et al*, 1997; 2002; 2003; Maalej *et al*, 2002).

L'acclimatation, phase finale et clef de tout le processus de clonage, est parfois difficile et délicate à réaliser vu que le changement des conditions physiques de la culture peut avoir des effets néfastes sur les jeunes plantules. Ainsi, il a été démontré que plusieurs structures et paramètres peuvent changer d'une façon abrupte pendant les premiers jours du transfert de l'in vitro et, par conséquent, influencer le taux de survie des vitroplants (Kozai *et al*, 1997; Pospisilova *et al*, 1999, Moncaleán *et al*, 2007). Ainsi, la densité des stomates et leur morphologie sont modifiés par l'augmentation de l'intensité lumineuse et la diminution du taux d'humidité relative pendant la phase d'acclimatation (Blanke et Belcher, 1989 ; Capellades *et al.*, 1990 ; Pospisilova *et al.* 1998). Selon Pospisilova *et al*, (1999), le taux de la photosynthèse nette dépend des conditions de l'environnement de l'acclimatation, en faisant savoir qu'une augmentation de la concentration de CO<sub>2</sub> provoque une hausse importante dans l'activité photosynthétique des vitroplants de *Nicotiana tabacum* . Quant à la transpiration, elle augmente généralement au début d'acclimatation, puis diminue progressivement après l'adaptation avec l'environnement extérieur (Pospisilova *et al*, 1999).

Dans ce papier nous présentons quelques caractéristiques morphologique et physiologique des feuilles de 4 variétés d'olivier in vitro et lors de l'acclimatation des vitro-oliviers.

## 2. Matériel & Méthodes

### 2.1. Conditions de l'acclimatation

Une fois enracinées, les vitro-plantules de 4 variétés d'olivier à huile (Chemlali de Sfax, Chemchali Gafsa, Zarrazi et Oueslati) sont prélevées des milieux d'enracinement et leurs racines sont soigneusement rincées. Les plantules sont ensuite transplantées dans des pots contenant du terreau et placées sous serre où l'humidité relative est en moyenne de 80% assurée par un système de nébulisation et la température ambiante de  $25^{\circ} \pm 5^{\circ}\text{C}$ . Elles sont couvertes pendant les premiers jours de la transplantation. Après un mois, 80 % des plantules restent vivantes.

### 2.2. Comptage des trichomes et des stomates

Pour les trichomes, la méthode consiste à les prélever à l'aide de rubans de scotch collés à la face supérieure ou à la face inférieure des feuilles. Pour enlever les empreintes des stomates, on étale du vernis à ongle transparent à la surface inférieure de la feuille après avoir éliminé les trichomes. Une fois séchée, la couche de vernis est enlevée par du scotch et placée sur une lame porte objet. L'observation et le comptage de ses structures sont réalisés sous microscope avec un logiciel approprié.

### 2.3. Mesure de la photosynthèse, de la transpiration et de la conductance stomatique

La mesure de la photosynthèse (en  $\mu\text{mol}(\text{CO}_2)/\text{m}^2/\text{s}$ ) et de ses paramètres (la transpiration en  $\text{mmole}(\text{H}_2\text{O})/\text{m}^2/\text{s}$  et la conductance stomatique en  $\text{mmole}(\text{H}_2\text{O})/\text{m}^2/\text{s}$ ) sont réalisées à l'aide d'un appareil de mesure de photosynthèse LCI (ADC Bioscientific Ltd).

### 2.4. Détermination de la concentration chlorophyllienne

Les feuilles issues de culture in vitro et au cours de l'acclimatation sont prélevées, pesées, ensuite mises dans des tubes à essai contenant 5ml de N-N-Diméthylformamide ( $\text{C}_3\text{H}_7\text{NO}_2$ : 0.95Kg/l). Après quelques jours à l'obscurité, on mesure la densité optique (DO) de l'extrait à 652nm. Pour calculer la teneur en chlorophylle totale (chl) de la suspension, on a recours à la formule d'Arnon (1949):

$$\text{Chl Totale} = \frac{DO.652}{34.5} \text{ mg/ml.}$$

## 3. Résultats et Discussions

### 3.1. Comptage des stomates et trichomes

Nos résultats montrent, qu'in vitro, la densité des stomates est plus élevée que celle comptabilisée au cours de l'acclimatation pour les 4 variétés (fig1).

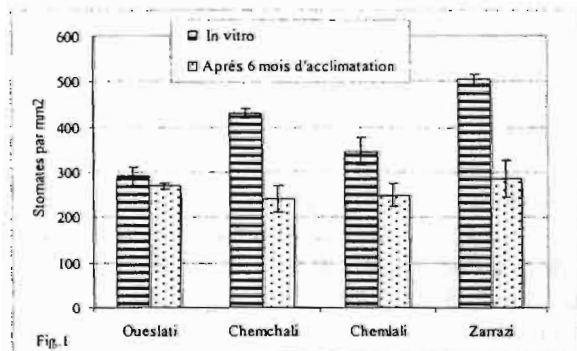


**Figure 1 :** Densité des stomates in vitro et après 6 mois d'acclimatation pour les 4 variétés d'olivier.

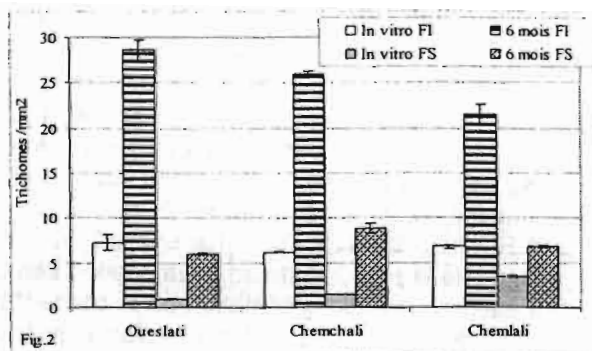


**Figure 2 :** Densité des trichomes in vitro et 6 mois après l'acclimatation à la face supérieure (FS) et inférieure (FI) des feuilles de 3 variétés d'olivier.

Ceci peut être expliqué par l'augmentation de la surface foliaire ce qui a pour conséquence la diminution du nombre de stomates par unité de surface. Cette même diminution a été constatée chez les feuilles de pommier et de rosier après leur transfert ex vitro (Blanke et Belcher, 1989 ; Capellades et al, 1990). Par opposition aux stomates présents uniquement sur la face inférieure de la feuille, chez

l'olivier, les trichomes colonisent aussi bien la face supérieure que la face inférieure des feuilles. Au cours de l'acclimatation, on constate une augmentation du nombre des trichomes sur les deux faces de la feuille (Fig.2), dont ils en assurent la protection, ce qui pourrait être attribué aux conditions plus stressantes de cette phase. Des résultats similaires ont été signalés par Donnelly *et al* (1986) sur les feuilles de Silvan Blackberry.

### 3.2. Mesure de la photosynthèse nette et ses paramètres

Le suivi de l'assimilation chlorophyllienne des vitropousses durant la culture in vitro nous a permis d'enregistrer des taux de photosynthèse assez importants (Tableau 1). Ce qui infirme le fait que les plantules in vitro ont une capacité photosynthétique faible pour fournir une balance de carbone positive (Serret *et al*, 2001). La diminution de la photosynthèse nette, constatée chez les vitroplants de l'olivier, pendant les premiers mois de l'acclimatation a été signalée chez d'autres espèces, elle serait attribuée au stress que subit les vitroplants suite aux changements des conditions environnementales. L'activité photosynthétique des vitroplants de l'olivier reste pratiquement égale à celle enregistrée in vitro au 3<sup>ème</sup> mois après le transfert ex vitro. Ce n'est qu'aux termes du 7<sup>ème</sup> mois que la photosynthèse devient plus importante et proche des valeurs enregistrées chez l'olivier adulte (Msalle, 2002). Estrada-Luna *et al* (2001) trouvent que la photosynthèse des vitroplantules de *Capsicum annum*, espèce annuelle, s'accroît dès le 6<sup>ème</sup> jour de l'acclimatation.

**Tableau 1:** Evolution de la photosynthèse, la transpiration et la conductance stomatique chez les vitroplants de différentes variétés d'olivier durant la culture in vitro et au cours de l'acclimatation ( $\pm$  Ecart Type).

|                                                                                                       | Période          | Chemlali Sfax   | Oueslati Alaa   | Chemchali Gafsa | Zarrazi Zarzis  |
|-------------------------------------------------------------------------------------------------------|------------------|-----------------|-----------------|-----------------|-----------------|
| Photosynthèse en $\mu\text{mol} (\text{CO}_2) \cdot \text{m}^{-2} \cdot \text{s}^{-1}$                | Culture in vitro | 4.55 $\pm$ 0.3  | 4.75 $\pm$ 0.1  | 4.75 $\pm$ 0.3  | 4.6 $\pm$ 0.1   |
|                                                                                                       | Après 3 mois     | 5.6 $\pm$ 0.3   | 5.05 $\pm$ 0.1  | 4.6 $\pm$ 0.2   | 4.75 $\pm$ 0.2  |
|                                                                                                       | Après 7 mois     | 8.05 $\pm$ 0.4  | 7.62 $\pm$ 0.1  | 8.20 $\pm$ 0.1  | 8.15 $\pm$ 0.5  |
| Transpiration en $\text{mmole} (\text{H}_2\text{O}) \cdot \text{m}^{-2} \cdot \text{s}^{-1}$          | Culture in vitro | 2.86 $\pm$ 0.05 | 3.2 $\pm$ 0.05  | 4.20 $\pm$ 0.05 | 4.27 $\pm$ 0.1  |
|                                                                                                       | Après 3 mois     | 2.62 $\pm$ 0.2  | 4.63 $\pm$ 0.05 | 2.5 $\pm$ 0.1   | 3.15 $\pm$ 0.05 |
|                                                                                                       | Après 7 mois     | 1.2 $\pm$ 0.1   | 0.6 $\pm$ 0.02  | 0.15 $\pm$ 0.05 | 0.8 $\pm$ 0.1   |
| Conductance stomatique En $\text{mmole} (\text{H}_2\text{O}) \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ | Culture in vitro | 0.46 $\pm$ 0.01 | 0.45 $\pm$ 0.1  | 0.55 $\pm$ 0.05 | 0.61 $\pm$ 0.05 |
|                                                                                                       | Après 3 mois     | 0.86 $\pm$ 0.05 | 0.85 $\pm$ 0.1  | 0.35 $\pm$ 0.05 | 1.15 $\pm$ 0.2  |
|                                                                                                       | Après 7 mois     | 0.09 $\pm$ 0.01 | 0.05 $\pm$ 0.1  | 0.06 $\pm$ 0.01 | 0.12 $\pm$ 0.1  |

En ce qui concerne la transpiration et la conductance stomatique, nos résultats montrent une augmentation importante de ces deux paramètres après la transplantation des vitroplants d'olivier. Ceci est en accord avec les travaux de Pospisilova *et al*, (1998) qui trouvent que la transpiration chez les vitroplants de *Nicotiana tobacum* s'accroît deux semaines après le début de l'acclimatation. Ce phénomène devrait être en relation avec le degré d'ouverture des stomates. D'après Pospisilova *et al*. (1999), l'augmentation des taux de transpiration chez les vitroplants juste après leur transfert ex vitro est due au développement tardif de la cuticule, de la cire épicuticulaire ainsi qu'au non fonctionnement des stomates durant la culture in vitro. L'augmentation de la transpiration et de la conductance stomatique pendant les premiers jours de l'acclimatation trouverait aussi son explication dans la diminution du potentiel hydrique de l'atmosphère autour des plantules. En effet, dans les tubes de culture in vitro, elles étaient en présence d'une humidité relative saturante ce qui limitait considérablement leur transpiration.

### 3.3. Variation de la chlorophylle totale

L'examen de la variation de la concentration chlorophyllienne dans les feuilles des vitroplants, au cours de l'acclimatation et in vitro montre à peu près la même évolution pour toutes les variétés (Tableau 2). En condition de culture in vitro, la teneur en chlorophylle totale des feuilles varie de 1,9 mg/g à 2,9 mg/g selon la variété. Les premiers mois d'acclimatation pourraient être marqués par une baisse de la concentration chlorophyllienne totale ce qui serait corrélé avec une réduction de l'activité photosynthétique. Au bout de 8 mois d'acclimatation, on observe une augmentation progressive de la concentration chlorophyllienne chez les feuilles des vitroplants de toutes les variétés étudiées (de 2.5 à 3.3 mg/g). Pospisilova *et al* (1998) montrent que les quantités de chlorophylle a et b des plantules de *Nicotiana tobacum* diminuent juste après la transplantation ex vitro, puis reprennent deux semaines après. Cette évolution de la teneur en chlorophylle tout au long de l'acclimatation pourrait

être expliquée par l'augmentation du rapport tissu palissadique / tissu lacuneux au cours de ce processus.

**Tableau 2:** Evolution de la chlorophylle totale (en mg/ g) chez les vitroplants des différentes variétés d'olivier durant la culture *in vitro* et après 3 et 8 mois d'acclimatation ( $\pm$ Ecart type).

| Période                 | Chemlali Sfax  | Oueslati Alaa | Chemchali Gafsa | Zarrazi Zarzis |
|-------------------------|----------------|---------------|-----------------|----------------|
| Culture <i>in vitro</i> | 2.9 $\pm$ 0.1  | 2.2 $\pm$ 0.2 | 2.9 $\pm$ 0.1   | 1.9 $\pm$ 0.1  |
| Après 3 mois            | 2.9 $\pm$ 0.1  | 2.1 $\pm$ 0.1 | 2.9 $\pm$ 0.15  | 2.1 $\pm$ 0.2  |
| Après 8 mois            | 3.2 $\pm$ 0.21 | 2.5 $\pm$ 0.1 | 3.3 $\pm$ 0.2   | 3.0 $\pm$ 0.2  |

Enfin, nos résultats permettent de conclure qu'au vu des changements enregistrés chez les vitro-oliviers au delà du 3<sup>ème</sup> mois d'acclimatation, ceux-ci sont considérés comme bien acclimatés étant donné que leur système photosynthétique fonctionne d'une façon normale et leur capacité autotrophique est supposée bien acquise.

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## Aptitudes variétales en culture intensive de l'olivier en Tunisie (région de Sfax)

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### Résumé

Le présent travail a été entrepris dans le but d'évaluer les potentialités végétatives et productives de sept cultivars d'oliviers locaux et étrangers en régime intensif irrigué en goutte à goutte cultivés sous un climat aride (Région de Sfax). Cette étude a été menée sur les variétés à huile Chemlali, Chétoui, Koroneiki et Coratina, et les variétés de table Picholine, Meski et Manzanille. Ces arbres sont âgés de six ans, issus de boutures herbacées et plantées avec une densité de 204 pieds par hectare (7 m x 7 m). Les résultats ont révélé des différences entre les variétés et les types variétaux par rapport à l'architecture de l'arbre, aux caractéristiques morphologiques de la feuille, à la croissance végétative et à la biologie florale ainsi qu'à l'évolution du diamètre et du poids frais des fruits. Ces différences ont été aussi affirmées à partir des relations établies entre la surface foliaire et le produit de la longueur et de la largeur des feuilles et entre la croissance végétative primaire et secondaire des rameaux.

**Mots clés:** olivier, variétés, culture intensive, caractéristiques végétatives et fructifères, croissance primaire et secondaire

### Olive trees characteristics (*Olea europaea* L.) under intensive condition cultivated in Tunisia (Sfax site)

### Abstract

This work has been undertaken with the aim of evaluating the vegetative and productive potentialities of seven local and foreign varieties of olive trees cultivated under an arid climate in an intensive mode and drop-to drop irrigated, in goblet form. The study has been carried out on the varieties of oil: Chemlali, Chétoui, Koroneiki and Coratina, and the varieties of table Picholine, Meski and Manzanille. These trees are six years old and planted with a density of 204 trees per hectare (7 m X 7 m). The results have revealed differences between varieties compared to the architecture of the tree, to the morphological characteristics of the leaf, to the vegetative growth, to the floral biology and the evolution of the diameter and fresh weight of the fruits. These differences have been also confirmed by the relations established between the leaf area and the product of leaf length and width and between the primary and secondary vegetative growth of the branches.

**Key words:** Olive varieties, intensive cultivation, vegetative and fruiting characteristics, primary and secondary growth.

### 1. Introduction

La stratégie oléicole Tunisienne actuelle est la maîtrise de la production par une meilleure gestion de la fluctuation inter-annuelle. Ceci ne peut être réalisé que par une maîtrise de l'alternance et une amélioration de la productivité du secteur et de la qualité du produit. Ainsi, parallèlement au rajeunissement des anciens vergers et l'installation de nouvelles exploitations, une extension de la conduite de la culture en intensive a été enregistrée ces dernières années. Actuellement, les superficies des oliveraies irriguées représentent 2.5 % des superficies totales et le nombre de pieds 5.8% du nombre total de pieds (DGPA, 2006).

L'adoption de la conduite en intensif a été aussi accompagnée par un élargissement de la gamme variétale existante à travers l'introduction de nouvelles variétés étrangères dont leur degré d'adaptation aux conditions édaphiques et climatiques tunisiennes n'est pas encore bien établi. Afin d'évaluer les performances végétatives et reproductives et les caractéristiques pomologiques aussi bien des variétés locales qu'étrangères dans des conditions édaphiques et climatiques soumises à conduites différentes, plusieurs études ont été réalisées (Aïachi et al., 2008; Mehri et al., 2001).

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L'objectif de ce travail est d'évaluer les potentialités végétatives et productives de sept cultivars d'oliviers locaux et étrangers âgés de 6 ans, conduits en régime intensif irrigué et cultivés sous un climat semi-aride du sud de la Tunisie, dans la parcelle expérimentale de l'Institut de l'olivier Ettaoues.

## 2. Matériel et Méthodes

L'expérience a lieu dans le Sud de la Tunisie Gouvernorat de Sfax, Lat. 34°N, Long. 21°E (domaine expérimental de L'Institut de l'olivier). Le Climat est semi-aride ( $P = 250$  mm/année et  $ET_o = 1325$  mm/an). La parcelle d'étude est occupée par de jeunes arbres âgés de 6 ans (plantation en Mars 2002), issus de boutures herbacées et composée de 7 variétés d'olivier dont quatre à huile *Chemlali*, *Chétoui*, *Koroneiki* et *Coratina* et trois de table : *Meski*, *Picholine* et *Manzanille*. Ces variétés sont conduites en intensif en forme gobelet avec une densité de plantation de 204 pieds/ha (7 m x 7 m). Le système d'irrigation adopté est le goutte à goutte.

Les valeurs journalières de l'évapotranspiration de la culture  $ET_c$  ont été calculées à partir de l'évapotranspiration de référence  $ET_o$  estimée selon la méthode de la FAO-Penman-Montheith (Allen et al., 1998) à l'aide du logiciel Mabilia- $ET_o$  (Jaboun et Sahli, 2004).

$$ET_c = K_c K_r ET_o$$

Où  $K_c$  représente le coefficient culturel de l'olivier, variant entre 0,45 et 0,65 durant l'année. Étant donné que la moyenne des indices de couverture (IC) dans la parcelle expérimentale est égale à 20% ( $IC < 30\%$ ), le coefficient de réduction  $K_r$  utilisé est de 0,7. L' $ET_c$  est estimée à 449 mm/an.

Les mesures ont porté aussi bien sur paramètres caractérisant la biomasse de l'arbre, sur le suivi des stades phénologiques, sur l'évolution de la dynamique de croissance des rameaux aussi bien en longueur qu'en épaisseur et enfin sur la production et la composition acide des huiles obtenues à partir des différentes variétés.

## 3. Résultats

Au niveau du développement de la frondaison, le rapport HA/DM (hauteur de l'arbre/diamètre de la frondaison) montre que la *Koroneiki* est à tendance plus érigée, le rapport est égal à 1,15. Les variétés *Meski*, *Manzanille* et *Chétoui* montrent une frondaison plus ou moins sphérique car le rapport est proche de 1. Enfin, *Chemlali* montre un rapport inférieur à 1 signifiant un port plus ou moins étalé. Le volume de frondaison varie entre 26 m<sup>3</sup>/arbre soit 5370 m<sup>3</sup>/ha chez la *Picholine* et la *Coratina* et entre 13 et 15 m<sup>3</sup>/arbre soit un volume de 2650 et 3060 m<sup>3</sup>/ha (*Meski*, *Koroneiki* et *Chétoui*). L'indice de couverture indiquant le taux de recouvrement du sol soit l'occupation du sol, varie entre 15 et 24% (Tableau 1).

**Tableau 1:** Paramètres caractéristiques de la frondaison

| Variétés   | HA* (m) | DM (m)  | HA/D     | VF (m <sup>3</sup> ) | IC (%) |
|------------|---------|---------|----------|----------------------|--------|
| Picholine  | 4,0±0,6 | 3,9±0,4 | 1,04±0,2 | 26,64±7,1            | 23±5   |
| Coratina   | 4,0±0,2 | 3,8±0,3 | 1,04±0,2 | 26,64±8,1            | 24±4   |
| Koroneiki  | 3,4±0,4 | 3,0±0,3 | 1,15±0,4 | 13,68±0,7            | 15±3   |
| Meski      | 3,4±0,1 | 3,3±0,2 | 1,03±0,1 | 15,23±3,4            | 17±3   |
| Manzanille | 3,6±0,3 | 3,4±0,4 | 1,06±0,2 | 19,28±6,76           | 19±5   |
| Chétoui    | 3,3±0,3 | 3,1±0,2 | 1,06±0,4 | 14,56±3,7            | 15±2   |
| Chemlali   | 3,2±0,2 | 3,6±0,2 | 0,88±0,1 | 21,66±3,33           | 21±2   |

\*HA : hauteur arbre, DM, SF et VF, diamètre, surface et volume de la frondaison, IC : indice couverture :



Au niveau du suivi des différents stades phénologiques, les variétés *Manzanille*, *Chemlali*, *Meski* et *Koroneiki* sont à floraison précoce tandis que la *Coratina* et *Chétoui* sont à floraison plus tardive. L'accumulation du froid durant cette année estimée a débuté le 11 décembre pour atteindre son maximum au 20 février avec 427 UF puis elle a chuté par la suite pour atteindre 244 UF vers le 31 mars en raison du réchauffement du climat durant cette période. Ainsi, il apparaît que les variétés *Coratina* et *Chétoui* (floraison plus tardive) ont des besoins en froid plus importants que les autres variétés (Figure 1).

| Dates                 | Picholine      | Coratina | Manzanille     | Chétoui        | Chemlali       | Meski          | Koroneiki |
|-----------------------|----------------|----------|----------------|----------------|----------------|----------------|-----------|
| 12/2                  | B              | A        | A              | A              | B              | A              | B         |
| 1/3                   | C              | A        | B              | B              | C              | B              | C         |
| 24/03                 | D              | B        | D              | C              | D              | C              | C         |
| 01/04                 | E              | C        | E              | D              | E              | D              | D         |
| 10/04                 | E              | D        | E              | D              | E              | E              | E         |
| 14/04                 | F (DF)         | E        | F (DF)         | E              | F (DF)         | F              | F         |
| 17/04                 | F1 (PF)        | F (DF)   | F1 (PF)        | F (DF)         | F1 (PF)        | F1             | F1        |
| 21/04                 | F1 (PF)        | F1 (PF)  | G (FF)         | F1 (PF)        | G (FF)         | G              | F1        |
| 29/04                 | G (FF)         | F1 (PF)  | H              | G (FF)         | G (FF)         | H              | F1        |
| 04/05                 | H              | G (FF)   | I              | H              | H              | I              | G (FF)    |
| 10/05                 | I              | H        | I <sub>1</sub> | I              | I              | I <sub>1</sub> | H         |
| 22/05                 | I <sub>1</sub> | I        | I <sub>1</sub> | I <sub>1</sub> | I <sub>1</sub> | I <sub>1</sub> | I         |
| Durée de la floraison | 20 jrs         | 18 jrs   | 15 jours       | 18 jours       | 21 jours       | 15 jours       | 26 jours  |

**Figure 1:** Stades Repères de différentes variétés en conditions intensives (Ettaaoues, régions de Sfax, 2008).

Le suivi de la dynamique de croissance des rameaux fructifères (années on) a montré que l'allongement primaire est variable selon les variétés. En effet, la croissance est monophasique et la période active de croissance se situe entre la mi-Avril et début Juillet (début de sclérification de l'endocarpe) pour les rameaux fructifères. Elle ralentit par la suite, due à une compétition au niveau des assimilats au profit du fruit (Bignami et al., 1993). L'allongement cumulé total varie entre 2,5 cm (*Chemlali*) et 6,1 cm (*Meski*). Le nombre d'entre-nœuds édifiés varie entre 2,1 et 4,81 chez ces mêmes variétés (Figure 2).

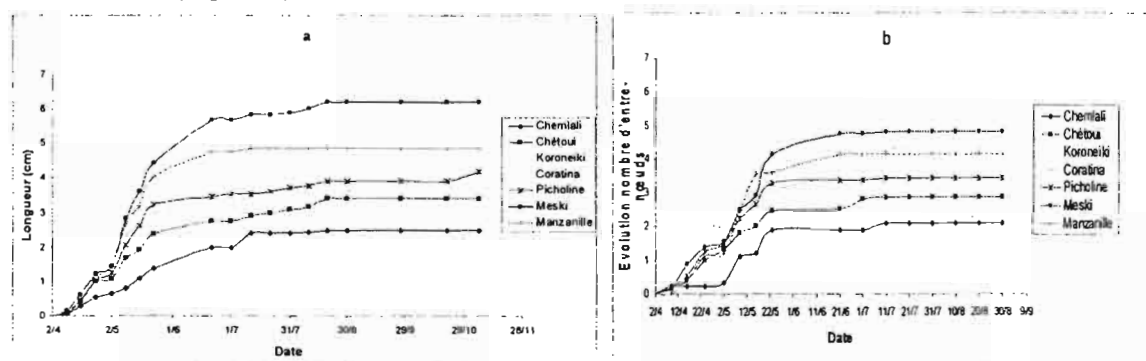


**Figure 2:** Evolution de l'allongement des rameaux (a) et du nombre d'entre-nœuds édifiés (b) chez différentes variétés.

L'étude de la croissance en épaisseur des rameaux montre une courbe composée de deux sigmoïdes quelque soit la variété étudiée: une première croissance ayant lieu entre début Avril et début juillet et une seconde croissance ayant lieu à l'entrée de l'automne entre début Septembre et la deuxième décennie d'Octobre (Figure 3a). Mezghani Aïachi (2009) montre que la croissance en épaisseur est bimodale même en condition de culture pluviale, pouvant être expliquée par une théorie hormonale ou par une théorie source-puits. La Figure 3b montre qu'il existe une relation très étroite entre la croissance en longueur et en épaisseur des rameaux tout au long du cycle de végétation.

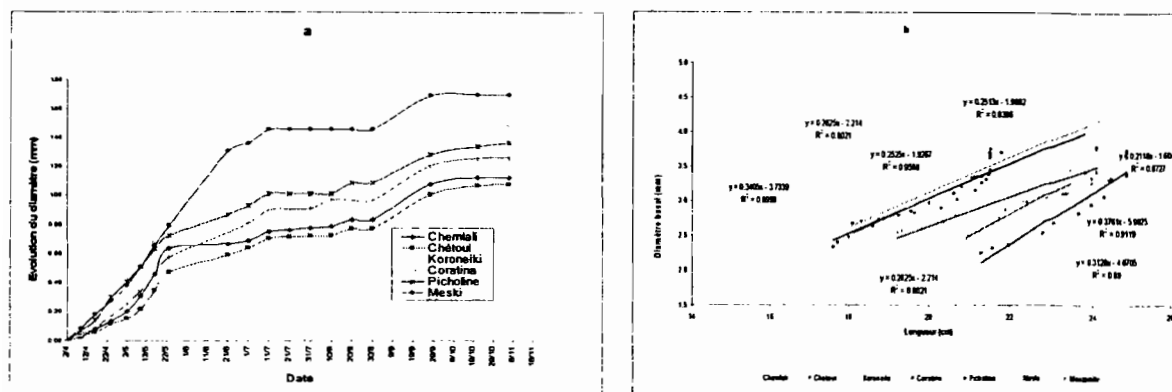


**Figure 3:** Evolution de la l'accroissement en diamètre du rameau et relations entre la longueur et le diamètre du Rameau.

En culture intensive, la *Chemlali* montre la production moyenne la plus importante; elle est de l'ordre de 6925 Kg.ha<sup>-1</sup>.an<sup>-1</sup> suivie par les variétés *Picholine*, *Coratina* et *Koroneiki* qui notent une production de l'ordre de 3000 kg/ha. La *Chétoui* montre la production la plus faible (1032Kg/ka) ainsi que l'efficacité de production la plus faible 0,37 confirmant les résultats de Larbi et al., indiquant que la *Chétoui* conduite en hyper-intensif montre un faible ratio et plutôt une tendance à la végétation. La *Chemlali* indique une efficacité de production soit la quantité d'olives par m<sup>3</sup> la plus importante (2,89Kg.m<sup>-3</sup>) qui montre que l'équilibre production/biomasse végétative est favorable chez cette variété d'où la bonne réponse de cette variété à l'intensification.

La matière fraîche du fruit varie de 1,2g chez la *Koroneiki* (variété à huile) à 6,2g chez la *Meski* (variété de table). Des différences significatives sont observées entre les variétés au niveau de la composition acide. L'acide oléique varie entre 55% pour *Meski* et 75,2 % chez *Koroneiki*. L'acide palmitique varie entre 11,7% (*Coratina*) à 16,6% (*Chemlali*) et l'acide linoléique de 7,9% (*Koroneiki*) à 24% (*Meski*). Les variétés *Koroneiki*, *Manzanilla*, *Luccio* et *Coratina* ont un taux élevé d'acide oléique (>65%) et un faible taux d'acide palmitique (11,7-13,5%). La *Chemlali* et la *Meski* montrent les plus fort taux d'acide palmitique autour de 16,6 et le plus faible taux d'acide oléique 55% (Tableau 2).

**Tableau 2:** Production et composition acide des différentes variétés.

| Variétés   | Prod. Moy de 2 années (on et off) Kg/ha | Efficience Product Kg/m <sup>3</sup> | Poids du fruit gr | Composition acide       |                      |                         |
|------------|-----------------------------------------|--------------------------------------|-------------------|-------------------------|----------------------|-------------------------|
|            |                                         |                                      |                   | A. palmitique C16:0 (%) | A.Oléique C18 :1 (%) | A.Linoléique C18 :2 (%) |
| Picholine  | 3131                                    | 0,70                                 | 4,68              | 14                      | 67,7                 | 13,9                    |
| Coratina   | 3117                                    | 0,89                                 | 2,64              | 11,7                    | 76                   | 9                       |
| Koroneiki  | 3166                                    | 0,81                                 | 1,21              | 12,9                    | 75,2                 | 7,9                     |
| Meski      | 1983                                    | 0,79                                 | 6,17              | 16                      | 55                   | 24                      |
| Manzanilla | 2139                                    | 0,99                                 | 4,73              | 13                      | 68                   | 14                      |
| Chétoui    | 1032                                    | 0,37                                 | 2,68              | 12                      | 64                   | 18                      |
| Chemlali   | 6925                                    | 2,89                                 | 0,94              | 16,6                    | 58,3                 | 19,4                    |

**Stade A:** stade hivernal, **Stade B:** réveil végétatif, **Stade C:** Formation des grappes florales, **Stade D:** Gonflement des boutons floraux, **Stade E:** Différentiation des corolles, **Stade F:** Début de floraison, **Stade F1:** Pleine floraison, **Stade G:** Chute des pétales, **Stade H:** Nouaison, **Stade I:** Grossissement des fruits (1<sup>er</sup> stade), **Stade I<sub>1</sub>:** Grossissement des fruits (2<sup>ème</sup> stade).

#### 4. Conclusions

Les résultats obtenus montrent des potentialités et des aptitudes variétales différentes entre les sept variétés cultivées en culture intensive dans la région de Sfax. Les variétés *Picholine*, *Coratina* et *Chemlali* montrent une biomasse végétative aérienne plus importante induisant un meilleur taux de recouvrement de la parcelle. Par ailleurs, l'étude de la chronologie des deux processus de croissance primaire et secondaire, permet d'apporter des informations sur le déroulement des deux phénomènes le long de la saison de végétation et la possibilité de l'existence d'une compétition entre eux. La



variabilité du développement des bourgeons floraux montre des exigences différentes en terme de besoins en froid. La variété Chétoui montre une floraison plus tardive qui se reflète plus tard aussi au niveau d'une production plus faible. Cette variété semble non adaptée aux régions du centre et du sud même en conditions de culture irriguée. Par contre, les deux variétés *Koroneiki* et *Coratina* peuvent montrer des potentialités très intéressantes dans cette région de point de vue de production (3 tonnes/ha) et de point de l'huile montrant des caractéristiques très intéressantes. En effet, ces deux variétés montrent un pourcentage d'acide palmitique inférieure à 13 et un taux d'acide oléique supérieur à 75%.

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## Lutte biologique contre les principaux agents phytopathogènes de l'olivier par l'intermédiaire des Actinobactéries (Actinomycètes)

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### Résumé

A partir de différents échantillons de sols provenant de la rhizosphère de trois variétés locales (Meski, Chetoui et Chemlali) de la région de Nabeul, on a procédé à l'isolement d'une collection de 40 souches d'actinobactéries, afin de les tester contre certains agents fongiques pathogènes de l'olivier, notamment des souches de *Phoma*, de *Fusarium* et de *Colletotrichum*. Les 40 isolats ont fait l'objet d'une étude enzymatique (catalase, cellulase, chitinase...) et les résultats ont montré que certaines souches présentent des activités assez intéressantes d'où notre intérêt à les sélectionner en tant qu'agents de lutte antifongique. Les tests d'antagonisme (tests de confrontation, technique des puits...) effectués, nous ont indiqué que les souches BA1, BA3, BA6, et BA10 ont stoppé la croissance des pathogènes visés. Dans une seconde étape, les tests d'inoculation « *in vivo* », qui sont actuellement en cours, nous ont préliminairement montré des conclusions très similaires. A la lumière de ces résultats, nous avons entrepris l'identification biochimique ainsi que la caractérisation moléculaire de ces isolats (PCR 16S, et séquençage partiel de l'ADNr 16S).

**Mots clés:** *Olea europaeae*, pathogens, biological control, actinobacteria, inoculation test.

### Abstract

A collection of 30 Rhizobacteria and Actinomycetes strains was isolated from different soils' samples of the rhizosphere of three olive tree varieties: *Meski*, *Chetoui* and 231, a hybrid issued from a crossbreeding between *Meski* and *Picholine*. After morphologic identification and biochemical tests (catalase, oxydase) these strains were tested using "in vitro" techniques, against some fungal pathogens such *Phoma sp* and *Fusarium sp*. Results showed that B2 strain stopped the development of two *Fusarium sp* strains'.

**Keywords:** Rhizosphere, Rhizobacteria, Actinomycetes, Test "in vitro"

### 1. Introduction

Malgré le développement de molécules de synthèse très efficaces, l'histoire des pesticides n'aura démontrée qu'une gestion strictement basée sur l'emploi des produits chimiques resterait toujours fragile à plusieurs égards, car l'acquisition de résistance chez les nuisibles, la pollution environnementale et les résidus de ces molécules chimiques sur les aliments demeurent possibles. La lutte biologique constitue une alternative à la lutte chimique afin d'un meilleur respect de l'environnement des cultures et de la santé des agriculteurs et des consommateurs. La rhizosphère compte parmi les régions du sol où l'activité microbienne est très intense. Cette dernière est stimulée par les exsudats racinaires qui représentent une source d'énergie pour les micro-organismes rhizosphériques (Rengel 1997). Les interactions microorganismes-plantes au niveau de la rhizosphère sont d'une influence capitale sur leur état sanitaire et sur la qualité du sol puisque leur association aux racines peuvent aider la plante hôte à lutter contre les stress dus à la sécheresse, aux problèmes nutritionnels ou encore aux pathogènes du sol. Parmi les micro-organismes antagonistes, les rhizobactéries en général et les actinomycètes, en particulier sont de plus en plus utilisés par les microbiologistes et constituent des agents prometteurs de lutte intégrée contre les agents phytopathogènes, grâce à leur très grande capacité à coloniser les racines (Crawford et al. 1993). Certaines rhizobactéries et notamment celles stimulant la croissance des plantes (PGPR) peuvent être considérées comme des micro-organismes utiles de la rhizosphère et des agents de lutte, en effet, plusieurs études prouvent que ces bactéries diminuent l'impact de plusieurs maladies racinaires en plus de promouvoir la croissance des plantes.

Dans le cadre de la protection de l'oléiculture, pivot de l'agriculture tunisienne, le présent travail englobe une investigation de la biodiversité de la microflore rhizosphérique de l'olivier, à la recherche de bactéries et d'actinomycètes, agents de contrôle des principales maladies de cet arbre.

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## 2. Matériel et Méthodes

### 2.1. Echantillonnage

Pour la collection des actinomycètes : les prélèvements des échantillons de sols ont été effectués à travers différentes régions de la Tunisie : Tunis, Siliana, et Nabeul, et à partir de la rhizosphère de plusieurs variétés d'olivier : *Meski* et *Chétoui* (variétés locales) et un hybride issu du croisement entre la variété *Meski* et *Picholine* : 23I.

Pour la collection des pathogènes fongiques: l'échantillonnage consiste en des prélèvements de feuilles, rameaux, fruits, et racines présentant plusieurs anomalies : Feuilles (dépôts grisâtre), Rameaux (blessures, échancrures, brunissement), Fruits (affaissement, momification, pourriture), Racines (pourriture, dessèchements).

### 2.2. Isolement des souches rhizobactéries et des Actinomycètes

Les Actinobactéries sont isolées suite à la préparation de solutions mères en mélangeant séparément chaque échantillon de sol (1,5g) avec 10 ml d'eau physiologique stérile. Ces solutions sont par la suite mises sous agitation pendant au moins une heure, on effectue alors une série de dilutions allant de  $10^{-1}$  à  $10^{-7}$ . Un volume de 100µl est étalé sur des boîtes contenant du milieu Lüedemann (Lüedemann, 1968), additionné d'un antifongique, le cycloheximide (0,01%). Les boîtes sont par la suite mises pour incubation à 28°. Les actinomycètes, étant à croissance lente, l'incubation dure entre deux semaines et un mois. Les colonies ayant les morphologies typiques sont sélectionnées pour les études ultérieures.

### 2.3. Isolement des phytopathogènes cibles:

Les champignons sont isolés sur des boîtes de pétri contenant du milieu PDA additionné d'un antibiotique. Après désinfection à l'eau de javel 3% pendant 3min et rinçage avec de l'eau distillée stérile, les échantillons sont découpés en de petits morceaux puis déposés sur les boîtes qui sont alors mises pour incubation à 28°C

Tous les isolats sont par la suite, sélectionnés, repiqués sur milieux solides, et placés à 28°C. La pureté des colonies est confirmée après analyse macroscopique et microscopique des cultures. Ces dernières sont ensuite conservées à court et à long terme. A court terme, la conservation se fait dans des cryotubes à gélose inclinée. A long terme, dans du milieu Lüedemann ou PDA à 30% de glycérol et conservés -80°C.

### 2.4. Identification des rhizobactéries

Les isolats étudiés ont fait l'objet d'une analyse macroscopique. Nous avons en effet, noté la forme, la texture, le diamètre ainsi que les pigmentations des colonies. Cette première analyse a été suivie par une observation microscopique sous microscope optique. Un examen à l'état frais afin de vérifier la morphologie cellulaire et une coloration de Gram ont été ainsi entrepris.

L'identification morphologique a été secondée par une caractérisation biochimique, nous avons de ce fait étudié quelques activités enzymatiques de certains isolats

### 2.5. L'activité antagoniste des rhizobactéries

Le pouvoir antifongique des souches isolées a été testé « *in vitro* » en utilisant la technique de confrontation directe qui consiste à déposer sur des boîtes de pétri contenant 15 à 20 ml de milieu PDA, un disque faisant 7 à 8 mm de diamètre provenant des cultures du champignon pathogène, face à cet explant, une strie de la bactérie à tester est effectuée à l'aide d'une anse de platine. Trois répétitions ont été retenues pour chaque test. Les témoins sont représentés par des boîtes de Pétri contenant uniquement le champignon pathogène, et l'ensemble des boîtes est placé à une température de 28°C. L'évolution de la croissance mycélienne est effectuée par la mesure du diamètre de la colonie mycélienne du champignon pathogène.

### 3. Résultats et Discussions

#### 3.1. Isolement des souches actinomycètes :

Suite aux prélèvements effectués, une collection importante de souches a été établie, seulement une trentaine d'isolats n'a été retenu (tableau1).

#### 3.2. Isolement des pathogènes :

Les prélèvements nous ont permis d'obtenir une collection intéressante englobant des souches pouvant toucher la partie racinaire (*fusarium*, *verticilium*), les fruits (*trichotecium*) et le bois de l'olivier (*phoma*)

#### 3.3. Observation macroscopique des Actinomycètes :

Les différents isolats, présentent des morphologies variées. Les colonies peuvent être rondes et régulières mais plus généralement dentelées et étoilées, présentant des textures lisses mais bien souvent rugueuses signe de l'apparition d'un mycélium aérien assez intense. Leur diamètre varie entre 3 à 5 mm. Le plus souvent, elles sont convexes, et très ancrées dans la gélose, témoignant ainsi de l'intensité de croissance du mycélium végétatif. Les colonies libèrent des pigmentations diverses, dont les plus importantes sont de couleur blanche, marron, orange et rouge. Certains isolats ont la capacité de changer de pigmentation avec la sporulation. Les propriétés morphologiques notées, ont été déjà reportés par (Urzi et al. 2004) et (Gorbushina 2007).

#### 3.4. Observation microscopique des Actinomycètes

**Coloration de Gram :** La coloration de Gram a indiqué que tous les isolats Actinomycètes sont des Gram-positives.

**Examen frais :** Sous microscope optique, plusieurs colonies présentent une structure hyphale, extrêmement filamenteuse et ramifiée rappelant celle des champignons. Avec la sporulation, les hyphes se fragmentent engendrant de petites cellules aussi bien en forme de petits bâtonnets que de structures conoïdes, pouvant être isolées, groupées en paire ou même en amas cellulaires.

#### 3.5. Tests de la catalase et de l'oxydase

Suite au test de la catalase, un dégagement très intense de bulles d'air a été observé sans exception chez toutes les colonies testées. De ce fait, on conclue que tous nos isolats sont catalase positives. Contrairement, au test de la catalase, celui à l'oxydase, s'est montré négatif pour la quasi totalité des souches. En effet, seulement la ch10, a montré une coloration violette qui n'est d'ailleurs pas très prononcée. Ces résultats démontrent que ces souches sont bien des Actinobactéries.

#### 3.6. Tests de confrontation directe in « vitro »

Parmi les isolats, certains ont été sélectionnés, pour être testés contre les souches fongiques obtenues. Les tests de confrontations directs entre la souche B6 et deux souches de *Fusarium* a montré que la croissance mycélienne des souches témoins est plus importante par rapport à celle obtenue avec la confrontation. En effet, une action inhibitrice exercée par la souche B2 a été observée. L'apparition d'une zone d'inhibition suivie par un arrêt total de la croissance mycélienne témoigne de l'efficacité de cette souche. Le calcul du taux d'inhibition confirme ces résultats, l'inhibition est en effet, à plus de 20%. (Figure 1).

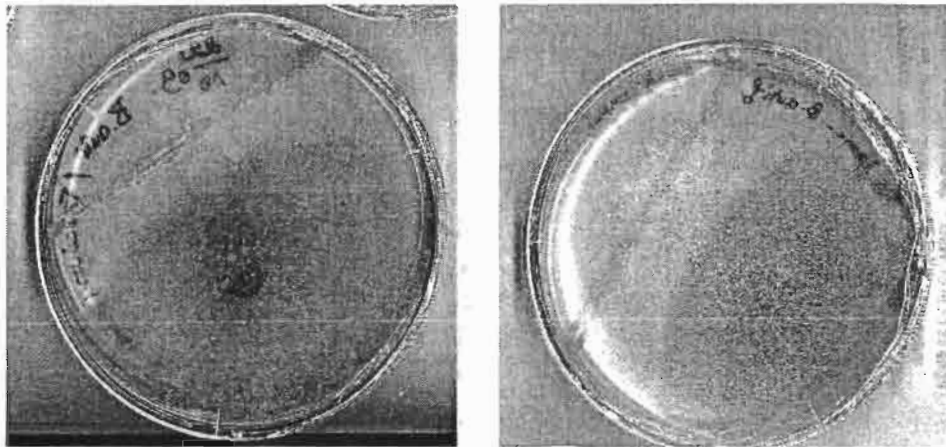


**Figure 1:** test de confrontation entre la souche B2 et deux types de *Fusarium*

#### 4. Conclusion

Le travail présenté dans cette étude, consiste à tester l'effet *in vitro* de quelques souches actinomycètes et d'une rhizobactérie B2 sur la croissance mycélienne de différents pathogènes et notamment deux isolats de *Fusarium sp.*

La confrontation directe de B2 et des *Fusarium sp.*, s'est traduite par un arrêt à distance de la croissance mycélienne du pathogène et la formation d'une zone d'inhibition entre les deux colonies confrontées. Dans une étape prochaine, il serait intéressant d'étudier le pouvoir antagoniste de ces souches « *in vivo* ».

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## Water relations and use of six years olive trees cultivated under deficit irrigation regimes

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### Abstract

Field experimentation has been performed in 2008 to study the effect of irrigation schedules on water relations for young olive trees cultivated at 7mx7m spacing at the experimental field 'Taoues' (Lat.34°, Long.21°) of *Institut de l'Olivier*, southern Tunisia. Plants were irrigated from March to October with sprinkler irrigation system, considering three water irrigation amounts (IA): 20%ET<sub>c</sub>, 50%ET<sub>c</sub> and 100%ET<sub>c</sub>. The effect of IA on midday leaf water potential ( $\Psi_{leaf}$ ), stomatal resistance ( $R_s$ ) and conductance ( $g_s$ ), soil ( $H_v$ ) and relative leaf (RWC) water contents, production and water use efficiency (WUE) was studied. Results showed controversial responses following to variety, large variations all over the season and a great sensitivity of the measurements to microclimatic conditions, which, apparently, produced variations larger than those produced by treatments. We have observed that further increases in IA did not systematically increased production and WUE or improved water potential. However, in spite of the different responses, significant correlations have been found between  $\Psi_{leaf}$  and  $g_s$ ,  $\Psi_{leaf}$  and RWC and  $\Psi_{leaf}$  and  $H_v$ .

**Key words:** Young olive trees, variety, deficit irrigation, crop evapotranspiration, correlation.

## Relations hydriques et utilisation de l'eau chez des oliviers ages de six ans et cultivés sous regimes d'irrigation déficitaire

### Résumé

L'expérimentation a été initiée en 2008 en vue d'étudier l'effet de l'irrigation sur les relations hydriques pour de jeunes oliviers cultivés selon l'écartement de 7mx7m au domaine expérimental 'Taoues' (Lat.34°, Long.21°), de l'*Institut de l'Olivier*, Sud de la Tunisie. Les plants ont été irrigués de Mars à Octobre au moyen d'un système goutte à goutte, considérant trois traitements hydriques (IA): 20%ET<sub>c</sub>, 50%ET<sub>c</sub> and 100%ET<sub>c</sub>. L'effet du traitement sur le potentiel hydrique foliaire ( $\Psi_{leaf}$ ), la résistance ( $R_s$ ) et la conductance ( $g_s$ ) stomatiques, l'humidité du sol ( $H_v$ ), le contenu relatif en eau (RWC), la production et l'efficacité de l'utilisation de l'eau (EUE) a été étudié. Les résultats ont montré des réponses variétales différentes, une grande variation des paramètres mesurés tout au long de la saison et une sensibilité importante des mesures aux conditions microclimatiques, qui, apparemment, ont produit une variation plus importante que celle qui a été induite par le traitement. Nous avons observé que l'augmentation de IA ne se traduit pas systématiquement par un rendement ou une EUE plus élevés, ni par une amélioration de l'état hydrique de la plante. Cependant, en dépit des réponses différentes, des corrélations significatives ont été trouvées entre  $\Psi_{leaf}$  et  $g_s$ ,  $\Psi_{leaf}$  et RWC et  $\Psi_{leaf}$  et  $H_v$ .

**Mots clés:** Jeune olivier, variété, irrigation déficitaire, évapotranspiration de la culture, corrélation.

### 1. Introduction

Conscious that irrigation is an obvious option to increase and stabilize olive production, and because olive tree is a hardy crop that respond positively to additional water supply (Michelakis, 2000; Fernandez *et al.*, 2006; Guggi *et al.*, 2007), the Tunisian government tried since the nineteenth to encourage orchadists to use localized irrigation in the intensive orchards as a compulsory practice (Masmoudi-Charfi *et al.*, 2004; Masmoudi-Charfi, 2006). However, as water resources are limited (Hamza, 2009), and in order to save more water and money, a prudent water management program based on efficient irrigation methods is found to be essential for satisfactory yields and efficient water

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use. Studies on *Deficit irrigation* carried out during the last decade, provided interesting results for olive crop, cvs. Meski and Picholine (Chehab, 2007). But, these results can't be generalized to all orchards because of the large diversity. We attempt from this work to determine the adequate seasonal irrigation amount (IA) for some commercial olive cultivars in the case of water scarcity for the semi-arid region of Sfax, which is considered as the most important olive tree area of Tunisia. The specific objective is to investigate water relations through the physiological responses of these varieties to sustained *Deficit irrigation*.

## 2. Materials and Methods

Experimentation was carried out since 2008 at the *Institut de l'Olivier* Experimental Field 'Taoues' (Lat. 34°N, Long. 21°E), located 40km from Sfax, southern Tunisia. Climate is semi-arid with annual rainfall and reference evapotranspiration of 250mm and 1400mm, respectively. Trees were planted at 7mx7m spacing, cvs. Chétoui, Chemlali, Coratina, Picholine and Manzanille, on silty clay soil and arranged in 5 plots of 3 trees each. They were drip irrigated from March to October. Crop evapotranspiration ( $ET_c$ ) and IA were determined following to Allen *et al.*, (1998) for non-standard conditions (Masmoudi-Charfi *et al.*, 2004). Three irrigation treatments were involved: 20% $ET_c$  (control), 50% $ET_c$  and 100% $ET_c$  (fig.1) with three replications for each variety. To check the influence of irrigation effect, soil water content ( $H_v$ ), leaf water potential ( $\Psi_{leaf}$ ), stomatal resistance ( $R_s$ ) and conductance ( $g_s$ ) and relative water content (RWC) measurements were made during the irrigation period. Correlations between these parameters were investigated.

## 3. Results and Discussion

### 3.1. Water need recovery and effect of IA on soil water content

Plants received during the irrigation season -including 97 mm of rainfall-, about 583mm of water for treatment 100% $ET_c$  (0.42 $ET_o$ ), 340mm for 50% $ET_c$  (0.24 $ET_o$ ) and 194mm for the control (0.14 $ET_o$ ). At 100% $ET_c$ , about 83% of water was met from irrigation, 71% at 50% $ET_c$  and 50% at 20% $ET_c$ . As a result, IA had a significant effect on  $H_v$ : as depth increased,  $H_v$  increased to reach maximums of 35-42% at 40-60cm depth in the watered sides (fig.2), resulting in (1) lower soil moisture in the topsoil layer, which is exposed to higher evaporative loss and (2) deep and rapid percolation as a consequence of the silty clay soil. However,  $H_v$  variations didn't follow systematically increases of IA. During the fruit set period,  $H_v$  values were lower at 50% $ET_c$  than at 20% $ET_c$  as a result of increasing water availability, which favoured root absorption. This result is concordant with that of Michelakis (1995 and 2000) which indicated that irrigation at higher soil water availability resulted in a water use, markedly increased than irrigation at lower values. It provided higher root water absorption rates, lower soil water content and a better plant water status. Grattan *et al.*, (2006) indicated that in the more heavily irrigated plots, large soil water changes occurred much closer to the tree and to the soil surface; plots receiving less water showed modest water changes.

### 3.2. Physiological responses

Midday leaf water potential ( $\Psi_{leaf}$ ) values showed significant seasonal variations and slight spring differences between treatments and varieties. At this time, soil water content was still enough to prevent significant  $\Psi_{leaf}$  decreases. Then, as the season progresses,  $\Psi_{leaf}$  decreased. Significant differences between treatments were observed for Coratina, Chétoui and Chemlali cvs., with lowest  $\Psi_{leaf}$  (-4.1MPa) recorded in October (fig.3). These results corroborate Ben Rouina *et al.*, (2007) which observed similar values for mature Chemlali trees cultivated in the same area. In our case, a threshold  $\Psi_{leaf}$  of -3MPa seems to be appropriate for all cvs. to manage more efficiently irrigation in the multi-variety orchards under *DI* regimes. Leaf stomatal resistance ( $R_s$ ) values showed significant differences between treatments for Chemlali, Chétoui and Coratina cvs., with higher values observed at lower IA (fig.4).  $R_s$  decreased quickly afterward to reach around 16h values varying from 1.14s/cm to 3.20s/cm following to treatment and variety. This decrease results from a partial stomatal closure, as it was stated by Deidda *et al.*, (1990), for which the maximum  $R_s$  values were recorded between midday and the first hours of the afternoon, *i.e.* during hours of intense radiation and high evaporative demand. The larger variations that we have observed were those of Chemlali cv., which seems to be the most able to regulate stomata aperture.



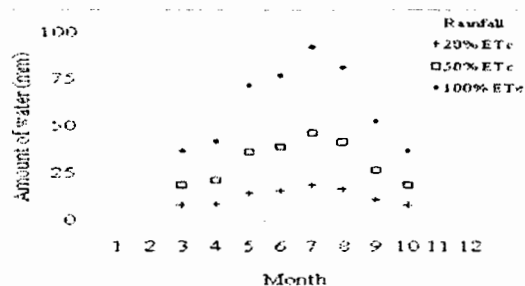


**Figure 1:** Monthly IA (mm) applied during the campaign 2007/2008 to six years olive trees cultivated in the semi-arid region of 'Taoues'.

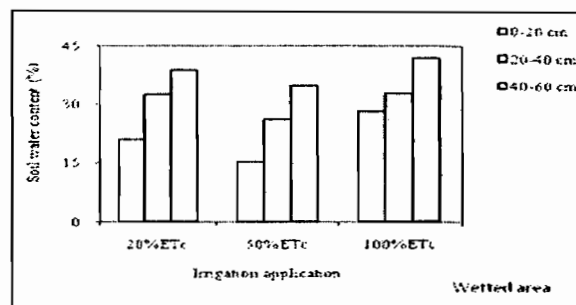


**Figure 2:** Soil water content (%) determined for the watered and non-watered sides at different depths and IA.

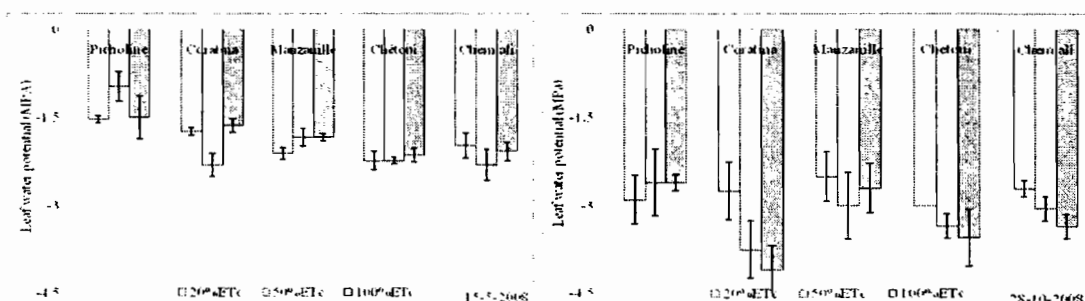


**Figure 3:** Leaf water potential (MPa) measurements made on May (a) and October (b) at different IA.

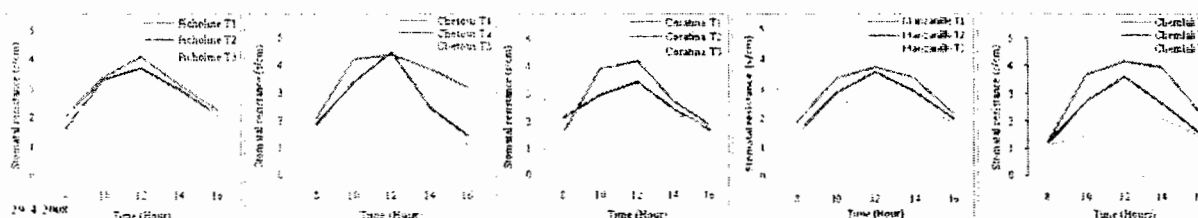


**Figure 4:** Daily variations of stomatal resistance (s/cm) measured on mature leaves at different IA.

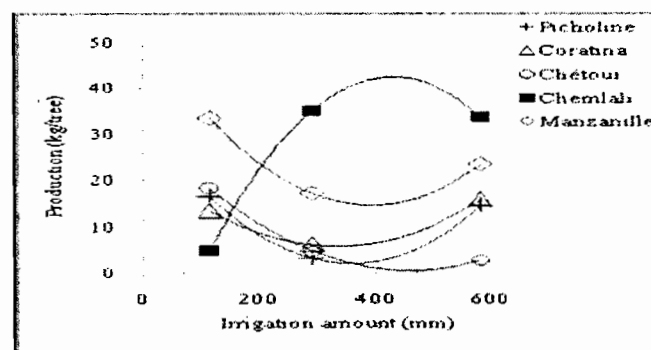


**Figure 5:** Correlation between olive production (Kg/tree) and irrigation amount (mm).

### 3.3. Water relations

Close coordination between  $\Psi_{\text{leaf}}$  and  $g_s$  were found (fig.5), but the response varied following to treatment;  $g_s$  was linearly and positively correlated to  $\Psi_{\text{leaf}}$  at 10%ET<sub>c</sub> ( $r=0.84$ ) and at 50%ET<sub>c</sub> ( $r=0.96$ ). At 100%ET<sub>c</sub>, polynomial function was found with optimum  $g_s$  of 450mmole/m<sup>2</sup>/s.  $\Psi_{\text{leaf}}$  was also found to be correlated to RWC (unpublished data). Minimum potential was registered at RWC of 82%. These results are coherent with those reported by Wullschlegel *et al.*, (1998) and Wahbi *et al.*, (2005), which observed significant linear correlations between  $\Psi_{\text{leaf}}$ ,  $R_s$  and RWC. Irrigation supply was also found to be significantly correlated to yield (fig.6). However, further increase in IA wasn't systematically followed by an increase of yield. Better responses were observed at IA ranging between 20%ET<sub>c</sub> and



50%ET<sub>c</sub> for most cvs., except the Chemlali cv., which seems to have higher water needs, probably because of its known higher potential and vigour.

#### 4. Conclusion

Irrigation affected significantly most physiological parameters; it can be inferred that for the experimental area, IA ranging between 20%ET<sub>c</sub> and 50%ET<sub>c</sub> can meet adequately *real and effective* water requirements for the experimented area and cvs.. However, there is a need for a more accurate scheduling of water application in the intensive orchards because of the large diversity (region, cultivar). Further research should be carried out on *DI* to elaborate an adequate program for the main locations and cvs. in response to the aims.

#### Acknowledgments

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## The olive mechanical harvesting by trunk with reversed umbrella

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### Abstract

The harvesting and pruning mechanisation is fundamental for olive culture improvement. The trunk shaker with mechanised interceptors showed high efficiency, but they need of suitable olive plantings for productivity, only one trunk, canopy volume, training system, variety, optional harvesting period, tree distance, tree age and vocational zone. Trunk shaker with upside-down interceptor umbrella had a high harvest yield for the Ortice and Ortolana cultivars. The work productivity has reached elevated values, for the lower labour employment, the high quantity of fallen fruits and the short harvest times. The trunk shakers with upside-down interceptor umbrella are an efficient solution for olives the mechanical harvesting of "Colline Beneventane" and "Sannio Caudino Telesino" P.D.O. (Protected Denomination of Origin) areas.

**Key words:** Harvest, *Olea europaea*, mechanization, shaker, reverse umbrella

## Récolte mécanique des olives avec un vibreur de tronc équipé d'un parapluie intercepteur

### Résumé

La mécanisation de la récolte et de l'élagage est fondamentale pour améliorer la culture de l'olivier. Le vibreur de tronc avec parapluie intercepteur pour la récolte des olives a démontré son efficacité mais il est nécessaire de tenir compte des dimensions des drupes, de la productivité des oliveraies, du volume du feuillage, de la distance entre les plants, de la variété de olives, de l'olivaison, de l'âge de l'exploitation et de la zone de culture. Le parapluie intercepteur pour la récolte des olives est d'un excellent rendement pour la culture des variétés d'olives de la Campanie, Ortice et Ortolana. Cependant, il est possible d'obtenir une meilleure productivité, des coûts moins élevés de main d'œuvre, une récolte plus importante en un temps plus court. Par conséquent, le vibreur de tronc avec parapluie intercepteur représente une solution efficace pour la récolte mécanique des olives de la Campanie en AOP (Appellation d'Origine Protégée): "Colline Beneventane" et "Sannio Caudino Telesino".

**Mots clés:** Récolte, *Olea europaea*, Mécanisation, Vibreur de tronc avec parapluie intercepteur.

### 1. Introduction

The harvesting represents one of the most important operations of the whole cultivating cycle of olive grove, both for the achievement of high quality oils and for the reduction of cultural costs (Tombesi, 1990 and 1996). The aim of the trial was to value the efficiency of a trunk shakers with reversed umbrella interceptor for the mechanical harvesting (Tombesi *et al.*, 1998) of two autochthonous cultivars, "Ortice" and "Ortolana", of Southern Italy.

### 2. Materials and methods

The experimentation was carried out in 2006 in an olive grove situated in Southern Italy (41°15' N, 14°38' E) (Benevento) constituted by adult plants of "Ortice" and "Ortolana" cultivars, trained to vase and planted at 6 x 6 m spacing. For the mechanical harvesting, occurred on 30 November 2006, was used a machine of self moving type, with three integral motive wheels, "F3" model, with a reversed umbrella interceptor produced by SICMA. The main characteristics of the trunk shaker were: 77 KW (105 CV), very-high-frequency vibration head, self-braking and umbrella opening of 6 metres in diameter.

The work force was constituted by 2 workers, one employed for the leading of the harvesting machine, the other one for the olives transport. During drupe maturation the following parameters were noticed: drupe coloration (Jaén index included between 0 and 7), drupe fresh weight, olive detachment force, pulp firmness, fruit drop pre-harvesting and the fruit oil content. At the moment of harvesting, for each plant, the drupes removed by the trunk shaker are weighed while the ones still on the plant are harvested by hand and then weighed, calculating the shaker efficiency (%). After the canopy volume, the trunk cross section area and the work productivity were calculated. On samples of mono-variety oil, obtained by micro oil mill, were carried out analysis of free acidity, peroxides numbers, spectrophotometric rates and sensorial characteristics by panel test. Data were submitted to analysis of variance (ANOVA) using MSAT-C software and mean separation was performed by Multiple Range Duncan test at 5% significance level and calculated standard error (ES) of the mean.

### 3. Results and Discussion

During the maturation, the cv. "Ortice" showed a resistance to drupe detachment that decreased at the beginning of November till to reach, at the moment of harvesting, the value of 309.17 g. The cv. "Ortolana", instead, showed a rather constant course, and at the moment of harvesting the registered value was of 455.83 g (Fig.1 and Tab.1). The pulp firmness, instead, decreased constantly during the maturation, till the values of 267.00 g/mm<sup>2</sup> and 231.58 g/mm<sup>2</sup> for the "Ortice" and the "Ortolana", respectively (Fig.2 and Tab.1).

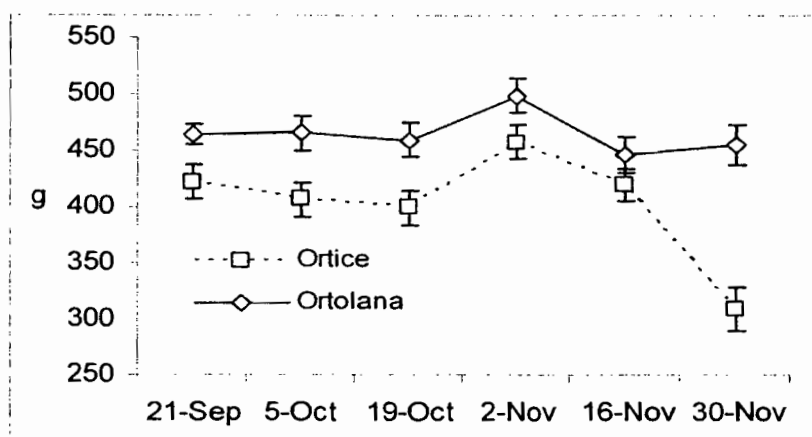


Figure 1: Evolution of olive detachment force during maturation (mean ± ES).

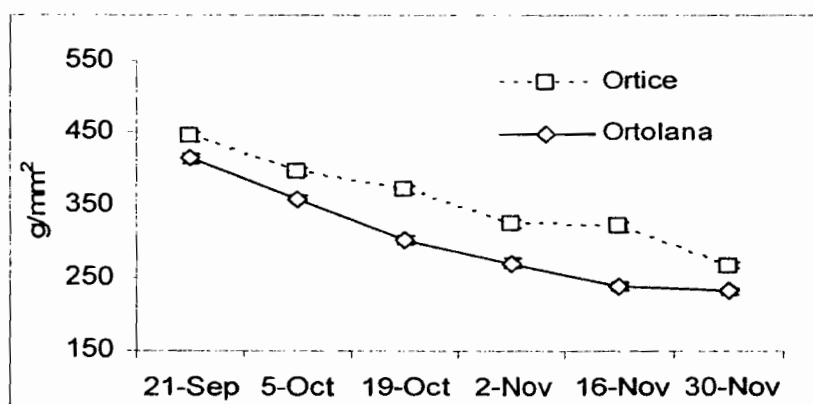


Figure 2: Evolution of pulp firmness during maturation (mean ± ES)

The accumulation of oil in the drupe is resulted to mostly grow in the "Ortice" reaching at the moment of harvesting, 26.09% of content in oil, while for the "Ortolana" it reached 16.51% (Fig.4). The phase of veraison, valued according the Jaén index, is resulted different and rather late both for the "Ortice" and the "Ortolana", reaching at the moment of harvesting, carried out on 30 November, a value of 2.10 and 4.35 for the "Ortice" and the "Ortolana", respectively (Tab.1).

The canopy volume on which the trial of mechanical harvesting was carried out was of 71.01 m<sup>3</sup> for the "Ortice" and of 84.47 m<sup>3</sup> for the "Ortolana", with an productive efficiency of plant respectively of 0.37 e 0.25 (kg/m<sup>3</sup>) (Tab.1). Both cultivars showed a good production for plant (26.06 and 21.18 kg) and a high adaptability to the mechanical harvesting as you can notice by the low ratio between the detachment force and the olives weight at the moment of harvesting (1.11 e 1.21 N/g) (Fig. 5 and Tab.1).

**Tab. 1:** Characteristics of plants and fruits at harvest, machine efficiency and work productivity of trunk shakers.

|                                                     | Cv "Ortice" |        | Cv "Ortolana" |        |
|-----------------------------------------------------|-------------|--------|---------------|--------|
| Olive weight (g)                                    | 2.79a ±     | 0.06   | 3.77b ±       | 0.01   |
| Olive detachment force (g)                          | 309.17a ±   | 19.30  | 455.83b ±     | 18.20  |
| Ratio olive detachment force/olive weight (N/g)     | 1.11a ±     | 0.08   | 1.21a ±       | 0.09   |
| Pulp firmness (g/mm <sup>2</sup> )                  | 267.00a ±   | 5.11   | 231.58b ±     | 0.45   |
| Drupe coloration - Jaën index (0-7)                 | 2.10a ±     | 0.05   | 4.35b ±       | 0.17   |
| Fruit drop (%)                                      | 13.95a ±    | 2.27   | 10.36a ±      | 2.49   |
| Canopy volume (m <sup>3</sup> )                     | 71.01a ±    | 8.65   | 84.47b ±      | 9.71   |
| Trunk cross section area (cm <sup>2</sup> )         | 1587.31a ±  | 337.96 | 613.43b ±     | 172.04 |
| Total olive yield for plant (kg)                    | 26.06a ±    | 7.72   | 21.18a ±      | 5.02   |
| Olive mechanical harvest for plant (kg)             | 25.17a ±    | 7.21   | 20.49a ±      | 5.03   |
| Productive efficiency of plant (kg/m <sup>3</sup> ) | 0.37a ±     | 0.17   | 0.25a ±       | 0.13   |
| Shaker efficiency for plant (%)                     | 96.58a ±    | 1.15   | 96.74a ±      | 0.88   |
| Work productivity (kg/h/worker)                     | 993.45a ±   | 351.65 | 823.87a ±     | 315.19 |

(mean ± ES). Rows with the same letter are not significantly different by Duncan multiple range test (P<0.05).

At the moment of harvesting the weight of fruits was of 2.79 g for the "Ortice" and 3.77 g for the "Ortolana" (Tab. 1), while the fruits drop pre-harvesting interested 13.95% of the "Ortice" and 10.36% of the "Ortolana" fruits. (Fig.3 and Tab.1).

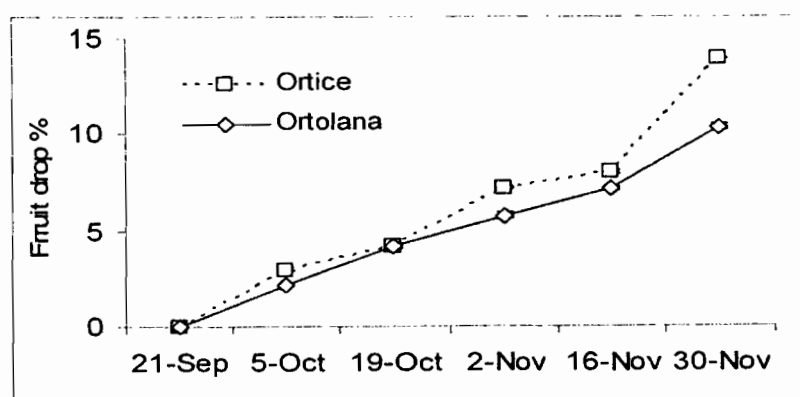


**Figure 3:** Percentage evolution of pre-harvest fruit drop during maturation.

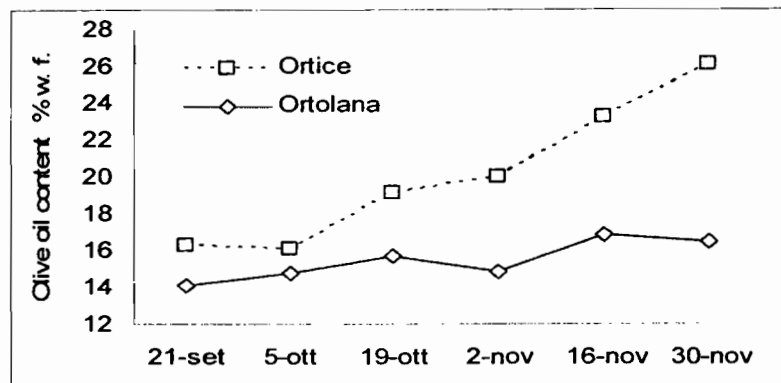


**Figure 4:** Evolution of olive oil content (% w.f.) during maturation.

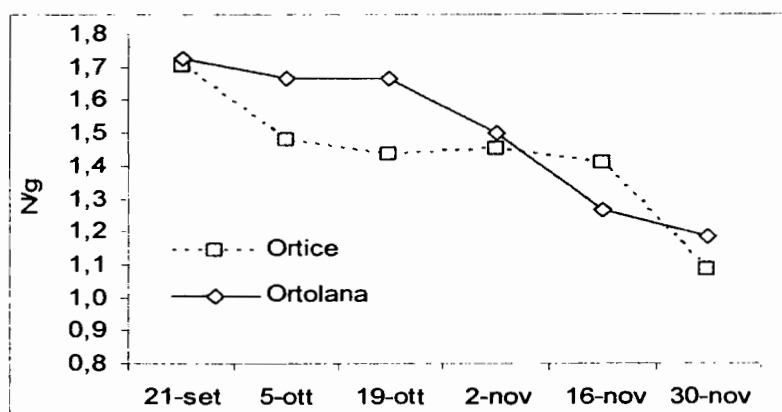


**Figure 5:** Evolution of ratio between olive detachment force and drupe weight during maturation (mean  $\pm$  ES).

Despite the high canopy volume and the trunk dimension, the mechanical harvesting yield by trunk shakers with reversed umbrella interceptor was very high, reaching a shaker efficiency equal to 96.58% and 96.74% of harvested fruits, respectively for the "Ortime" and the "Ortolana" (Tab.1). Moreover, considered the low require of work (only two workers), the reduced time for the operation (2 minutes/plant) and the quantity of harvested fruits, the productivity of work has reached very high values, 993.45 kg/h/worker for the "Ortime" and 823.87 kg/h/worker for the "Ortolana" (Tab.1). The trunk shakers with interceptor, protecting the drupes integrity, has not reduced the qualitative characteristics of oil, on the contrary, increasing the quantity of harvested fruits for unity of time, it allows to concentrate the harvesting in optimal time and to reduce the duration of olives storage, influencing positively on the oil quality. In fact, both samples of mono-variety oil that were analysed, were classified as extra virgin olive oil having largely satisfied the analytical (free acidity, peroxide number, spectrophotometric rates) and sensorial (panel test) quality parameters.

#### 4. Conclusions

Therefore, the obtained results show that the trunk shakers with reversed umbrella interceptor, causing the drop of more than 95% of olives and reaching a high productivity of employed labour, is to consider an interesting solution for the mechanical harvesting of two autochthonous cultivars, "Ortime" and "Ortolana", of Southern Italy.

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**Photo 1:** The olive mechanical harvesting by trunk shakers with reversed umbrella interceptor.

This work was carried out with MiPAAF financing, in the ambit of R.I.O.M. Project – Research and innovation for the Olive growing of Southern Italy.

## **A self-propelled radio-controlled shaker for mechanical harvesting of olives: Technical and economical evaluation**

Giuseppe Z., Bernardi B., Morabito S.

### **Abstract**

The harvest of olives is generally considered the most demanding phase of field operations from an economic-organizational aspect as a result of high manpower demands, high work costs, the limited period available for carrying out the operation and the often small farm dimensions. The objective of the present study is to value the performances, and the economic aspects, of a self-propelled radio-controlled shaker to evaluate its suitability in "difficult" olive groves. A rational mechanization is able to reduce production costs and find technical solutions that can solve the particular problems of traditional olive groves and of those on sloping ground which characterize a significant part of olive growing in the Mediterranean countries.

**Key words:** Mechanization, Radio-Controlled Shaker, Sloping Area.

### **Résumé**

Dans le cadre de la production oléicole en super intensif, la disponibilité limitée des variétés à basse vigueur et l'absence de porte-greffes capables de contrôler efficacement la croissance des plants, implique la nécessité d'effectuer d'importantes opérations de taille sur la frondaison, dans le but de garantir un niveau suffisant de luminosité, de réduire le phénomène de compétition, et de préserver une dimension des plants permettant le passage des machines à vendanger. Le présent article expose les résultats relatifs à la première année d'expérimentation, sur la taille manuelle et mécanique, effectuées dans le cadre de la conduite en super intensif de la culture d'olivier, en vue d'évaluer les performances effectuées à travers deux différents systèmes de taille, et d'établir la comparaison en terme de productivité et de qualité avec les travaux antérieurs.

**Mots clés:** Taille, mécanisation, production oléicole en super intensif, productivité.

## Influence of cover crops management on soil predators arthropods of olive orchards

D. Paredes<sup>1</sup>, S. Franco-Micán<sup>1</sup>, J. Castro<sup>2</sup>, M. Campos<sup>1</sup>

### Abstract

A two years experiment was carried out to compare the impact, on soil predators, of three different treatments: T<sub>1</sub>.- *Vicia sativa* planted, cut and incorporated to the soil; T<sub>2</sub>.- Natural and spontaneous cover incorporated to the soil; T<sub>3</sub>.- *Vicia sativa* planted, cut and laid on the surface. Samples were taken with "pitfall traps" (three traps per plot; four plot per treatment) two dates a year (June and October). Statistical analyses were made with Kruskal-Wallis test at  $p < 0.05$ . Ants (Formicidae) did not show any statistical difference between years and treatments. Spider (Araneae) showed differences between treatments in June 2008. Beetles (Coleoptera) showed statistical differences between treatments in October in both years, being *Vicia sativa* planted, cut and incorporated to the soil the treatment that maintains higher populations of coleopterans.

**Key words:** Soil predators; Olive orchards; Cover crops; *Vicia sativa*; Pitfall traps.

## Influence de la gestion des couvertures végétales sur les arthropodes prédateurs des sols des cultures d'oliviers

### Résumé

Des essais ont été développés pendant deux années pour comparer l'impact de trois traitements différents, sur les prédateurs du sol: T<sub>1</sub>.- *Vicia sativa* plantée, coupée et incorporée au sol; T<sub>2</sub>.- Couverture végétale naturelle et spontanée incorporée au sol; T<sub>3</sub>.- *Vicia sativa* plantée, coupée et étalée sur la surface. Les échantillons ont été récupérés avec des pièges (trois pièges par parcelle; quatre parcelles par traitements) deux dates par an (Juin et Octobre). Les analyses statistiques ont été faites par le test Kruskal-Wallis à  $p < 0.05$ .

Les fourmis (Formicidae) n'ont pas montré de différence statistique entre les années et les traitements. Les araignées (Araneae) ont montré des différences entre les traitements en Juin 2008. Les scarabées (Coleoptera) ont présenté des différences statistiques entre les traitements en Octobre pendant les deux années. *Vicia sativa* plantée, coupée et incorporée au sol est le traitement qui maintient la plus grande population des coléoptères.

**Mots clés:** Prédateurs du sol, Oliviers, Couverture végétale, *Vicia sativa* Pitfall traps.

### 1. Introduction

Cover crops are being studied in olive orchard because may have substantial effects on soil protection, soil fertility, groundwater quality and pest management (Gómez et al., 2009; Rodríguez et al., 2009). As in others agroecosystem, cover crops can provide alternatives hosts or food sources for natural enemies and pests, reason why cover crops may have a large impact on the complexity of arthropods population dynamics (Altieri, 1999). Many populations of pests can be managed by enhancing the efficacy and local abundance of the existing community of natural enemies through modification of the environmental or existing practices (Barbosa, 1998).

In olive groves, soil predators represent a prospect of complex ecological connections (Morris & Campos, 1999; Morris et al., 1999; Ruiz & Montiel, 2000; Santos et al., 2007) that could be affected by the way we manage the habitat (González et al. 2004; Cotes et al., 2009).

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Land maintenance systems in organic olive groves are based on the use of cover crops which are mechanically controlled with trimmers. The plant remains are either left on the surface or buried, thereby eliminating the cover crops (Guzmán & Alonso, 2004).

The purpose of this work is to determinate how different managements of cover crops affect the abundance and diversity of the main soil predatory groups and establish the more suitable management criteria.

## 2. Material and methods

The study was carried out during the years 2007 and 2008 in the locality of Deifontes (Granada-Spain; UTM: X 448861.03; Y: 4130932.13) in an organic olive orchard that was restructured 15 years ago with trees at 8 x 8 m spacing and Picual variety.

A randomly assay was performed to determinate some differences between three different treatments:

- T1: *Vicia sativa* planted, cut and laid on the surface.
- T2: *Vicia sativa* planted cut and incorporate to the soil.
- T3: Natural and spontaneous cover cut incorporated to the soil.

"Pit-fall" traps, left in the country 24 hours, were used to collect the arthropods. A total of 36 traps were provided in 12 different plots (three traps per plot and four plots per treatment). Samples were taken in June and October. The plots were adjacent and had a surface of 1024 m<sup>2</sup>.

The statistical analyses were carried out with the non-parametric test Kruskal-Wallis.

## 3. Results and discussion

### 3.1. Abundance

A total of 7090 individuals were caught during the experiment. In both years the captures in June were higher (85%) than in October. Ants were the most abundant group (87.9%), followed by beetles (7.1%) and spiders (5.0%).

#### 3.1.1. Predatory beetles (Coleoptera)

Staphylinidae (54%) and Carabidae (44%) were the most representative families. In June there is not any statistically significant difference between treatments. In October of 2007 the number of coleopterans collected is significantly lower ( $p < 0.05$ ) in treatment 3 than in the others, however, in 2008 this treatment maintains the highest ( $p < 0.05$ ) population. Others studies in olive orchards have shown that carabids and staphylinids populations are favored by non-managed soil (Castro et al., 1996), but Cotes et al. (2009) indicated that carabids populations were favored by the removal vegetation. These results could be explained because crop management activities may affect population of beneficial arthropods in different ways, either directly through mortality and emigration, or indirectly through habitat disruption (Thorbek & Bilde, 2004).

#### 3.1.2 Spiders (Araneae)

In 2007 the spider population was similar in the three treatments in June and October. However, in June 2008 in the plot with cover of *Vicia sativa* planted, cut and laid on the surface, the number of captured spider was significantly ( $p < 0.05$ ) higher (Figure 1). It is difficult to explain these results, but the olive agroecosystem is complex and the combination of many factors influences the densities of the arthropods. According to Cardenas (2007) in the olive growing the spiders abundance and diversity are benefited by the development of the vegetal cover, in size and species composition. Another aspect to consider is the great dispersal capacity of spiders (Foelix, 1996) and the fact that the plots in this study were adjacent.

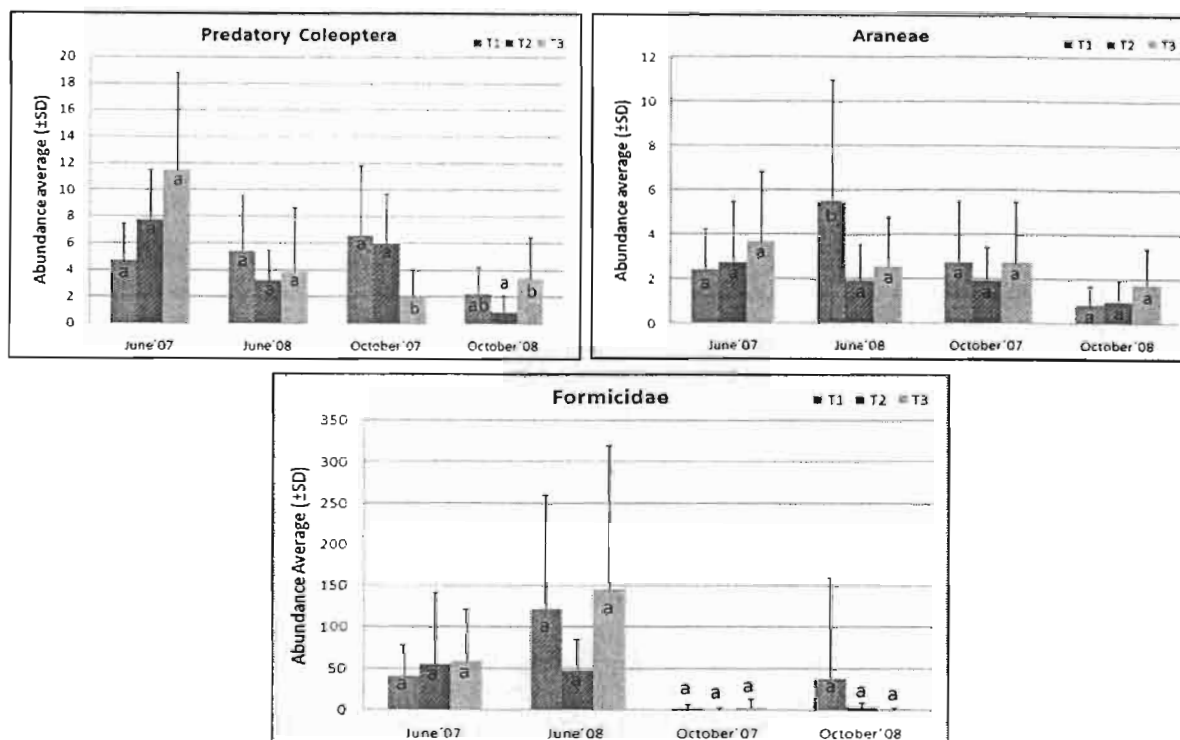


Figure 1: Abundance average of the taxons Coleoptera, Araneae and Formicidae.

### 3.1.3 Ants (Formicidae)

The number of ants trapped in June was 1877 in 2007 and 3647 in 2008, nevertheless, in October, captures descended noticeably (Figure 1). Throughout all the period of study, the abundance of ants was similar in the three treatments (Figure 1), which could be explained because the availability of resources were suitable for them in all the treatments and because the ants' nests were not disturbed during cover crops management. In addition, it is possible that the great variability in captured ants between traps reduced the statistical significances.

### 3.2. Dominance and Diversity

To have a higher view of the differences between treatments, dominance and diversity were measured. These indexes can give us an idea about the state of the agroecosystem in relation with every treatment.

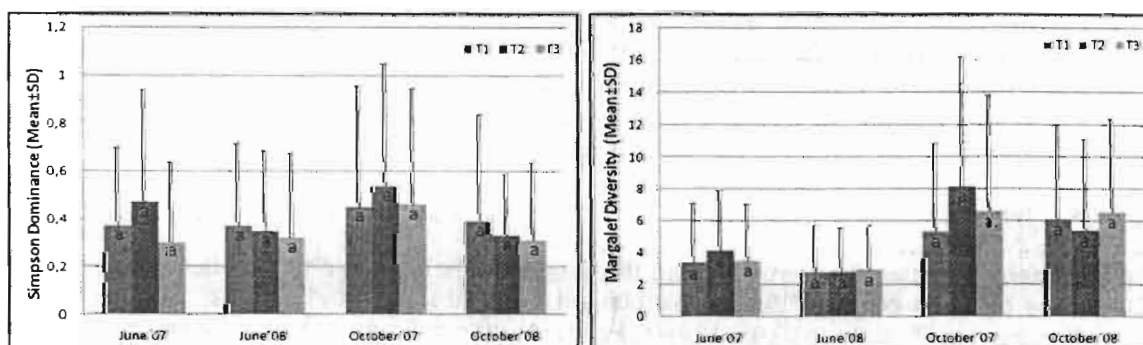


Figure 2: Simpson dominance index & Margalef diversity index respectively.

Simpson dominance index maintains the same levels in every treatment, season and year. No statistical differences were found on it. Margalef diversity index shows statistical differences between seasons but no differences were found between treatments or years (Figure 2). It is possible that the spatial scale selected for the study was not large enough to be able to find significant differences on diversity between sampled plots.

In summary, the results indicate that the cover crops management affected the different predatory groups but it would be advisable to carry out other studies with a greater time and space scale to verify the specific responses and implement knowledge on olive orchard-environment relationships.

### Acknowledgments

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## Security of the application of the herbicide Goal Supreme® (Oxifluorfen) under olive tree canopy in harvesting

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### Abstract

The use of herbicides in olive grove causes worry in the farmers for the possibility of contamination of the olive oils, but to harvest the fruits efficiently it's primordial that the area under canopy it remains free of weeds, during the harvesting time. Possible contamination of the fruits of the olive tree is evaluated by the herbicide Goal Supreme<sup>®</sup> (Oxifluorfen 48%) applied in the area under canopy and after the past among trees of the fruits in the crop plastic blankets. We simulate the whole process of harvesting, later the oils are extracted before and after past the fruits + blankets among trees. The obtained results show that if we apply the herbicide oxifluorfen according to good agricultural practice code and recommendations of the label, contamination of olive oils is not observed, although the fruits were dragged by the soil with applied herbicides.

**Key words:** Olive tree, herbicides, Oxifluorfen, olive oil.

## Sécurité de l'application de l'herbicide Goal Supreme® (oxifluorfen) sous l'olivier dans la récolte des arbres

### Résumé

L'utilisation d'herbicides dans l'olivieraie cause une inquiétude des agriculteurs pour la possible contamination des huiles d'olive par des produits chimiques. Mais pour récolter les fruits de manière efficace il est primordial que la zone sous la canopée de l'olivier reste libre de mauvaises herbes, pendant la période de récolte. La possible contamination des olives par l'herbicide Goal Supreme<sup>®</sup> (Oxifluorfen 48%) a été évaluée par son application dans la zone sous la canopée avant l'installation entre les arbres d'un filet en plastique pour la récolte des olives. Nous avons simulé l'ensemble du processus de récolte, plus tard, les huiles ont été extraites avant et après passage des fruits sur filets de récolte parmi les arbres. Les résultats obtenus montrent que, si l'on applique l'herbicide oxifluorfen en fonction de code de bonnes pratiques agricoles et les recommandations de l'étiquette, la contamination des huiles d'olive n'est pas enregistrée, bien que les fruits sont tombés sur le sol avec application d'herbicides.

**Mots clés:** Olivier, herbicides, Oxifluorfen, huile d'olive.

### 1. Introduction

Herbicides in farming systems are used to control weeds, but also used on uncultivated terrain, where the aim is to limit the growth of these undesired plants (Guzzella et al. 2006). In olive orchards, herbicides are fundamental tools in soil-management systems, both to control all weeds under the canopy as well as to manage plant covers. Pre-emergent herbicides are applied under the canopy in autumn before the harvest. Contamination problems have been detected when the pre-emergent is applied under the canopy of the olive trees. These problems may be due to the bad practices in herbicide application and/or fruit harvest. The Spanish government has established recommendations and regulations, as provided in the Manual of Good Agricultural Practices (CAP, 2006), which establish safe practices in managing the chemical, recommending the harvest of the fruit directly from the tree or collecting it in containers or on clean tarps. For the collection of the fruits by hand or machine, collection tarps are used to gather the olives from the ground. The tarps are dragged from tree to tree until full of olives and then emptied. Thus the tarps, and therefore the olives, make contact with the soil treated with herbicides.

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The origin and scope of this contamination problem is treated in several works, such as that of Guardia-Rubio et al. (2006), who detected fruit contamination, with differences between that collected directly from the tree and that gathered from the soil without using any sort of collection tarp, as the olives are simply knocked down and raked from the soil. The fruits are sampled on their arrival to the oil mill, and many cases of contamination are registered in olives gathered from the ground and less in those picked from the tree. In the case of not separating the fruits from the two forms of harvest, the action of combining the two lots in a sense dilutes the contamination, thereby lowering the high values of contamination from the soil. It is suggested that the fruits, on being knocked from the tree and fall directly to the ground make contact with the soil and thus become contaminated. This indicates the importance of the harvest method. In later works, Guardia-Rubio et al. (2007 and 2008) demonstrated that washing the fruit could eliminate certain pesticides from the skin after having been sprayed with these chemicals, but continue to point out that fruits can become contaminated directly from contact with soil treated with herbicides when the fruits are knocked down and collected from the soil with sweepers and vacuums.

The herbicide oxifluorfen is used in other tree crops. Alister et al. (2009) and Kogan et al. (2007), studying the persistence and leaching of oxifluorfen in vineyards, consider this a low-risk herbicide with regard to surface-water pollution, although they advise that crops planted on slopes are a factor to be considered, since this herbicide lodges in the first few centimetres of the soil (2.5 cm), which are susceptible to water erosion. In olive orchards, pre-emergence herbicides is applied to olive orchards in order to maintain the area under the tree canopy free of vegetation that could interfere at harvest time.

The aim of the present work is to detect whether there is indirect contamination by contact with soil treated with the herbicide Goal Supreme® when fruits are shaken from the tree by mechanical shakers and collected in tarps that are dragged from tree to tree.

## 2. Materials and Methods

For this study, we used an olive-orchard plot of cv. Picual 40 years old planted on 11 x 11 m centres, at the research centre IFAPA Venta del Llano de Jaén (Mengíbar, Jaén). Prior to the treatments, the orchard floor was raked of all vegetal remains. The commercial herbicide Goal Supreme® (Dow Agrosciences Ibérica S.A.), composed of oxifluorfen (48% p/v) was used, this chemical being registered for olive-orchard use to control annual weeds at commercial application rates of 1-2 L ha<sup>-1</sup> with a safety lag of 21 days between application and harvest.

The treatments with Goal Supreme® were applied with a hydraulic sprayer at two application rates, 1 and 2 L ha<sup>-1</sup>, with 4 replicates. The herbicide treatments were made on 11 November 2008 and the harvest on 12 December 2008, 33 days after the application. Prior to the test, rainfall of 165.5 mm was registered, while 64.4 mm was measured from the application of the herbicide to the harvest of the olives.

The plot was formed by two contiguous rows of trees for a set of 5 trees, the first being control (untreated with herbicide) and collected completely and dragged in a tarp under the tree canopy contiguous to the one that had received the herbicide treatment. The length of the dragging from the point of harvest under a tree and the outside turn around the tree to return to the starting point was 25-30 m. The control harvest was by total shaking of the fruit by the mechanical shaker and collection in new tarps. The tarps were made of polyethylene (HDPE) of 70 g m<sup>-2</sup> and measured 6x10 m.

For each of the plots, two samples of fruits were taken (8 Kg). In the first, fruit were knocked onto the tarp without dragging afterwards, while in the second sampling, after the fruit was knocked down and the tarp was dragged among the trees. For the fruit samples, they retire the fallen leaves in harvest, next the olive oil was extracted with manual equipment (ABENCOR), the fruits were not washed before the oil extraction. The oxifluorfen contents in the oils were determined by gas chromatography with a mass detector and a detection limit of 0.05 mg Kg<sup>-1</sup> in assays accredited in the Agrofood Laboratory of Cordoba, Spain (Junta de Andalucía). Currently, the Maximum Limits on Residues (MLR) for oxifluorfen is 1 mg Kg<sup>-1</sup> in olives fruit grown for oil.

### 3. Results and Discussion

The analytical results are presented in Table 1, observing that in no case was herbicide contamination detected in the oils from the fruits before or after the harvest tarps were dragged through the orchard. It should be pointed out that the soil was damp (19% gravimetric moisture) and under the worst harvesting conditions, the fruits were collected with soil particles on their skins after the dragging. At harvest, the farmers must walk over the tarps with poles to help the shakers knock down all the fruits, and in almost all cases, fragments of mud were left from the workers' boots, which mixed with the fruits during the dragging.

**Table 1:** Levels of oxifluorfen in oils of olive fruits before and after being dragged in tarps during harvest over soils treated with herbicide. Detection limit 0.05 mg Kg<sup>-1</sup>.

| Replicate | Dosage p.c. L ha <sup>-1</sup> | Oxifluorfen mg Kg <sup>-1</sup> |       |
|-----------|--------------------------------|---------------------------------|-------|
|           |                                | Before                          | After |
| 1         | 1                              | 0                               | 0     |
| 2         | 1                              | 0                               | 0     |
| 3         | 1                              | 0                               | 0     |
| 4         | 1                              | 0                               | 0     |
| 1         | 2                              | 0                               | 0     |
| 2         | 2                              | 0                               | 0     |
| 3         | 2                              | 0                               | 0     |
| 4         | 2                              | 0                               | 0     |

c.p.: commercial product

In this experiment, it was fundamental to take into account the phenomenon "dilution" of the possible contamination, mentioned by Guardia-Rubio et al. (2006) referring to the mixture of the olives from the soil and those picked by the tree directly. In our case, for the entire harvest of the tree, following the recommendations of the Manual of Good Agricultural Practices (CAP, 2006), any direct contamination by herbicides (oxifluorfen in our case) was avoided, this result being applicable to other pre-emergent herbicides used and registered for olive orchards.

The data recorded in this experiment were clarified with respect to the possibility of direct contamination of the fruits from the treated soil, when harvest tarps are used for collection. This represents a safe collection model and compatible with the use of herbicides under the tree canopy.

### 4. Conclusions

According to the results found, we have established points of attention in the use of pre-emergent herbicides in the production system of the olive orchards used for oil. With respect to contamination by herbicides applied to the soil, if tarps are used to collect the olives by the traditional systems of knocking the olives onto the tarps and dragging the tarps from tree to tree, no contamination of the olives was detected.

The herbicides are tools meant to help the sustainability of the olive orchard, and responsible maintenance is needed to avoid contamination episodes. Continued field work is necessary to develop a model compatible with the high quality of the olive oil produced.

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## Production of plants of *Dittrichia viscosa* (L.) Greuter for installation in olive orchard in hedgerow

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### Abstract

The species *Dittrichia viscosa* (L.) Greuter is a weed of the mediterranean olive orchards, which shelters a parasitoid of *Bactrocera oleae* Gmel. The installation of hedgerow or field break of *Dittrichia* would increase the development of the parasitic complex and therefore the control of the *Bactrocera* populations. To obtain plants of *D. viscosa* and to use them in the creation of the hedges, different techniques of obtaining were tested. The most effective method consists on the obtaining plants for germination of seeds in trays with peat. The obtained plants can be used directly in field or transplanted to black plastic bags to increase their size.

**Key words:** olive orchard, *Dittrichia viscosa* (L.) Greuter, *Bactrocera oleae* Gmel., hedgerow, biological control.

## Production de plants de *Dittrichia viscosa* (L.) Greuter pour une installation dans les oliveraies et de former des haies

### Résumé

L'espèce *Dittrichia viscosa* (L.) Greuter est une mauvaise herbe dans l'oliveraie en Méditerranée, qui abrite un parasitoïde de *Bactrocera oleae* Gmel.. L'installation de haies de *Dittrichia* aux limites d'un champ d'oliviers serait favorable au développement du complexe parasitaire et, par conséquent pour le contrôle des populations de *Bactrocera*. Pour obtenir des plantes de *D. viscosa* et à les utiliser dans la création de haies, différentes techniques d'obtention ont été testées. La méthode la plus efficace consiste en l'obtention de plantes par germination des graines dans des bacs de tourbe. Les plants obtenus peuvent être utilisés directement pour la transplantation dans des sacs en plastique, noir pour augmenter leur taille.

**Mots clés:** oliveraie, *Dittrichia viscosa* (L.) Greuter, *Bactrocera oleae* Gmel., haie, contrôle biologique.

### 1. Introduction

In the agroecosystem of the olive orchard, there are many interrelations between organisms. Since ancient times, relations have been described between olive pests, parasites, and weeds. In the present work, we centre on the relationship between *Bactrocera oleae* Gmelin, *Eupelmus urozonus* Dalman, and *Dittrichia viscosa* (L.) Greuter. Isaakies (1957, cited by Warlop 2006) described the parasite complex in Greece, and there are references also for Gerona, Spain in 1961 (Askew and Nieves-Aldrey, 2000), followed by Pujade i Villar (1989). For the cultivation conditions in Andalusia (S Spain), this parasite complex is being studied by Franco-Micán et al. (2008). The complex is formed by the Hymenoptera *Eupelmus urozonus* (family Eupelmidae), which is an entomophagous parasite of the olive fly *Bactrocera oleae* as well as *Myopites stylata* F.. The latter develops in the capitulum of the *Dittrichia viscosa* (Compositae), where it forms galls in which its larvae develop, these in turn being parasitized by *Eupelmus*, which overwinters in these galls. In recent works, Boccaccio and Petacchi (2009) have highlighted the importance of this parasite complex (the second in importance) and the influence of the natural stands of vegetation and of their connectivity to favour the parasites, the connection between the patches of vegetation being more important than plant abundance. *Dittrichia viscosa* (L.) Greuter is a forb or a shrub, densely pubescent and glandular, with erect stems, woody at least at the base of the shoot (Blanca et al. 2009). The flowers are yellow and open from August to November. An individual of this species is formed by a set of independent branches from

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the base, from which new branches sprout each year that can remain functionally active for more than one vegetative cycle. The shoot usually dies back in the winter from the cold, although some green stalks can survive in the lower parts. This plant, containing essential oils, is not consumed by grazing animals, and it also escapes routine weeding controls of the olive orchard for its later sprouting and for its physiological resistance to the penetration of herbicides. Its appearance in olive orchards is owed primarily to non-tillage of the areas under the olive trees. Lopez Soria (1980) studied the dynamics of this plant, detailing its capacity to colonize abandoned terrain, taluses, and banks, indicating its decline in growth around the tenth year. With regard to its planting in the field, Warlop (2006) described how to grow plants by sowing, plants reaching flowering adulthood in 3–4 years.

The way to encourage this parasite complex and the connectivity between stands or patches of vegetation in the olive orchard, precise methods are needed to plant hedgerows of *Dittrichia* in a rapid and precise way. The aim of the present work is to establish reliable and rapid methods of growing *Dittrichia viscosa* plants to be used as hedges in olive orchards (ecological or conventional).

## 2. Materials and methods

### 2.1. Producing seedlings in greenhouses

The plants of this study were sown in a glass greenhouse with active temperature control, located at the IFAPA Centro Camino de Purchil in Granada (Spain). The greenhouse propagation began with pregerminated seeds in paper filter placed in an oven as well as seeds directly planted. The seeds were planted in Jiffy pots and in polyethylene (PE) germination trays containing peat and placed in water baths in the winter months. The peat used was Kekkila® Mix420 with the slow-release fertilizer Plantacote® plus12/14 applied at a rate of 3 kg m<sup>-3</sup>. For field growing, 18x25cm root bags with 75 µ, perforations were used (Oliveres y Pallares S.A., Córdoba, Spain).

In the greenhouse, the combinations of methods tested were:

- Pregerminated seeds transplanted to Jiffy pots in the greenhouse.
- Seeds sown directly in Jiffy pots in the greenhouse.
- Seeds sown directly in PE trays maintained floating in a water bath in the greenhouse table.

All the plants grown in the greenhouse were transplanted to root bags with peat fertilized and maintained growing in the field until planted in the orchards.

### 2.2. Planting of *Dittrichia* hedges

For the planting of *Dittrichia* in commercial olive orchards, different methods and farms were tested. Seeds were directly sown in the field, adult plants removed from the field with their root balls intact were replanted, seedlings from germination trays were planted, and plants 3–4 months old were transplanted to the field and grown in greenhouse bags.

The following orchards were used for the experiment:

- La Reja (Bobadilla, Málaga), an ecological olive orchard. For planting, a cultivator was passed through the lanes of the orchard and a localized irrigation system was installed.
- Dehesa del Tobazo (Alcaudete, Jaén), an ecological olive orchard. For planting, two passes were made by a subsoiler; the plants were grown without irrigation, although they were hand watered with buckets.
- Los Torcales (Los Villares, Jaén), a conventional olive orchard. The plants were planted after one pass with a cultivator, and the plants were watered with buckets, as the olive orchard was rainfed.

The design was random plot with three replicates, the basic plot being formed by a row of 100 plants in the centre of the lane of the olive orchard. In each orchard, the habitual production means, irrigation, machinery, etc. were used, as the aim was to simulate a real orchard situation. The result was that each orchard applied a different methodology.

### 3. Results and Discussion

For the greenhouse plants grown, the results show that the sowing of seeds in PE trays (using a large number of seeds per well) maintained in floatation baths was the simplest, fastest, and most economic for producing seedlings. The plant thus grown can be transplanted in the field where irrigation is available. The transplanting of greenhouse bags is a necessary step for growing a more vigorous plant in rainfed olive orchards, but this transplanting has the drawback of an added economic cost in labour when the plants are transplanted to the bags. These plants can be maintained for a long period of time in floatation baths for growth. The baths can be depressions in the field lined with plastic. The plants tolerate the flooding well and in this way avoid the need for daily irrigation. The plants should be planted in the same season as the one in which they germinate.

The results according to the methods of sowing and planting in the field were:

#### 3.1. Direct sowing in the field

Seed collected in the field in 2006 were sown in the following orchards:

- La Reja, with localized irrigation, sown 25-04-07; germination at 50 days after sowing (dds), with a final survival of 5 plants per row.
- Dehesa del Tobazo, rainfed, sown 27-03-07; germination at 60 days dds, seedlings bucket watered, with a final survival of 9 plants per row.

#### 3.2. Adult plants with root ball, removed from the field and later transplanted in the orchard

- Dehesa del Tobazo, rainfed; plants planted 27-03-07 in soil worked with a subsoiler. Survival 11%. New plants were added 12-12-07 and 4 times in 2008. In June 2008, only 35% of the plants survived.

#### 3.3. Seedlings from germination trays

Pregerminated seeds placed in trays were directly transplanted in soil recently tilled with a cultivator.

- Finca La Reja, with localized irrigation, planted on 23-05-07, with survival of 87-99%. The first flowers appeared in July 2007, and the following year all plants flowered.

#### 3.4. Plants in root bags

Mature plants of 4 months old from the greenhouse were planted in the orchard.

- Los Torcales (27-05-08), after one pass with the cultivator, plants were bucket watered after planting and twice later. Survival was 99%. The first flowers appeared in 2008, and the following year all plants flowered.

### 4. Conclusions

To grow plants in a greenhouse, the best method is to sow seeds in PE trays maintained in floatation baths in a temperature-controlled greenhouse, sowing a large number of seeds per well. This simple, fast method provides seedlings with a high survival rate.

For growing *Dittrichia* hedges, a cultivator is recommended to prepare the terrain. Where irrigation is available, plants from PE trays can be used, whereas in rainfed orchards, transplanting 3- to 4-month-old plants with root bags is recommended, with temporary watering at the time of transplanting in the field.

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## Les composés organométalliques: Produits novateurs dans la lutte contre les maladies de l'olivier

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### Résumé

La surface oléicole couvre en Tunisie le tiers des surfaces agricoles utiles. Ce patrimoine agricole est confronté à plusieurs contraintes d'ordre phytosanitaire, qui s'accroissent avec l'intensification de la culture oléicole, comme les agents fongiques de pourriture de racine et de dépérissement et les maladies bactériennes.

Ce travail s'inscrit dans le cadre de la lutte chimique et a consisté dans la synthèse d'un composé organométallique, le 1,1-bis[4-(3-Diméthylaminopropoxy)phényl]-2-ferrocenyl-but-1-ène, puis de prospecter son effet antimicrobien par la méthode d'antibiogramme.

Notre produit, ayant démontré un fort potentiel d'inhibition, a fait l'objet d'une détermination de la concentration minimale inhibitrice (CMI) et de la concentration minimale bactéricide (CMB) afin de comparer son efficacité par rapport à la doxycycline (antibiotique de référence) et au sulfate de cuivre. Notre produit, qui s'est révélé aussi efficace que les antibiotiques (interdit en agriculture) et meilleur que le sulfate de cuivre, a été formulé (citrate de 1,1-bis [4-(3-Diméthylaminopropoxy) phényl]-2-ferrocenyl-but-1-ène), dans le but de sa solubilisation dans l'eau pour servir aux essais de lutte *in vivo* et faciliter sa future utilisation dans les traitements agricoles au champs.

**Mots clés:** Maladies bactériennes et fongiques, Lutte chimique, Composés organométalliques, 1,1-bis [4-(3-Diméthylaminopropoxy) phényl]-2-ferrocenyl-but-1-ène, CMI et CMB

### Abstract

Olive tree is affected by several plant pathogenic fungi and bacteria which can have reduce the production as well as the oil quality.

In the present study, we tried to test the antimicrobial activity of an organometallic compound (1,1-bis[4-(3-Diméthylaminopropoxy)phényl]-2-ferrocenyl-but-1-ène) lately synthesized by the antibiogram method.

We have determined the minimal inhibiting concentration (MIC) and minimal bactericidal concentration (CMB) for the best efficient compound in order to compare its effectiveness compared to the doxycycline (antibiotic of reference) and with copper sulphate.

The organometallic product was found more efficient than the commercial product. It was formulated (1,1-bis[4-(3-Diméthylaminopropoxy)phényl]-2-ferrocenyl-but-1-ène citrate) to allow its solubilization in water in order to be used in open field conditions to control olive tree pathogens.

**Key words:** Olive tree pathogens (Fungi and Bacteria), antimicrobial activity, organometallic Compounds, 1,1-bis[4-(3-Diméthylaminopropoxy)phényl]-2-ferrocenyl-but-1-ène, MIC and MBC

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## Les cochenilles de l'olivier dans le Nord Tunisien

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### Résumé

Les données collectées concernant les cochenilles de l'olivier dans le Nord Tunisien montrent la présence principalement de six espèces : deux appartiennent à la famille Coccidae, trois espèces appartiennent aux Diaspididae et une aux Pseudococcidae. La prospection dans les oliveraies Tunisiennes en relation avec l'abondance et la densité des populations des cochenilles a montré que *Saissetia oleae* Olivier est l'espèce prédominante et qui cause le plus de dégâts sur le plan phytosanitaire dans le Nord Est tunisien, alors que la cochenille farineuse *Peliococcus cycliger* Leonardi est l'espèce commune du Nord Ouest. Cependant les cochenilles Diaspines *Parlatoria oleae* Colvée, *Aspidiotus nerii* Bouché *Lepidosaphes fava* Signoret, et la cochenille lecanine *Phillipia follicularis* Targioni Tozzetti n'ont pas d'importance économique significative malgré leur abondance. La surveillance de la faune auxiliaire des cochenilles a reporté la présence d'une coccinelle *Chilocorus bipustulatus* L. (Coleoptera) occasionnellement prédatrice des cochenilles lecanines. Nombreux parasitoïdes attaquent la cochenille noire de l'olivier ; les espèces collectées depuis la majorité des sites sont *Scutellista cyanea* (Hymenoptera) Mots et *Metaphycus spp* (Hymenoptera) et doivent être considérés comme les parasitoïdes par excellence de *S. oleae* en Tunisie. Le rôle d'*Eublemma scitula* Rbr (Lepidoptera) en tant que facteur de mortalité de *S. oleae* est peu considérable.

**Mots clés:** L'olivier, cochenilles, dégât, parasitoïdes, prédateurs.

## Scale insects fauna of olive in Tunisia

### Abstract

Field surveys of scale insects fauna living on olive trees in Northern Tunisia were carried out in 2009 showing the presence mainly of six species: two belong to the Coccidae family, three to Diaspididae and only one species to the Pseudococcidae family. The analysis of the results prove as *Saissetia oleae* is the dominant species and, in the North East investigated area it is responsible of high levels of damage to the cultivations. On the contrary, in the North-Western part, *Peliococcus cycliger* is the most common species mostly present at low population densities. Moreover, *Parlatoria oleae*, *Aspidiotus nerii*, *Lepidosaphes fava*, and *Phillipia follicularis* do not have significant economic importance in spite of their abundance. The monitoring of the auxiliary fauna reported the presence of the Moth *Eublemma scitula* and *Chilocorus bipustulatus*, an occasional predator of the soft scales. Many parasitoids attack the black olive scale; the species collected from the majority of the sites are *Scutellista cyanea* and *Metaphycus spp.* that have to be considered as important parasitoid of *S. oleae*.

**Key words:** Tunisia, scale insects, olive crop, parasitoid, predators.

## **Tentative de correction d'eau saline d'irrigation par ajout de potassium et calcium : Effets sur quelques paramètres écophysologiques et nutritionnels des plants d'olivier (var. Arbequina).**

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### **Résumé**

Dans la présente étude, nous avons procédé à l'évaluation de l'effet de l'application d'un supplément de potassium ( $\text{KNO}_3$ ) et de calcium ( $\text{CaCl}_2$ ) à différentes doses sur le comportement physiologique de jeunes plants d'olivier irrigués avec une solution saline contenant 100 mM et 200mM de NaCl. La variété d'olivier étudiée est Arbequina I18. Les plants d'olivier âgés d'un an ont été cultivés dans des pots en plastique d'une capacité de 8 litres et contenant un mélange de perlite et de sable (1:1). La culture a duré onze mois et l'irrigation a été réalisée avec une solution  $\frac{1}{2}$  Hoagland additionnée de NaCl à une concentration de 100 mM et 200mM. L'apport de potassium et de calcium (10 et 40 mM  $\text{KNO}_3$  et  $\text{CaCl}_2$ ) a été appliqué 4 mois après l'installation de l'essai. Les résultats obtenus montrent que tous les paramètres étudiés (croissance, concentration chlorophyllienne, photosynthèse et transport électronique) ont été négativement affectés par la salinité. Par addition, l'intégrité membranaire a augmenté par la salinité. L'application d'un apport de potassium a été bénéfique dès la dose de 10mM de  $\text{KNO}_3$ . En effet, nous avons enregistré suite à l'application de cette dose, une réduction des effets nocifs de la salinité avec une nette amélioration de la photosynthèse, la concentration chlorophyllienne ainsi que du transport électronique. L'application d'un apport de calcium n'a été bénéfique qu'à la dose de 40mM de  $\text{CaCl}_2$ . Pour l'intégrité membranaire, l'ajout de calcium aux doses de 10 et 40 mM de  $\text{CaCl}_2$  a provoqué une nette amélioration dudit paramètre.

L'étude de la nutrition minérale montre que l'apport de potassium et du calcium à une dose de 40mM de  $\text{KNO}_3$  et de  $\text{CaCl}_2$  a provoqué une accumulation importante de potassium et du calcium dans les tissus foliaires ainsi que dans les fruits.

**Mots clés:** Salinité, Potassium, Calcium, écophysologie, *Olea europaea*.

### **Abstract**

This study was conducted to evaluate the effect of potassium and calcium application on physiology behaviour of Arbequina olive treated with a saline solution (200mM). one-year old plants were grown in 8 litres pots containing sand perlite mixture(1 :1) for 11 months and were irrigated with half-strenght Hoagland solution plus 200 mM de NaCl . Supply potassium and calcium were applied 4 months after imposition of study.

Results obtained showed that all measured parameters were affected by salinity (Photosynthesis and stomatal conductance). Application of potassium 40mM  $\text{KNO}_3$  and Calcium 10 mM  $\text{CaCl}_2$  had a beneficial effect on photosynthesis and stomatal conductance. The study of mineral nutrition showed in important accumulation of potassium and calcium in foliar tissues and fruits in plants treated with a saline solution by addition of 40 mM of  $\text{KNO}_3$  and 10 mM of  $\text{CaCl}_2$ .

**Key words:** Salinity, potassium, calcium, *Olea europaea*

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## 1. Introduction

Les effets nutritionnels de la salinité incluent les deux actions primaires du sel sur les plantes : la toxicité directe due à l'accumulation excessive des ions dans les tissus et un déséquilibre nutritionnel provoqué par l'excès de certains ions. Des concentrations salines trop fortes dans le milieu provoquent une altération de la nutrition minérales des plantes (Levigneron et al., 1995). Ceci provoque une inhibition du transport et l'accumulation de potassium et de calcium et par conséquent une inhibition de la croissance des plantes. Le potassium joue un rôle fondamental dans l'absorption des cations, dans l'accumulation des hydrates, de protéines, l'organisation de la cellule, le maintien de la turgescence de la cellule et la régulation de l'économie de l'eau des plantes (régulation des stomates). Il intervient dans la synthèse de protéines et dans le processus de la photosynthèse. Le calcium est impliqué dans plusieurs processus tel que l'élongation cellulaire, la division cellulaire, la perméabilité membranaire, il joue aussi un rôle de régulateur dans le maintien de l'équilibre de la balance cation anion. L'excès de sel cause néanmoins des effets dépressifs comme l'inhibition de la photosynthèse, synthèse des pigments photosynthétiques, endommagement de la perméabilité de la membrane plasmique.... C'est la raison pour laquelle les études s'orientent actuellement vers l'amélioration de la tolérance des plantes à la salinité. Plusieurs mécanismes de tolérance a la salinité ont été étudiés et un de ces mécanismes est l'amélioration de la croissance et le métabolisme des plants stressés avec du NaCl par l'application d'un supplément de potassium et/ou du calcium.

Le présent travail a pour objectif d'étudier les effets de différentes doses de potassium et de calcium sur le comportement des plants d'olivier (*Olea europaea* L.cv. Arbequina 118) traités par une solution saline.

## 2. Matériels et Méthodes

Les plants d'olivier (variété Arbequina118) âgés d'un an ont été cultivés dans des pots en plastique d'une capacité de 10 litres et contenant un mélange de perlite et du sable (1:1). La culture a duré onze mois et l'irrigation s'est faite avec une solution nutritive additionnée de NaCl à une concentration de 200mM de NaCl. L'apport de potassium (10 et 40 mM de  $\text{KNO}_3$ ) et du calcium (10 et 40 mM de  $\text{CaCl}_2$ ) ont été appliqués 4 mois après l'installation de l'essai. Durant cette étude, l'effet de sel combiné aux deux doses de potassium et de calcium sur le comportement des variétés d'olivier a été suivi par un certain nombre de paramètres physiologique. Nous avons procédé à la mesure de la photosynthèse et la conductance stomatique réalisée avec un analyseur de type CID301ps. Pour étudier l'effet du sel sur le statut nutritionnel, nous avons effectué le dosage de sodium et potassium avec un photomètre à flamme, les chlorures avec la méthode de Mohr et le calcium par ionimètre.

## 3. Résultats et Discussion :

### 3.1. Effet sur la photosynthèse

Les résultats illustrés dans la Figure 1 montrent que la salinité a affecté négativement la photosynthèse. En effet nous avons enregistré une diminution dudit paramètre de 59,38% par rapport aux témoins. Nos résultats sont en accord avec plusieurs études (Chartzoulakis, 2005, Chartzoulakis et al., 2006). L'application de potassium et de calcium sous forme de  $\text{KNO}_3$  et de  $\text{CaCl}_2$  a un effet positif sur la photosynthèse. Avec la dose 40 mM de  $\text{KNO}_3$  ajoutée, l'augmentation de la photosynthèse a été de 58,7% en comparaison aux plants irrigués seulement avec du NaCl. Pour le supplément de calcium, l'augmentation de la photosynthèse a été de 54,72% à la dose 10 mM de  $\text{CaCl}_2$  et 50,49% à la dose 40mM de  $\text{CaCl}_2$  (figure1). Chartzoulakis et al, 2006 ont montré que l'application du potassium à une dose de 100 mM contribue à l'amélioration de la photosynthèse des plants d'olivier irrigués avec une solution contenant 100 mM de NaCl. Tattini et Traversi (2009), ont indiqué que l'ajout de calcium à une dose de 9 mM produit une amélioration de la photosynthèse des feuilles des plants d'olivier cultivés sur un milieu salin (200mM de NaCl).

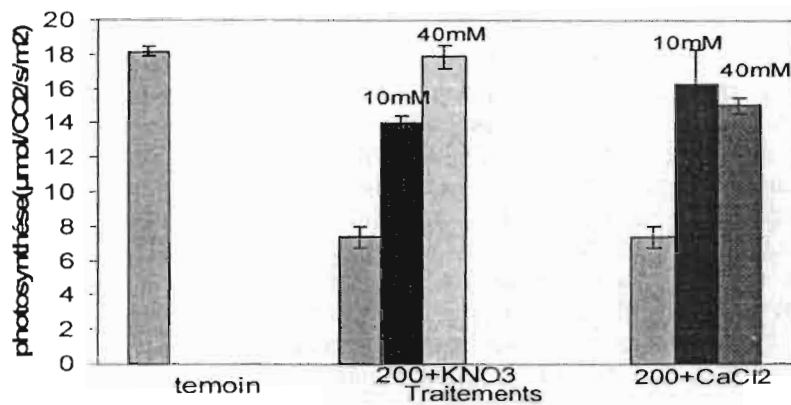


**Figure 1:** Effet de la salinité (200mM de NaCl) et de l'application du potassium (10 et 40mM de KNO<sub>3</sub>) et de calcium (10 et 40mM de CaCl<sub>2</sub>) sur la photosynthèse des plants de la Variété Arbequina irrigués avec un solution saline après onze mois de traitements

### 3.2. Effet sur la conductance stomatique

L'analyse de la Figure 2 a permis de noter que la salinité a provoqué une diminution de la conductance stomatique. Cette diminution a été de 27,2 % à 200mM de NaCl. Nos résultats sont en concordance avec d'autres études (Chartzoulakis, 2005, Chartzoulakis et al, 2006, Kchaou, 2006).

L'application de potassium sous forme de KNO<sub>3</sub> à la dose 40 mM a engendré une augmentation significative de 25,9% de la conductance stomatique en comparaison aux plants traités uniquement avec 200mM de NaCl (figure 2). L'application de calcium aux plants traités avec une solution saline, a généré aussi une augmentation dudit paramètre avec la dose 10 mM de CaCl<sub>2</sub> (24,6%) par rapport aux plants irrigués avec une solution saline. Tattini et Traversi (2009), ont montré que l'addition de 9 mM de calcium engendre une amélioration de la conductance stomatique des feuilles des plants d'olivier cultivé sur un milieu salin (200mM de NaCl).

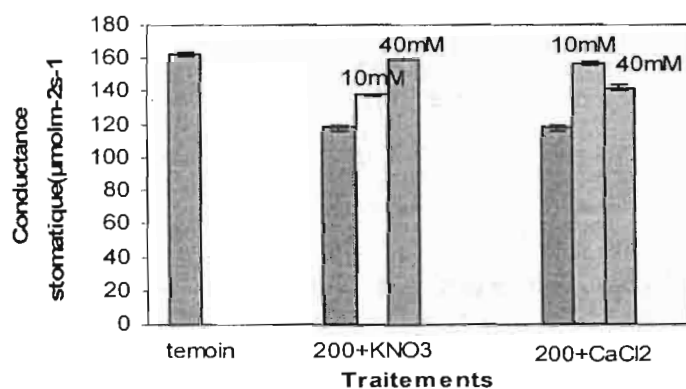


**Figure 2:** Effet de la salinité (200mM de NaCl) et de l'application du potassium (10 et 40 mM de KNO<sub>3</sub>) et de calcium (10 et 40mM de CaCl<sub>2</sub>) sur la conductance stomatique des plants de la variété Arbequina irrigués avec un solution saline après onze mois de traitements

### 3.3. Effet sur le statut nutritionnel

La salinité a provoqué une augmentation de la concentration de sodium et de chlorures dans les feuilles et les fruits. En effet, cette augmentation dans les feuilles et fruits a été 92%, 89% et 93%, 89,41% respectivement (tableau1). En ce qui concerne le potassium, nous avons trouvé que la concentration dudit élément diminue sous l'effet de la salinité. Au niveau des feuilles, la diminution de potassium a été 3,76 fois inférieure à celle obtenue dans les feuilles des plants témoins. De même, la concentration de potassium dans les fruits a diminué de 64% par rapport aux plants témoins. La salinité a provoqué une diminution de la concentration de calcium aussi bien au niveau des feuilles qu'au niveau des fruits. En effet, nous avons enregistré une diminution de la teneur en calcium de



61,7% au niveau des feuilles et 47,2% au niveau des fruits (tableau1). Des résultats similaires ont été rapportés par plusieurs auteurs (Chartzoulakis et 2002, Chartzoulakis, 2005, Chartzoulakis et al, 2006, kchaou 2006 ; Loupassaki et al., 2002). L'application de potassium sous forme de  $\text{KNO}_3$  a engendré une diminution de la concentration de sodium et de chlorures dans les feuilles et les fruits par rapport aux plants irrigués avec seulement une solution saline. Toutefois, il est à signaler que la diminution la plus importante a été obtenue avec la dose 40mM de  $\text{KNO}_3$  (tableau 1). En ce qui concerne l'application du calcium, la diminution la plus importante de la teneur du sodium et des chlorures dans les feuilles et les fruits a été obtenue avec la dose de 10 mM de  $\text{CaCl}_2$ . L'application de potassium à différentes doses a engendré une augmentation progressive de la concentration du potassium dans les feuilles et les fruits. Cette augmentation a été plus accentuée chez les plants ayant reçu une dose de 40 mM de  $\text{KNO}_3$ . En effet, nous avons enregistré une augmentation de la teneur du potassium de 74,7% dans les feuilles et de 61,3 % dans les fruits en comparaison avec les plants irrigués uniquement avec une solution saline. L'application de calcium à 10 mM de  $\text{CaCl}_2$ , a provoqué une augmentation de la concentration de potassium dans les feuilles de 63,2% et de 55,5% dans les fruits en comparaison avec les témoins. Concernant le calcium, l'application de potassium a provoqué une augmentation de cet élément dans les feuilles des plants traités. A 40 mM de  $\text{KNO}_3$ , la concentration de calcium dans les feuilles a augmenté de 55% et de 36,13% dans les fruits. L'application de calcium à la dose de 10 mM de  $\text{CaCl}_2$  a engendré aussi une augmentation de la concentration de calcium aussi bien au niveau des feuilles qu'au niveau des fruits qui a été de 59% et 42,1% pour feuilles et fruits, respectivement (Tableau 1). Des résultats similaires ont été obtenus par Bohra et Doerffling (1993), Bahmaniar (2006), Kaya et al, (2003).

**Tableau 1:** Effet de la salinité (200mM de NaCl) et de l'application du potassium (10 et 40mM de  $\text{KNO}_3$ ) et de calcium (10 et 40mM de  $\text{CaCl}_2$ ) sur la nutrition minérale des plants de la Variété Arbequina irrigués avec une solution saline après onze mois de traitements

| Feuilles        |      |      |      |      | Fruits |      |      |      |      |
|-----------------|------|------|------|------|--------|------|------|------|------|
|                 | Na   | Cl   | K    | Ca   | Na     | Cl   | K    | Ca   |      |
| Temoin          | 0,05 | 0,08 | 0,98 | 0,94 | 0,04   | 0,09 | 1,01 | 0,91 |      |
| 200+            | 0    | 0,63 | 0,76 | 0,26 | 0,36   | 0,61 | 0,85 | 0,36 | 0,48 |
| $\text{KNO}_3$  | 10   | 0,23 | C    | 0,7  | 0,63   | 0,26 | 0,56 | 0,78 | 0,63 |
| (mM)            | 40   | 0,11 | 0,16 | 1,03 | 0,81   | 0,16 | 0,28 | 0,93 | 0,76 |
| 200+            | 0    | 0,63 | 0,76 | 0,26 | 0,36   | 0,61 | 0,85 | 0,36 | 0,48 |
| $\text{CaCl}_2$ | 10   | 0,18 | 0,31 | 0,71 | 0,88   | 0,23 | 0,38 | 0,81 | 0,83 |
| (mM)            | 40   | 0,37 | 0,48 | 0,51 | 0,71   | 0,33 | 0,4  | 0,77 | 0,7  |

#### 4. Conclusion

A partir des résultats obtenus, on peut conclure que

- La concentration 200mM de Cl additionnée à la solution nutritive a affectée négativement la photosynthèse et la conductance stomatique et a provoquée une déficience en potassium et en calcium dans les feuilles
- L'application de supplément de potassium (40mM de  $\text{KNO}_3$ ) et de calcium (10mM de  $\text{CaCl}_2$ ) a un effet bénéfique sur les paramètres affectés par la salinité

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## **Etude de l'impact des acariens ériophyides sur la croissance et le développement de quelques variétés d'olivier dans la région de Sfax**

Chatti Kolsi A., Ksantini M.

### **Résumé**

Notre travail s'intéresse à l'étude des acariens ériophyides nuisibles à l'olivier dont la multiplication anormale observée ces dernières années semble causer des dégâts très importants aussi bien à la végétation qu'à la production. L'évaluation de l'impact des acariens ériophyides sur la croissance de l'arbre et la production de fruits a porté sur 6 variétés d'olive à huile et 3 variétés d'olives de table, en comparant des rameaux sains à d'autres infestés à raison de 20 par arbres (10 traités et 10 non traités) sur un ensemble de 4 arbres par variété.

Les variables mesurées ont concerné des critères quantitatifs : l'allongement des nouvelles pousses, la chute des grappes et la nouaison des fruits. Pour mieux déterminer l'impact de ces parasites, on a eu recours au suivi de l'évolution de la plante hôte en mesurant mensuellement l'allongement des nouvelles pousses, en dénombrant les grappes florales et en estimant les niveaux de population des ériophyides. L'impact des acariens sur la croissance des pousses et la nouaison des fruits est variable selon les variétés. Ces phytophages semblent avoir un effet néfaste sur Koroneiki, Zalmati, Arbequina et Picholine en réduisant l'allongement des pousses jusqu'à 16 %, en augmentant la chute des grappes (peut atteindre 45 % de perte) et la nouaison des fruits avec un effet sur les rameaux infestés évalué à une perte de 46 %. Par contre pour les variétés Chemlali, Chemchali, Chetoui, Manzanille et Meski, la présence des phytophages semble plutôt bénéfique, dans le sens que ces acariens, à un seuil d'attaque bas, jouent le rôle de stimulateur par l'appel de sève.

**Mots clés:** Acariens ériophyides, Dégâts, Croissance végétative, Nouaison.

## **Study of the impact of eriophyid mites on growth and development of some olive varieties in the region of Sfax**

### **Abstract**

Our work focuses on the study of harmful eriophyid mites olive whose abnormal growth in recent years seems to cause major damage to both vegetation and production.

We are interested to study the impact of eriophyid mites on 6 varieties of olive oil and 3 varieties of table olives, considerable the influence on growth and production. By comparing healthy branches to other infested at a rate of 20 per trees (10 treated and 10 untreated) on a set of 4 trees per variety. The variables measured were for quantitative criteria: the elongation of new shoots, the fall in clusters and fruit set.

To better determine the impact of these parasites, we studied the evolution of host plant monthly by measuring the elongation of new shoots, calculating the load in inflorescences and describing its phonology and estimate population levels of eriophyid mites.

The impact of mites on shoot growth and fruit set varies by variety.

These herbivores seem to have an adverse effect on Koroneiki, Zalmati, Arbequina and Picholine in reducing shoot elongation up to 16%, increasing the collapse of clusters (up to 45% loss) and fruit set of fruit with an effect on infested branches evolved to a loss of 46%.

By contrary for the varieties Chemlali, Chemchali, Chetoui, Manzanille and Meski, the presence of herbivores is more beneficial in the sense that these mites at a low level of attack play the role of pacemaker by calling sap.

**Keywords:** eriophyid Mites, Damage, vegetative growth, fruit set.

## **Machines manuelles pour la récolte des olives : caractéristiques techniques et requis agronomiques**

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### **Résumé**

En Italie centrale, pendant quatre années de 2005 à 2008, des essais pour évaluer l'efficacité de la récolte avec plusieurs machines manuelles pour cueillir des olives, ont été effectués. Les épreuves ont été conçues afin d'étudier l'influence de la variété, du système de plantation, de la période de récolte, de l'intensité de la taille sur l'efficacité de différents type de machines à récolter tenues dans la main (c-à-d peigne pneumatique, fouetteur électrique, secoueur, etc). Les résultats donnent des indications utiles pour le choix et le meilleur usage de chaque machine par rapport à la structure de l'olivieraie et de l'objectif de production.

**Mots clés:** *Olea europaea* L., peignes pneumatiques, fouetteur électrique, secoueur.

## **Hand-held machines for olive harvesting: technical characteristics and agronomical requirements**

### **Abstract**

In central Italy, in the four-year-period 2005-08, trials to assess the harvest efficiency of several hand-held machines for olive harvesting were carried out. The trials were arranged in order to establish the influence of the cultivar, training system, time of harvesting, pruning intensity on the efficiency of different type of hand-held machines (i.e. pneumatic comb, electric whipper, hook shaker, etc.). The results give indications useful for the choice and the best use of each machine in relation to the structure of the olive orchard and the farm productive objective.

**Key words:** *Olea europaea* L., pneumatic combs, electric whipper, hook shaker.

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## Study of some parameters physic - chemic of the infestation olive oil with olive fruit fly *Bactrocera oleae*

Mraicha F<sup>(1)</sup>, Ksantini M<sup>(1)</sup>, Sayadi<sup>(2)</sup> S., Bouaziz M.<sup>(2)</sup>

### Abstract

The quality of virgin olive oil is strongly related to the health status of the fruit from which it is extracted. One of the most detrimental threats to the quality of olive oil is the olive fruit fly (*Bactrocera oleae*). The influence of olive fly attack on the quality of virgin olive oil (free acidity, pigment concentration, and polyphenols) has been studied. Analytical results showed that both attacks by *B. oleae* and maturity process affected the quantitative and qualitative composition of the oil. These analyses demonstrated that the degree of fly attack was positively correlated with free acidity and pigment concentration, and negatively related to the phenolic content.

**Key words:** Virgin olive oil; Olive fly; infestation; maturation; qualitative parameters

## Etude des quelques paramètres physico-chimiques de l'huile d'olive infestée par la mouche de l'olive *Bactrocera oleae* (Diptera – Tephritidae)

### Résumé

La mouche de l'olive *Bactrocera oleae* est considérée comme le phytophage le plus redoutable pour l'oléiculture méditerranéenne. Elle cause des pertes sérieuses au fruit et réduit la qualité de l'huile d'olive en affectant certains des caractéristiques chimiques.

Dans le but de déterminer l'impact de l'infestation par la mouche de l'olive sur la qualité de l'huile, nous avons suivi l'évolution des quelques paramètres physico-chimiques de l'huile en fonction du degré d'infestation et du processus de maturation des olives. Les paramètres étudiés sont : les composés phénoliques, l'acidité libre et les pigments. Ce travail a été effectué à région Bouakkazine (sud de Sfax), sur la variété Chemlali.

Les résultats obtenus montrent que le stade de maturité des olives et l'infestation par la mouche influe sur la composition chimique de l'huile. En effet, les teneurs en pigments (chlorophylle et carotène) et en polyphénols diminuent au cours du temps, et en augmentation d'infestation. Concernant l'acidité, les résultats obtenus permettent de conclure que l'infestation par *Bactrocera* n'a aucun effet pendant les mois d'Octobre et Novembre alors qu'elle est proportionnelle à l'infestation en hiver.

**Mots clés:** *Bactrocera oleae*, infestation, maturation, huile d'olive, caractéristiques chimiques.

### 1. Introduction

The olive tree (*Olea europaea*) has an important social and economical significance in the Mediterranean basin, which occupies 98% of the world's cultivated olive trees (Alfonso and Owen, 2002). The Tunisian olive growing counts about 67 millions of olive trees covering more than 1.7 millions of ha. *O. europaea* L. cv. Chemlali is the most abundant olive variety, which represents >60% of the total olive trees in Tunisia (Khlif et al., 2002). The quality of virgin olive oil is strongly related to the health status of the fruit from which it is extracted. One of the most detrimental enemies of the quality of olive oil is the olive fruit fly (*Bactrocera oleae*). The aim of this study was to assess different qualitative parameters of olive oil samples depending on the percentage of fly attack and stage of fruit ripening of Chemlali variety.

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## 2. Materials and methods

### 2.1. Free acidity

These parameters were determined according to the official methods described in European Regulation EC 2568/91 and amendments (Commission Regulation, 1991).

### 2.2. Chlorophylls and carotenoids contents

Following the procedures described by Minguez Mosquera et al. (1990) a sample of olive oil (7.5 g) was placed in a Falcon tube and filled until 25 ml with cycle-hexane. The chlorophyll fraction was measured in a UV spectrophotometer (Shimadzu Co., Kyoto, Japan) at (630, 670, and 710 nm) and the carotenoids fraction at 470 nm.

**Table 1:** Quality characteristics of olive oil samples obtained from olives infested with olive fly

| Date                  | 8/10/2008    |       |      |       | 4/11/2008    |      |      |      |      | 30/01/2009   |      |      |       |      |
|-----------------------|--------------|-------|------|-------|--------------|------|------|------|------|--------------|------|------|-------|------|
|                       | Infestation% |       |      |       | Infestation% |      |      |      |      | Infestation% |      |      |       |      |
| Paramètres            | 0            | 5     | 10   | 15    | 100          | 0    | 5    | 10   | 15   | 100          | 0    | 5    | 10    | 15   |
| Acidité               | 0,1          | 0,4   | 0,45 | 0,5   | 0,9          | 0,3  | 0,4  | 0,5  | 0,8  | 1            | 0,5  | 0,55 | 0,6   | 1,5  |
| Chlorophylles (mg/kg) | 12,51        | 10,50 | 9,4  | 6,024 | 3,28         | 5,39 | 4,56 | 4,11 | 1,3  | 1,11         | 1,18 | 0,86 | 0,643 | 0,5  |
| Carotenoids (mg/kg)   | 8,82         | 7,54  | 6,22 | 6,41  | 5,36         | 6,33 | 6,19 | 5,63 | 5,41 | 4,49         | 6,06 | 6,13 | 4,91  | 4,73 |

### 2.3. Total phenol measurement

Total phenol content of extracts was determined using the Folin–Ciocalteu technique (Singleton and Rossi, 1965).

## 3. Results and discussion

### 3.1. Free acidity

The free acidity values of the studied oils ranged from 0.1% to 3.4%. Taking into account the acidity of the oil samples (fifteen samples) there were eleven samples of oils with very low acidity values (60.8) that could be classified as extra virgin olive oils; three samples with an acidity between 0.8% and 2% that were defined as virgin olive oils, and finally one oil with an acidity higher than 2% that were considered as lampante olive oil (**Table 1**). The exit holes, made by the olive fruit fly favor the entrance and development of bacteria and fungi, which increase the damage and breakdown in the olive pulp (Pereira et al., 2004). In these conditions, acidity can increase due to the action of the hydrolytic enzymes and the lipolytic activity of those microorganisms (Pereira et al., 2004).

### 3.2. Change of chlorophyll and carotenoids contents

Table 1 shows a decrease in the chlorophyll and carotenoid contents during the ripening process. Attack of olives by *B. oleae* caused greater pigment loss as the degree of infestation increased.

### 3.3. Total phenols contents

The content of phenolic compounds presented a high variability depending on the degree of olives infestation. The *B. oleae* attack resulted in notable losses in the total amount of phenolic compounds in oils from olives in advanced stages of ripeness. Different samples with low, medium, and high percentages of fly attack were chosen (**Fig. 1**), and an evident decrease of phenolic content was observed when the percentage of fly attack increases. The reduction of the phenolic concentration in oils from olives totally attacked (100%) at harvest in October, November 2008, and January, 2009, in relation to corresponding samples from healthy fruit, was about 80.62% and 60%.

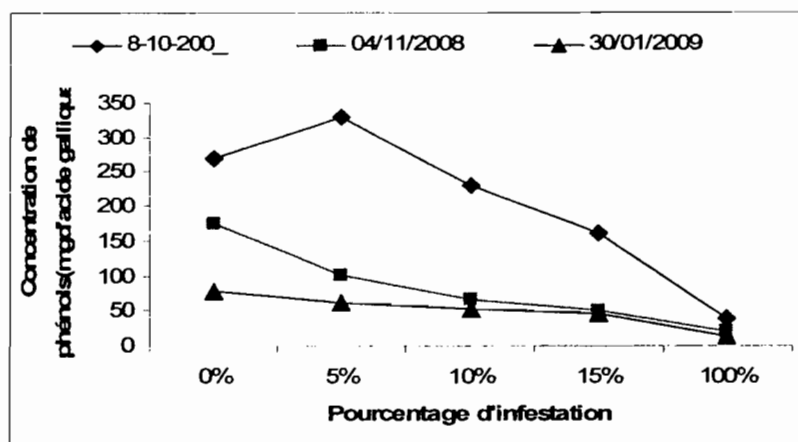


**Figure 1:** Phenolic content in oil during maturation of olives with infestation levels.

In conclusion, from the obtained results, Chemlali olive cultivar seemed to be largely influenced by the olive repining stage and *B. oleae* attack. The latter, have a negative repercussions on oil quality, it affected significantly the free acidity, pigments, total phenols content and consequently affects the sensory characteristics by diminishing most of the attributes.

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## Impact de l'infestation par la 3<sup>ème</sup> génération de *Prays oleae* sur la composition et la structure des feuilles d'olivier

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### Résumé

L'objectif de ce travail est d'étudier l'impact de l'infestation par *P. oleae* sur la composition minérale en phosphore et en potassium des feuilles infestées, ainsi que sur quelques caractéristiques tissulaires. L'échantillonnage a été réalisé à Melloulèche (Mahdia-Tunisie) et les feuilles sont prélevées d'une dizaine d'arbres avec 3 répétitions. Les paramètres mesurés sont les teneurs en phosphore et en potassium. Des échantillons similaires de feuilles saines et infestées sont prélevés des mêmes arbres, puis préparés à l'aide de la technique d'histologie par inclusion à la paraffine puis montés pour observation microscopique.

Les résultats d'observation microscopique des coupes histologiques montrent la destruction du parenchyme palissadique et l'élargissement de l'ouverture des stomates. Aussi, la comparaison des teneurs en phosphore dans les feuilles saines et infestées révèle une réduction de moitié de la quantité en phosphore totale des feuilles (de 176.6 ppm dans les feuilles saines contre 50.3 ppm chez les infestées).

En conclusion, il apparaît que l'infestation des feuilles par *P. oleae* présente un effet néfaste sur le déroulement normal du cycle végétatif de la plante du fait du rôle que joue le phosphore dans la photosynthèse. Par opposition, l'élément potassium ne semble pas être influencé par l'infestation du ravageur.

**Mots clés:** Infestation des feuilles, *Prays oleae*, composition minérale, phosphore, potassium, coupes histologiques, structure tissulaire.

### Third generation of *Prays oleae* impact on the composition and structure of olive tree leaves

#### Abstract

The present work aims to study the impact of *P. oleae* infestation on olive tree leave composition in phosphore and potassium elements as well as the infestation effect on some characteristics of leave tissues. The sampling of leaves has been achieved at Melloulèche (Mahdia-Tunisia) from about ten trees with 3 repetitions. The parameters which were examined are the amounts (ppm) of phosphore and potassium. From the same trees, samples of healthy and infested leaves were prepared using the technique of histology by paraffin inclusions than observed with microscope.

Microscopic observation of infested leave tissues shows the destruction of the palissadic parenchyma and the widening of stomata's. Also, the comparison between healthy and infested leaves reveals a reduction of half in total phosphorus amount (176.6 ppms in healthy and 50.3 ppms in infested leaves).

In conclusion, it appears that *P. oleae* infestation presents a negative effect on the vegetative cycle progress of the tree due to the role that plays the phosphore element in photosynthesis. However, the potassium doesn't seem to be influenced by the insect infestation.

**Key words:** infestation of leaves, *Prays oleae*, mineral composition, phosphorus, potassium, histology, tissues structure,



## 1. Introduction

L'arboriculture oléicole compte parmi les principales spéculations agricoles dans la méditerranée. Cette culture se trouve par ailleurs, menacée de l'attaque de plusieurs ravageurs d'importance primaire dont la teigne de l'olivier *Prays oleae* (Lepidoptera, Hyponomeutidae) qui s'attaque aux différents organes de l'olivier : feuilles, fleurs et olives au cours de ces trois générations annuelles. *P. oleae* trouve son origine dans les oliviers de l'Eurasie, la Syrie et la Grèce. En Tunisie, les populations importantes ne sont observées que dans les régions côtières allant du sahel de Sousse jusqu'à l'extrémité du sud où l'on marque une diminution progressive en s'éloignant de la côte. A l'encontre des deux premières générations de *Prays oleae* (Lepidoptera, Hyponomeutidae) qui causent des dégâts notables à la floraison et à la production des fruits, la 3<sup>ème</sup> génération, sur feuilles, passe souvent inaperçue. Très peu d'études sont faites pour évaluer les dégâts de la troisième génération sur les feuilles qui ne subit pas jusqu'à présent de traitements dans le cadre des campagnes nationales de lutte. Les seuls travaux ayant trait à l'évaluation des dégâts de cette génération remontent à Trigui (1983) qui a démontré ses effets néfastes sur les propriétés optiques des feuilles infestées. Les dégâts occasionnés sont dus aux phases de développement larvaire observables surtout à partir de la fin du mois d'octobre environ, où commencent à apparaître de longues galeries étroites, qui se transformeront plus tard, en mines rondes lobées, avec des orifices à la face inférieure des feuilles à partir desquelles sortent des excréments enchevêtrés (Arambourg, 1964 ; Bellido, 1975; Pralavorio, 1985). Vers la fin d'hiver, des dégâts de râpage au revers des feuilles peuvent être importants sans que la face supérieure de l'épiderme soit touchée. Entre fin février et fin avril, les jeunes pousses et les feuilles se trouvent reliées par des fils soyeux et présentent parfois des morsures pratiquées par les chenilles. Bien que les dégâts ne soient pas évalués économiquement, il importe de signaler le possible effet nuisible de *P. oleae* sur les paramètres biochimiques et structuraux des feuilles. En effet, les dégâts induits par une forte infestation des nuisibles à un effet direct sur les paramètres physiques et biochimiques dont notamment la destruction plus ou moins prononcée du tissu végétal (Heitefuss et Williams, 1976) ainsi que l'assimilation des éléments minéraux essentiels (Das et al, 1999).

La diversité et l'importance des fonctions remplies par les éléments minéraux confirment la nécessité de s'assurer de la disponibilité de ces éléments en quantités suffisantes pour une production optimale de l'arbre. En ce qui concerne les éléments minéraux essentiels, en plus de l'azote dont la carence des feuilles se traduit par un jaunissement et dressage des feuilles, le potassium est aussi essentiel pour la translocation de sucres et pour la formation de l'amidon. Il intervient dans la régulation osmotique et ionique, ainsi que dans le processus d'ouverture et de fermeture des stomates. Pour le phosphore, la carence se manifeste par une réduction générale de la croissance et les feuilles sont minces et se dressent. Le phosphore possède un rôle fertilisant majeur et joue un rôle important dans les phases juvéniles et intervient de façon quantitative dans la multiplication cellulaire et la croissance des organes et possède également un rôle essentiel dans la photosynthèse. Ainsi et dans le cadre de l'évaluation des dégâts occasionnés par la teigne phyllophage, il apparaît important de s'informer de l'effet de l'infestation par *P. oleae* sur la structure des feuilles et les rendements en éléments minéraux essentiels.

## 2. Matériels et méthodes

### 2.1. Biotope d'étude

Le biotope d'étude où sont conduits les différents échantillonnages est celui de Malloulech appartenant au gouvernorat de Mahdia situé au Sud de Sfax à 42 Km. Les arbres sont de la variété Chemlali Sfaxien assez vigoureux et en année de production.

### 2.2. Echantillonnage et analyse de la composition minérale des feuilles

#### 2.2.1. Echantillonnage

L'échantillonnage des feuilles a été réalisé en prenant des feuilles infestées par les stades larvaires L4-L5 à partir de 10 arbres choisis au hasard et à raison de 100 feuilles par arbre. Les feuilles saines sont prises des mêmes arbres et dans les mêmes conditions de prélèvement.

### 2.2.2. Paramètres analysés

Les paramètres mesurés pour l'analyse des feuilles sont l'humidité, les teneurs en phosphore :  $P \text{ (ppm)} = (L \times (1/D) \times (1/Re) \times \alpha)$  et en potassium :  $K^+ \text{ (ppm)} = (L \times (1/D) \times (1/Re))$  où selon les formules, D, est la dilution, L, est lecture de l'absorbance, Re, le rapport d'extraction (1/100) et  $\alpha$  : pente de la courbe d'étalonnage du colorimètre. Les méthodes d'analyses employées sont celles utilisées dans le laboratoire de l'institut de l'olivier de sfax selon (Pauweles et al 1992). Avant l'analyse, les échantillons sont bien lavés pour éliminer les débris de larves et excréments sur les feuilles.

### 2.3. Préparation des coupes histologiques des feuilles infestées et saines

A l'aide de la technique d'histologie par inclusion à la paraffine, les feuilles infestées (stade larvaire L4 et L5) et saines collectés depuis les mêmes arbres et en même temps sont ramenées au laboratoire, lavées à l'eau distillée et fixés pendant 48 heures dans la solution composé d'Alcool (80ml), Acide acétique (10ml) et de Formol (10ml) (Lu et Hu, 2001). Après un lavage à l'eau courant pendant 24 heures, les organes fixés sont déshydratés puis inclus dans les bains de paraffine avant d'être coupés à l'aide d'un microtome. Les coupes ainsi préparées sont fixées entre lame et lamelles par l'albumine puis colorées à l'hématoxyline et à l'éosine. Le montage de ces coupes se fait dans une goutte de baume de CANADA avant l'observation au microscope.

## 3. Résultats et Discussions

### 3.1. Impact de l'infestation des feuilles au cours de la génération phyllophage sur la composition minérale et la structure des tissus végétales

#### 3.1.1. Mesure de la quantité du phosphore des feuilles

La quantité du phosphore **[P] (ppm)** est obtenue à partir de la formule décrite dans la partie méthodologie. Les résultats relatifs aux quantités de phosphore et de potassium des feuilles saines et infestées dans le biotope de Melloulèche sont récapitulés dans les Figures 1 et 2.

D'après les résultats des analyses de la composition minérale des feuilles, il en résulte que les feuilles saines sont plus riches en quantité de phosphore P (ppm) que les feuilles infestées (Fig. 1) par contre, il apparaît que la quantité en potassium K (ppm) ne semble pas être influencé par l'infestation par la teigne de l'olivier (Fig.2).

Ceci montre que l'infestation par la teigne de l'olivier diminue d'une façon importante, presque de moitié, la quantité en phosphore totale des feuilles. Le phosphore possède un rôle fertilisant majeur et joue un rôle important dans les phases de multiplication cellulaire et de croissance des racines et des tiges. Comme élément régulateur de la végétation, le phosphore réduit les cycles végétatifs : c'est donc un facteur de précocité. D'où sa diminution pourrait avoir un effet accentuant de l'alternance chez l'olivier. En plus, dans les feuilles, le phosphore existe sous la forme phospho-esters comprenant les glucides phosphorylés qui jouent un rôle extrêmement important dans la photosynthèse et le métabolisme intermédiaire. De ce fait, il apparaît que l'infestation des feuilles par *P. oleae* a des conséquences immédiates sur le déroulement normal du cycle végétatif de la plante. Il en résulte des résultats de cette analyse que l'assimilation de l'élément potassium ne semble pas être influencé par l'infestation par la teigne de l'olivier au contraire de la composition en phosphore. Ceci peut être expliqué par le fait que le potassium est toujours abondant dans la matière sèche des feuilles et que ce dernier ne joue pas un rôle direct dans les processus biochimiques au niveau des feuilles. En effet, cet élément agit d'une façon indirecte, il active la photosynthèse et favorise la formation des glucides dans la feuille.

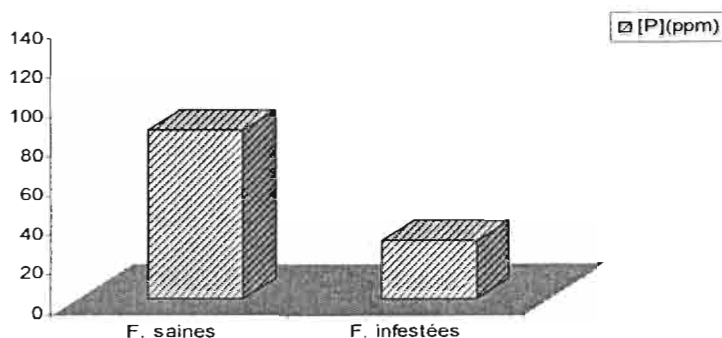


**Figure 1:** Comparaison des quantités de phosphore (ppm) dans les feuilles saines et infestées par *P. oleae*

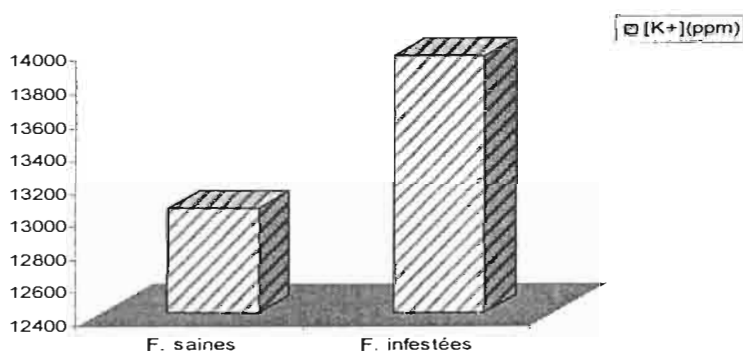


**Figure 2:** Comparaison des quantités de potassium (ppm) dans les feuilles saines et infestées par *P. oleae*

### 3.1.2. Coupes histologiques et structures des feuilles saines et infestées par *P. oleae*

L'observation microscopique des coupes histologiques des feuilles saines et infestées montrent une dégradation partielle plus au moins prononcée des tissus chez les feuilles infestées. Cette dégradation se manifeste par l'élargissement des stomates et le déchirement du parenchyme palissadique (Fig.3) mais aussi par la détérioration des pigments chlorophylliens suivi d'un dessèchement et perte de couleurs des zones infestées. Quelques recherches ont montré que la dégradation de la chlorophylle peut résulter d'un phénomène complexe lié de l'alimentation des feuilles des végétaux par les insectes (Ni et al., 2001). Par ailleurs, étant donné que la chlorophylle dans les feuilles est le composé déterminant dans le processus photosynthétique (Mao et al., 2007), il importe de signaler la possibilité de perte des rendements photosynthétiques suite à un dessèchement des feuilles infestées par *P. oleae*.

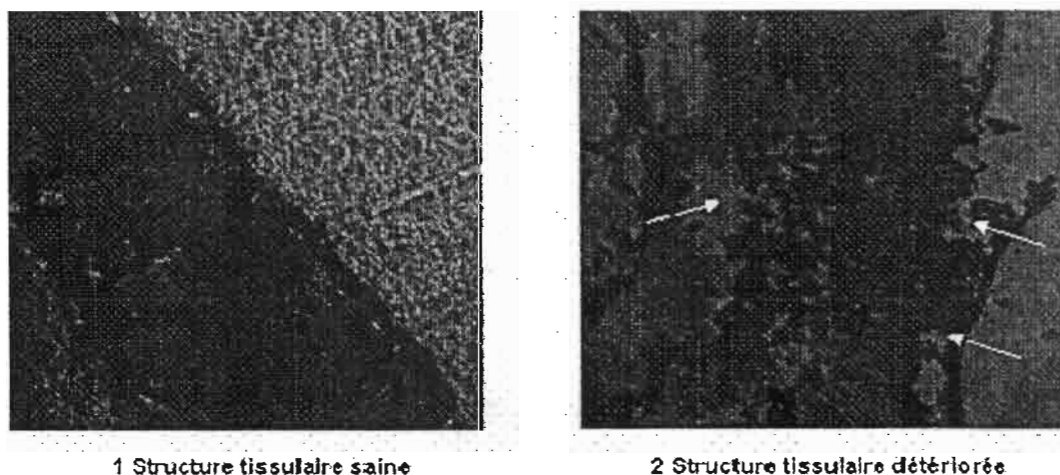


**Figure 3:** Coupe transversale de feuille d'olivier saine (1) et infestée (2) (microscope, Gross. 100)

#### 4. Conclusion

L'effet de l'infestation par la troisième génération de *P. oleae* se répercute sur quelques paramètres qualitatifs et quantitatifs des feuilles. Ces paramètres s'adressent principalement à la composition minérale en éléments minéraux essentiels dont notamment le phosphore qui se trouve significativement atténué par suite de l'infestation par les stades larvaires âgés de ce ravageur. Par ailleurs, une observation microscopique détaillée de l'assise cellulaire montre l'altération du parenchyme palissadique et l'élargissement des stomates ainsi que la perte de chlorophylle dans les zones attaquées. Il importe de signaler que ces paramètres étudiés sont en étroite corrélation avec le rôle essentiel que joue le phosphore dans les processus photosynthétique et de croissance végétale et il apparaît en conclusion la nécessité d'intervention contre la génération phyllophage surtout en des cas de forte infestation de jeunes plantules.

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## Preliminary evaluation of the status of olive-infecting viruses in Tunisia

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### Abstract

A virus survey was carried out in two Tunisian mother blocks located at Sfax and Mannouba. A total of 174 olive samples of 19 different olive varieties were selected. These plants were checked by RT-PCR for the presence of *Arabis mosaic virus* (ArMV), *Cherry leaf roll virus* (CLRV), *Cucumber mosaic virus* (CMV), *Olive latent ringspot virus* (OLRSV), *Olive latent virus 1* (OLV-1), *Olive latent virus 2* (OLV-2), *Olive leaf yellowing-associated virus* (OLYaV) and *Strawberry latent ringspot virus* (SLRSV). PCR results indicated that about 86% of the trees were infected. OLYaV was the prevailing virus (50.2%), followed by SLRSV (35.6%), CMV (27.7%), OLRSV (18%) and CLRV (11.2%). Less represented were the remaining three viruses. A high infection rate (84%) was obtained for the major cultivated oil variety "Chemlali".

**Key words:** Olive, mother blocks, viruses, PCR

### Résumé

Un inventaire des virus de l'olivier a été réalisé dans deux champs de pieds mère situés à Sfax et Mannouba, un total de 175 échantillons d'olivier prélevés de 20 différentes variétés ont été collectés. Les plants ont été analysés par RT-PCR pour vérifier la présence de l'*Arabis mosaic virus* (ArMV), du *Cherry leaf roll virus* (CLRV), du *Cucumber mosaic virus* (CMV), de l'*Olive latent ringspot virus* (OLRSV), de l'*Olive latent virus 1* (OLV-1), de l'*Olive latent virus 2* (OLV-2), de l'*Olive leaf yellowing-associated virus* (OLYaV) et du *Strawberry latent ringspot virus* (SLRSV). Les résultats de la PCR ont indiqué qu'environ 86% des arbres étaient infectés. OLYaV est le virus le plus répandu (48.5%), suivi par OLV-1 (37%), SLRSV (35.6%), CMV (27.7%), OLRSV (18%) et CLRV (13%). Les deux autres virus sont moins fréquents. Un taux élevé d'infection (84%) a été détecté dans la variété à huile "Chemlali" qui est la plus cultivée.

**Mots-clés:** olivier, virus, PCR, champs de pieds mère,

### 1. Introduction

Olive is the most important fruit tree crop in Tunisia, where about 65 millions trees are under cultivation and spreading throughout the country from North to South, with plantings covering around 1.67 million hectares. Tunisia represents the largest olive oil producer after the European countries and accounts for 8% of the total olive oil production in the World (Anonymous, 2008). Two main varieties of olive oil are cultivated, Chétoui prevailing in the north and Chemlali in all the country. Olive hosts a number of infectious pathogens including 14 viruses (Martelli, 1999; Al Abdullah *et al.*, 2005). The improvement of the sanitary status of this important crop becomes strategically important. To this aim a preliminary investigation on the occurrence of viruses in Tunisian olive groves was done.

A total of 175 samples was collected from two different mother plant plots located at Sfax and at Mannouba containing clonally selected olive plants coming from different Tunisian regions and some accessions of important olive varieties introduced from abroad potentially interesting for Tunisia. Cuttings about 25–30 cm in length were collected from 1- or 2-year-old twigs from the quadrant of each tree. Samples were labeled and stored at 4°C until using for the laboratory assays.

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## 2. Materials and methods

### 2.1. Reverse transcription-polymerase chain reaction (RT-PCR)

Samples were analyzed by RT-PCR for detecting the presence of eight olive viruses, the *Closterovirus Olive Leaf yellowing associated virus* (OLYaV), the *Sadwavirus Strawberry latent ringspot virus* (SLRSV), the *Nepoviruses Arabis mosaic virus* (ArMV), *Cherry leaf roll virus* (CLRV) and *Olive latent ringspot virus* (OLRSV), the *Necrovirus Olive latent virus 1* (OLV-1), the *Oleavirus Olive latent virus 2* (OLV-2) and the *Cucumovirus Cucumber mosaic virus* (CMV), which can be detected by using reliable sets of primers (Table 1).

**Table 1:** Sets of DNA primers used for the RT-PCR detection of olive-infecting viruses

| Virus | A.l (Pb) | A.r              | P.s                                                 | Reference                         |
|-------|----------|------------------|-----------------------------------------------------|-----------------------------------|
| OLV-1 | 747      | CA-s<br>HA-a     | CTCACCCATCGTTGTGTGG<br>TTTCACCCACCAAATGGC           | Grieco <i>et al.</i> , 1996a      |
| SLRSV | 293      | 3D-s<br>5D-a     | AGGCTCAAGAAAACACAC<br>CCCTTGTTACTTTTACCTCCTCATTGTCC | Faggioli <i>et al.</i> , 2005     |
| CMV   | 280      | CPN3-s<br>CPN5-a | AACATAGCAGAGATGGCGG<br>ACTCTTAACCCCCAACCTT          | Faggioli <i>et al.</i> , 2005     |
| ArMV  | 504      | 3A-s<br>5A-a     | TTGGTTAGTGAATGGAACGG<br>TCAACTCACCTCCAAATCCC        | Grieco <i>et al.</i> , 1996a      |
| CLRV  | 413      | 3<br>5           | TTGGCGACCGTGTAACGGCA<br>GTCGGAAAGATTACGTAAAAGG      | Grieco <i>et al.</i> , 1996a      |
| OLYaV | 346      | y1-s<br>y2-a     | CGAAGAGAGCGGCTGAAGGCTC<br>GGGACGGTTACGGTCGAGAGG     | Sabanadzovic <i>et al.</i> , 1999 |
| OLV-2 | 206      | C-s<br>H-a       | GCCAGGAGTTTGAGCTTTG<br>GAAGGTGGCTCGCCTAGAG          | Faggioli <i>et al.</i> , 2005     |
| OLRSV | 492      | 3'-terminal      | CTGCAAACTAGTGCCAGAGG<br>TGCATAAGGCTCACAGGAG         | Grieco <i>et al.</i> , 2000       |

A.l: Amplicon length, A.r: Amplified region, P.s: Primer sequence (from 5' to 3')

Total nucleic acids (TNA) were extracted from 0.2 g of phloem tissues according to Foissac *et al.* (2001).

A two step protocol was used for the reverse transcription (RT) and amplification (PCR) of target RNA. Amplified products were analyzed in 5% TBE polyacrylamide gel (PAGE) and visualized by silver nitrate staining.

## 3. Results and discussions

These preliminary results show that the most important olive-infecting viruses (OLYaV, CLRV, SLRSV, OLRSV, CMV, OLV-1 and OLV-2) occur in the country. Out of 175 samples submitted to RT-PCR, 151 (86.3%) were infected with at least one virus. The level of infection is relatively high if compared to those reported from Syria (50.7%) (Al Abdullah *et al.*, 2005), Lebanon (31.3 %) (Fadel *et al.*, 2005) and other Mediterranean regions (64.5%) (Saponari *et al.*, 2002).

OLYaV was the most widespread in Tunisian olive groves with an average infection rate of 49.1% (fig.1), reaching a peak of 100% incidence in Gerbouei and Ouesleti cultivars. Whereas its incidence was slightly lower for cv. Koroneiki (9%) and without infection for cv. Tounsi.

OLV-1 was the second virus in terms of incidence (34.3%), and it was detected mainly in imported cvs. Marsalline (75%), Ascolana (63.6%) and Picholine (50%). OLV-1 is considered as a soil-borne virus infecting crop directly without the intervention of vectors (Martelli, 2002).

CMV was detected in 25.7% of samples (fig.1). Its highest infections were seen in Tounsi (100%), Koroneiki (72.7%), Ascolana (45.5%). If the widespread occurrence of CMV can be due to a possible presence of vector(s), mainly aphids, in the Tunisian olive orchards is difficult to say, since no any relationship between the virus with potential vectors has been up to now demonstrated for this crop.

The infection by OLRSV was surprising high (16.6%) if compared with that recorded from other Mediterranean countries (Saponari *et al.*, 2002; Al Abdullah *et al.*, 2005). The infection was significant in cv. Manzanille (37.5%).

CLRV was present in 13.1% (fig.1) of the tested plants. The highest infection was in cv. Zarrazi injassi (66.7%).

SLRSV one of the few viruses associated to true diseases in olive (bumpy fruits) was found in 7.4% of samples.

The *Oleavirus* OLV-2 was present with a low rate (6.9%).

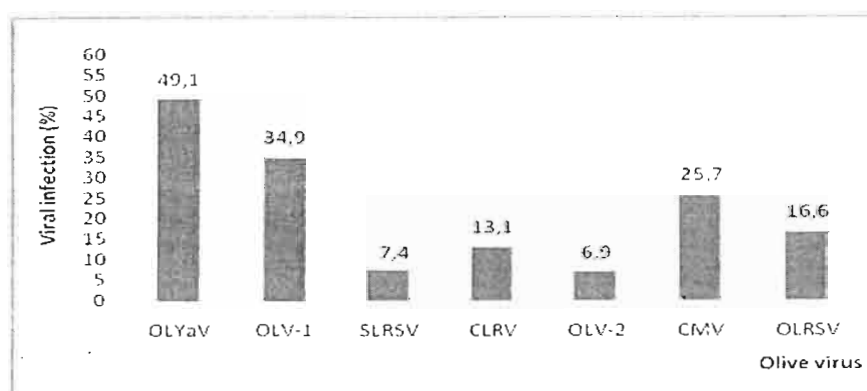


**Figure 1:** Extent of olive-infecting viruses.

The sanitary status of the two main Tunisian cultivars Chemlali (84.6%) and Chétoui (86.9%) (Fig. 2a and b) seemed much degraded. It was also the case of Chemchali and Meski, whose 100% and 89.5% (Fig. 2a and b) of tested plants resulted infected, respectively.

No better was the sanitary situation of the olive varieties introduced from abroad, for which the infection rates ranged from 63.6% of cv. Coratina to 81.8% of cv. Koroneiki.

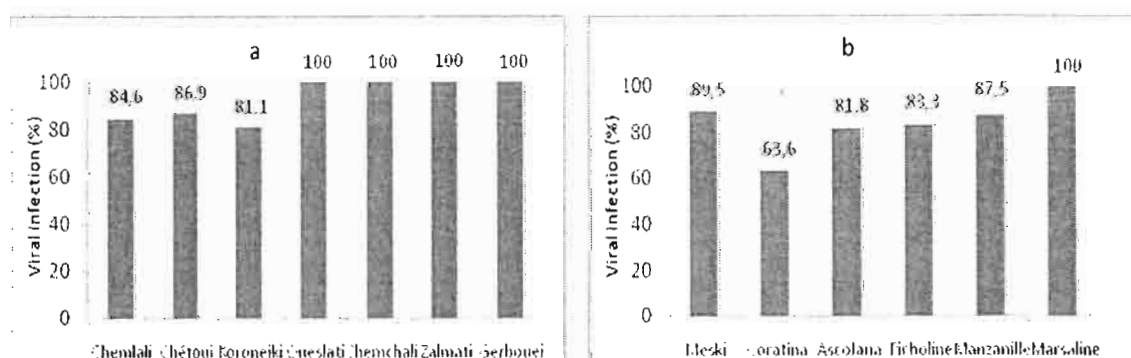


**Figure 2:** Extent of viral infection in different olive varieties.

The results obtained in this survey show the unsatisfactory sanitary status of Tunisian olive trees and the consequent urgent need to adopt control strategies. Sanitary selection is best performed in the framework of certification schemes encompassing also pomological selection for varietal conformity and superior quality traits.

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## Méthodes alternatives pour la lutte contre la mouche de l'olive *Bactrocera oleae* (Gmelin) (Diptera: Tephritidae) appliquées en Crète

Varikou Kyriaki and Venizelos Alexandrakis

### Résumé

Dans une plantation isolée d'oliviers contenant approximativement 5.000 arbres (variété de Koroneiki et de Tsounati) située dans un petit village d'Apokoronas (Macheroi) en Crète, un piège à papier (contenant des attractifs de nutrition, un attractif sexuel et imbibé d'un insecticide) a été installé dans chaque deuxième arbre fin mai pendant 2 années consécutives. En Septembre, le même piège a été installé aux oliviers en repos. Des traitements par appâts empoisonnés avec *Saccharopolyspora spinosa* (Success) ont été également appliqués si nécessaire. La population adulte de la mouche de l'olive dans la plantation d'oliviers avec le piégeage massif était significativement inférieure comparée aux plantations situées à l'extérieur qui ont été traitées par la méthode classique. L'infestation des fruits a été maintenue très basse dans toutes les oliveraies.

**Mots clés:** Mouche de l'olive, pièges, lutte biologique, *Saccharopolyspora spinosa*, Crète.

## Alternative methods for controlling olive fruit fly *Bactrocera oleae* (Gmelin) (Diptera: Tephritidae) applied in Crete

### Abstract

In an isolated olive grove of approximately 5.000 trees (Koroneiki and Tsounati variety) in a small village of Apokoronas (Machairoi), one paper trap (contains trophical and sexual attractive and is dipped into an insecticide) was hung in every second tree at the end of May for 2 consecutive years. In September, the same trap was hung to the rest olive trees. Bait sprays with *Saccharopolyspora spinosa* (Success) were also applied when necessary.

The olive fly population of the olive grove with the Mass trapping was significantly lower compared to the groves outside which was sprayed with the classical control. The infestation was kept very low in all olive groves.

**Keywords:** Mass trapping, olive fruit fly, bait sprays, *Saccharopolyspora spinosa*, Crete.

S1-P22

## Etude de l'impact de quelques paramètres physico-chimiques de l'écorce de quelques variétés d'oliviers sur la virulence de l'hylésine *Hylesinus oleiperda* (Coleoptera, Scolytidae) dans la région de Sfax

Zina Aouem<sup>1</sup> et Mohieddine Ksantini<sup>2</sup>

### Résumé

Cette étude a pour objectif de déterminer l'impact de certains paramètres physico-chimiques de l'écorce de quelques variétés d'olivier sur l'expression des attaques de l'hylésine *Hylesinus oleiperda* Fabricus (Coleoptera, Scolytidae). Toutes les variétés, objet de cette étude, appartiennent à la collection variétale de l'OTD Bouzouita. Il s'agit de Chetoui Tunis, Meski, Chemcheli Gafsa, Ouesleti, Chemleli Sfax, Zarrazi Zarzis et Picholine. Les observations ont porté sur le marquage et le dénombrement des anciennes et nouvelles plaques infestées par l'hylésine sur 4 arbres par variété. Alors que l'analyse physico-chimique a porté sur des fragments (2\*2 cm) d'écorce d'olivier en vue de déterminer: l'épaisseur de l'écorce, la teneur en eau, en azote, en phosphore, en potassium et en polyphénols. Les analyses statistiques effectuées sur les niveaux d'infestations anciennes et nouvelles à l'aide du test Duncan moyennant le logiciel SPSS 11.0, ont montré que les différentes variétés étudiées sont réparties en deux classes d'infestation :

\* Classe des variétés les plus infestées dont le nombre des plaques dépasse 500, représentée par la variété Chetoui Tunis

\* Classe des variétés les moins infestées dont le nombre des plaques est inférieur à 300 plaques, représentée par les variétés Chemcheli Gafsa, Meski, Ouesleti, Chemlali Sfax, Zarrazi Zarzis et Picholine.

Quant à l'impact des paramètres physico-chimiques de l'écorce seule la teneur en eau semble être déterminante de l'infestation. Plus cette teneur augmente moins le bois est infesté ; 42% d'eau semble être la teneur la plus favorable au développement de l'hylésine.

**Mots clés:** *Hylesinus oleiperda*, olivier, degré d'infestation, paramètres physico-chimiques, écorce.

## The study of some physico-chemical parameters in the bark of some olive tree varieties and their impact on the virulence of *Hylesinus Oleiperda* (Coleopetra, Scolytidae) in the region of Sfax

### Abstract

This study aims to determine the impact of certain physico-chemical parameters in the bark of some olive tree varieties on the expression of the infestation by *Hylesinus oleiperda* Fabricus (Coleopetra, Scolytidae).

All the studied varieties belong to the variety collection of Bouzouita OTD, as follows: Chetoui Tunis, Meski, Chemcheli Gafsa, Ouesleti, Chemleli Sfax, Zarrazi Zarzis and Picholine.

The method of survey consists in the marking and enumeration of the new and old plates infested by the insect on 4 trees by variety. Whereas the physico-chemical analysis was carried out about the olive tree bark's fragments (2\*2cm) in order to determine the thickness of the bark, the content of water, azote, phosphore, potassium and polyphenols.

The statistical analyses carried out on the levels of the old and new infestations, using the Duncan test, using the SPSS 11.0 program, showed that the infested varieties were distributed in 2 classes of infestation:

\* The class of the more infested varieties whose plates number exceeds 500, represented by Chetoui Tunis variety.

\* The class of the less infected varieties whose number of plates is inferior to 300 plates, represented by the varieties Chemcheli Gafsa, Meski, Ouesleti, Chemlali Sfax, Zarrazi Zarzis and Picholine.

Regarding the impact of the physico-chemical parameters in the bark, only the water content seems to determine the infestation. Also, this content increases when the wood is less infested; 42% of water seems to be the most favourable content for the development.

**Key words:** *Hylesinus Oleiperda*, Olive tree, infestation degree, physico-chemical parameters, bark.

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## 1. Introduction

Décrite comme la base de l'agriculture durable, la diversité génétique revêt une importance particulière en terme de mesure prophylactique dans l'entretien d'une culture saine et rentable. Une telle diversité procure aux agriculteurs une sécurité contre les maladies, les ravageurs, la sécheresse ainsi que d'autres stress environnementaux.

Partant de ce même principe, nous avons envisagé exploiter la richesse des oliveraies tunisiennes en variétés autochtones et étrangères pour déterminer celles qui sont résistantes aux agressions de l'hylésine *Hylesinus oleiperda* (Coleoptera, Scolytidae).

Cet insecte xylophage creuse des galeries dans l'écorce de l'olivier causant ainsi le flétrissement des rameaux infestés et parfois même la mort de l'arbre.

Les attaques de l'hylésine sur le bois sont facilement reconnaissables grâce à la formation de plaques rougeâtres tout autour des galeries. En fin du développement larvaire, ces plaques portent les trous de sortie des adultes de l'insecte et de ses parasitoïdes. Avec le temps, ces plaques finissent par se dessécher et se craqueler.

L'objectif de ce travail consiste, alors, en l'établissement de l'échelle de sensibilité variétale de quelques variétés d'olivier vis-à-vis des attaques de ce phytophage, et en la détermination des facteurs physico-chimique impliqués dans le phénomène de résistance.

## 2. Matériel et méthodes

### 2.1. Matériel végétal

Le matériel végétal, support de cette étude, consiste en 8 variétés d'olivier à huile et de table relevant tous de la collection variétale El Hanout (71 variétés) conduite en mode biologique et en sec.

Les variétés choisies sont les plus cultivées en Tunisie : Chetoui Tunis, Chemleli Sfax, Chemcheli Gafsa, Ouesleti, Zarrazi Zarzis, Meski et Picholine. Chaque variété est représentée par 4 pieds à l'exception de la Chemcheli Gafsa où l'on trouve trois seulement.

Les plants, âgés d'environ 15 ans, sont issus d'un greffage sur racines d'une vieille plantation (100 ans) et sont placés selon l'écartement: 24\*24m.

Pour les différents tests physico-chimiques on prélève sur chaque pied de chaque variété 8 plaques saines de l'écorce (2\*2 cm) au niveau des grosses charpentières. Après séchage, ces plaques subissent un broyage fin et la poudre végétale obtenue servira de substrat pour les analyses chimiques.

### 2.2. Méthodes

#### 2.2.1. Détermination du degré d'infestation des variétés d'olivier par l'hylésine

La détermination de l'infestation des variétés à l'hylésine s'est basée sur deux variables:

- Le nombre de plaques de ponte par arbre.
- Le nombre moyen d'adultes d'hylésine émergés par plaque par arbre par variété. Ceci renseigne sur la faculté de la variété à permettre la survie de l'insecte.

Ces deux paramètres sont déterminés par un dénombrement minutieux des plaques, tout en distinguant entre les nouvelles et les anciennes (plaques rouges, plaques en cours de craquelure ou craquelées). On compte aussi les trous de sortie des adultes en prenant soin de ne pas les confondre avec ceux des parasitoïdes (Figures 1 et 2, planche 1). Signalons qu'il faut, également, distinguer les plaques de ponte des plaques nutritionnelles. Ces dernières se caractérisent par la présence de la galerie de la femelle sans le brunissement des tissus corticaux tout autour (Figures 3 et 4, planche 1).

### 2.2.2 Suivi des stades préimaginaux de l'hylésine

L'hylésine étant un insecte endophyte, la seule technique fiable pour l'étude de la structure de la population préimaginale est celle du grattage des plaques moyennant un ciseau à bois de menuisier (Figure 5, planche 1). Chaque semaine, 10 plaques sont prélevées sur la variété Chemleli Sfax et examinées sous loupe binoculaire.

### 2.2.3. Détermination de quelques paramètres physico-chimiques de l'écorce des différentes variétés étudiées

#### - Epaisseur de l'écorce

L'épaisseur de l'écorce est mesurée par un pied-coulisse numérique avec une précision de 0,01 mm sur les échantillons frais, utilisés pour la détermination de l'humidité.

Pour chaque plaque, l'épaisseur est mesurée au niveau des quatre côtés.

#### - Teneur en eau de l'écorce

Les plaques prélevées sont placées dans des crachoirs à fermeture hermétique pour empêcher toute perte d'eau par évapotranspiration (Figure 6 et 7, planche 1).

Elles sont soumises, par la suite, à un séchage dans une étuve à 60°C. L'humidité de l'écorce a été calculée par la formule d'Abbot :

$$H (\%) = ((\text{poids frais} - \text{poids sec}) * 100) / \text{poids frais}$$

#### Analyses chimiques de quelques éléments minéraux

\* La composition biochimique de la sève peut influencer l'infestation de l'olivier par l'hylésine. Selon Loussert et al. (1978), l'azote, le phosphore, le potassium et les polyphénols sont des éléments connus pour être responsables de la résistance des plantes.

#### \* Dosage de l'azote total (méthode de KJELDAHL)

Un gramme de la poudre végétale obtenue est minéralisé par attaque d'acide sulfurique concentré à chaud, afin de détruire la matière organique et libérer l'azote sous forme de sulfate d'ammonium. Après minéralisation, la distillation par entraînement à la vapeur permet d'extraire l'ammoniaque qui sera dosé par acidimétrie avec l'acide chlorhydrique 0,1N.

#### \* Dosage du phosphore et du potassium (Pauwels et al., 1992)

La libération des éléments potassium et phosphore à partir de la poudre végétale se fait par calcination dans un four à 450°C puis par attaque par l'acide nitrique à chaud.

La détermination de la teneur en phosphore assimilable est effectuée selon la méthode d'OLSEN modifiée qui se base sur un dosage spectrophotométrique des ions orthophosphorés ayant acquis la couleur bleue suite à une complexion avec le molybdate d'ammonium.

Le dosage du potassium est assuré par la méthode d'absorption atomique. Le potassium est atomisé par soumission à la flamme à haute température. La population d'atomes est, ensuite traversée par une radiation monochromatique d'intensité  $I_0$  dont une partie sera absorbée. Selon la loi de Bur Lambert la lumière absorbée varie linéairement avec la concentration de l'élément dosé.

#### - Dosage des polyphénols totaux (Darné et al., 1972, Singh et al., 2002)

L'extraction des polyphénols totaux à partir de la poudre végétale est effectuée par addition de méthanol et de chloroforme avec centrifugation afin de séparer les composés phénoliques et les glucides des autres métabolites contenus dans l'écorce. On ajoute à l'extrait le réactif de Folin qui, en milieu basique, réagit avec les composés phénoliques en donnant la couleur bleu-violet.

Pour doser quantitativement les phénols, on a eu recours au spectrophotomètre ajusté à une longueur d'onde de 727 nm.

### 3. Résultats et discussions

#### 3.1. Biologie de l'insecte

La collecte régulière des plaques de ponte de l'hylésine durant le printemps 2007 a permis de suivre l'évolution de la structure de ses populations (Fig. 1) et d'observer le comportement d'accouplement de cet insecte dans les conditions naturelles du champ (Fig. 3).

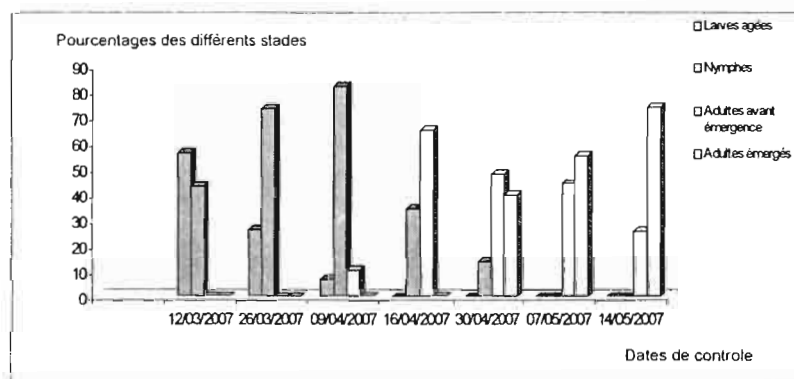


**Figure 1:** Suivi de l'évolution des stades de développement de l'hylésine durant la période au 15/05/2007 dans la parcelle El Hanout.

Il ressort de la Figure 1 qu'au cours des mois mars, avril et mai, le développement de l'insecte passe par trois phases : nymphose progressive (du 12 mars au 09 avril), émergence graduelle des adultes (du 09 au 30 avril) et envol de la première génération de l'année (à partir de 30 avril).

Le premier vol a été signalé pour la première fois vers la fin d'avril. L'envol a donc accusé un retard de trois semaines par rapport à celui observé par Jarda et al. (1997) selon lesquels l'envol commence au centre de la Tunisie au début du quatrième mois de l'année.

Le retard enregistré au cours de l'année 2007, peut être attribué aux facteurs climatiques. En effet, les deux mois mars et avril se sont caractérisés par des basses températures dont la moyenne journalière a chuté jusqu'à 10°C.

#### 3.2. Détermination du degré d'infestation des variétés d'oliviers par l'hylésine

Les degrés d'infestation par l'hylésine ont été étudiés à trois niveaux : ancienne et nouvelle infestation et nouvelle émergence des adultes afin de prouver que ce scolyte manifeste un comportement nutritionnel préférentiel pour certaines variétés constant dans le temps et non pas aléatoire.

##### 3.2.1. Importance des anciennes et des nouvelles attaques

Le suivi des attaques de l'hylésine sur olivier durant le printemps (2007) a montré que les niveaux d'infestation varient selon les variétés (Fig.2 et 3).

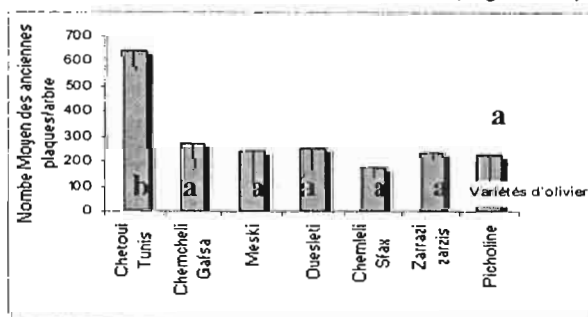


**Figure 2:** Variation des anciennes infestations par l'hylésine selon la variété d'olivier.

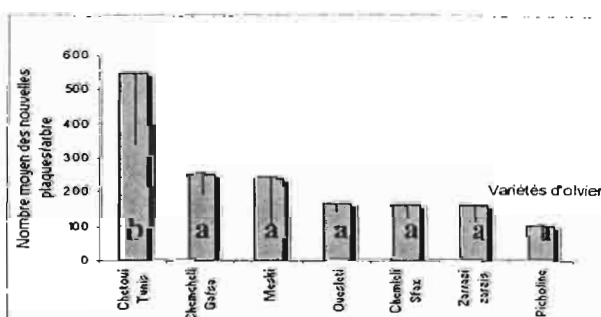


**Figure 3:** Variation des nouvelles infestations par l'hylésine selon la variété d'olivier.

\* Les variétés affectées par des lettres différentes sont significativement différentes au seuil de 1%.

L'examen détaillé des deux figures 2 et 3 ainsi que analyses statistiques effectuées sur les niveaux d'infestation moyens (anciennes et nouvelles plaques) de chaque variété à l'aide du test Duncan moyennant le logiciel SPSS 11.0 (Annexes 7 et 8), permettent d'avoir le même classement des variétés en deux groupes bien distincts

- Variétés les plus infestées dont la moyenne des plaques a dépassé les 600 plaques anciennes et 500 nouvelles plaques par pied. Cette catégorie comprend la variété Chetoui Tunis;

- Variétés les moins infestées ayant une moyenne de plaques inférieure à 300 par pied : Chemcheli Gafsa, Meski, Ouesleti, Chemlali Sfax, Zarrazi Zarzis et Picholine;

Toutefois, au sein du deuxième groupe nous notons une légère différence entre les six variétés.

Les résultats évoqués précédemment sont confirmés par la présence de craquelures très accentuées sur l'écorce de la variété Chetoui Tunis empêchant parfois même de dénombrer avec précision les anciennes plaques. Ces craquelures sont moins importantes sur les variétés de moindre infestation (Planche 3).

### 3.2.2. Importance des adultes d'hylésine et des parasitoïdes émergés par plaque

Les résultats obtenus lors de nos prospections sur le terrain sont relatifs à une fraction de la population imaginale car l'envol de l'insecte correspondant à la première génération, ne s'est pas achevé à l'arrêt de nos observations. Par ailleurs, les aléas du terrain (chutes de pluies) ont fait que le comptage a été effectué sur un seul pied pour chaque variété. Quoiqu'il en soit, les résultats du dénombrement sont consignés dans le Tableau 1. :

**Tableau 1:** Emergence des adultes de l'hylésine et des parasitoïdes en fonction de la variété d'olivier.

| Variétés        | Nombre de nouvelles plaques | TSA  | MTSA / plaque | TSP | Parasitisme (%) |
|-----------------|-----------------------------|------|---------------|-----|-----------------|
| Chetoui Tunis   | 798                         | 2352 | 2,947         | 21  | 0,88            |
| Chemcheli Gafsa | 295                         | 886  | 3,003         | 59  | 6,24            |
| Meski           | 326                         | 1087 | 3,334         | 19  | 1,72            |
| Ouesleti        | 201                         | 172  | 0,856         | 7   | 3,91            |
| Chemlali Sfax   | 219                         | 423  | 1,932         | 8   | 1,86            |
| Zarrazi Zarzis  | 219                         | 485  | 2,215         | 107 | 18,07           |
| Picholine       | 162                         | 408  | 2,519         | 18  | 4,23            |

TSA : trous de sortie des adultes

MTSA : moyenne des trous de sortie des adultes

TSP : trou de sortie des adultes des parasitoïdes

En se basant sur les moyennes des trous de sortie des adultes de l'hylésine, nous notons que la majorité des variétés une fois infestées, offrent un milieu favorable au développement de l'insecte. Ainsi le taux de survie dépasse 2,5 adultes par plaque, à l'exception des trois cultivars Ouesleti, Chemlali Sfax et Zarrazi Zarzis. Ceci pourrait être attribué à des facteurs endogènes caractéristiques de l'écorce qui seront abordés plus loin.

Le rôle de la faune parasitaire dans la réduction des populations du phytophage a été étudié en réalisant le comptage des trous de sortie des parasitoïdes. Les résultats obtenus montrent que le taux de parasitisme le plus élevé (environ 18%) a été enregistré sur la variété Zarrazi Zarzis malgré son faible taux d'infestation. D'autre part, la variété Chetoui Tunis présente le coefficient de parasitisme le plus bas (inférieur à 1%), bien qu'elle fût la plus infestée. Il en ressort que l'action parasitaire ne semble pas être liée à la présence de l'hylésine. La plante hôte pourrait par le biais de substances chimiques jouer le rôle d'attirant à l'égard des parasitoïdes.

En se référant aux résultats obtenus suite au comptage de l'ensemble des plaques indicatrices du développement de *H. oleiperda*, nous avons établi l'échelle du degré d'infestation des variétés étudiées à ce ravageur (figure 4).

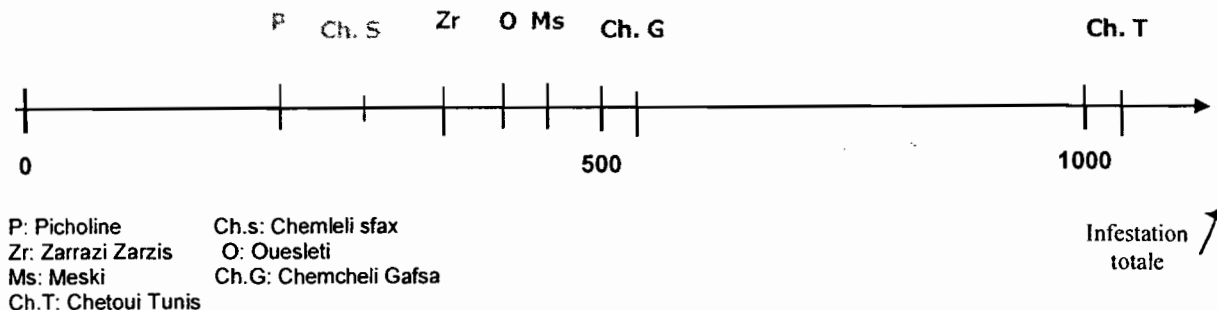


**Figure 4:** Echelle du degré d'infestation de 7 variétés d'olivier à huile et de table par *H. oleiperda*

Ce résultat est conforté par les travaux de Jardak et al. (1997) qui ont montré que la variété Chetoui Tunis est la variété la plus sensible suivie de la Manzanille puis de la Meski et enfin la Picholine.

### 3.2.3. Détermination de quelques paramètres physico-chimiques de l'écorce des différentes variétés d'olivier

#### - Epaisseur de l'écorce

Les mesures moyennes de l'épaisseur réalisées sur l'écorce de l'olivier se sont montrées légèrement variables pour la majorité des variétés étudiées. Elles sont comprises entre 5,24 et 5,52 mm exceptés les deux variétés Picholine et Chetoui Tunis elle est de l'ordre de 6,5 mm (Figure 5).

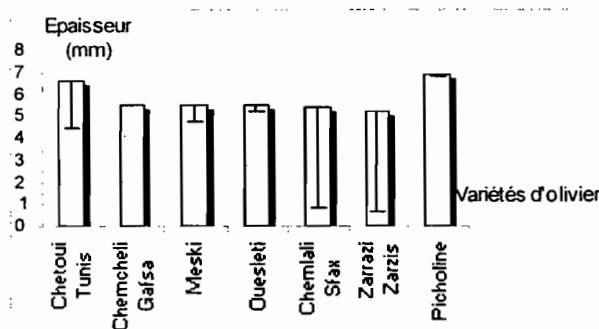


**Figure 5:** Variation de l'épaisseur moyenne de l'écorce en fonction de la variété d'olivier

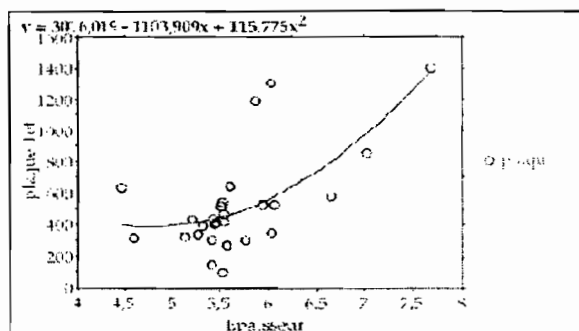


**Figure 6:** Courbe de régression de l'épaisseur de l'écorce des variétés d'olivier en fonction de leur infestation par l'hylésine

L'analyse de la variance par l'application du logiciel Stat View sur Macintosh, menée sur les 27 pieds étudiés, montre une légère variation qui n'est pas corrélée avec : la variation du niveau d'infestation par l'hylésine (Figure 6).

Ce résultat est conforté par celui de Jardak et al. (1997) qui ont montré que l'infestation par ce scolyte chez les variétés Meski, Picholine, Chetoui, Changloreal et Chemlali varie indépendamment de l'épaisseur de l'écorce.

#### - Teneur en eau

Les teneurs en eau au niveau de l'écorce de l'olivier varient graduellement entre 41,26 et plus que 45% (figure 7). Ces valeurs correspondent respectivement à la variété la plus infestée Chetoui Tunis (548,7 nouvelles plaques) et les moins infestées Chemlali Sfax, Zarrazi Zarzis et Picholine (moins de 200 nouvelles plaques par pied).

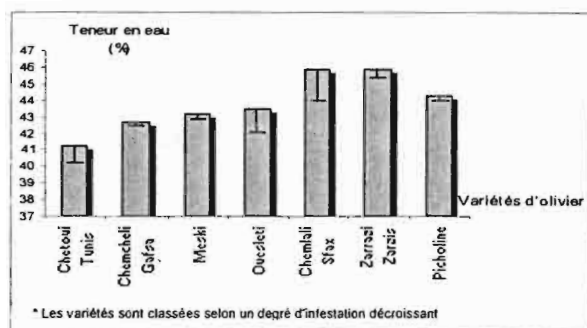


**Figure 7:** Variation de la teneur moyenne en eau de l'écorce en fonction de la variété d'olivier

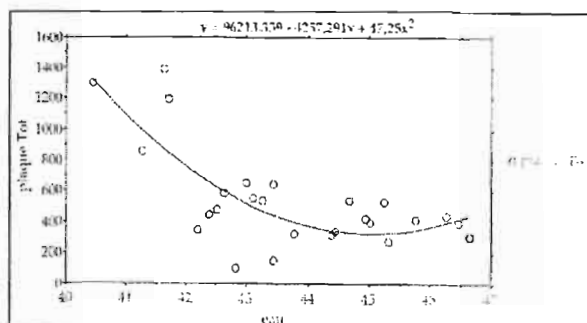


**Figure 8:** Courbe de régression de la teneur en eau des variétés d'olivier en fonction de leur infestation par l'hylésine

L'analyse de la variance moyennant le même logiciel montre une corrélation très étroite entre la teneur en eau et le degré d'infestation (Figure 8).

Ainsi, il apparaît clairement que les faibles humidités de l'écorce ont un effet positif sur les attaques de l'hylésine. Une humidité inférieure à 42% est probablement optimale au développement du scolyte à l'intérieur de l'écorce. En revanche, une humidité qui dépasse 44% devient gênante pour l'insecte. Ces résultats vont dans le même sens que ceux obtenus par Jardak et al. (1997). Ces auteurs ont montré que l'humidité optimale pour le développement de l'hylésine est située entre 40 et 45 %, valeurs au dessous et au-delà desquelles l'activité de l'insecte est entravée. De même, *Phoracantha semipunctata* (Coleoptera, Cerambycidae) se développe mieux sur des arbres d'*Eucalyptus globulus* soumis à un stress hydrique que sur des arbres irrigués (Cadeira et al., 2002).

#### - Teneur en éléments minéraux N, P, K

Selon Kumbasli (2005), l'azote, le phosphore et le potassium sont des nutriments nécessaires aux insectes phytophages. Les résultats auxquels ont abouti nos analyses sont illustrés par les Fig. 9, 11 et 13.

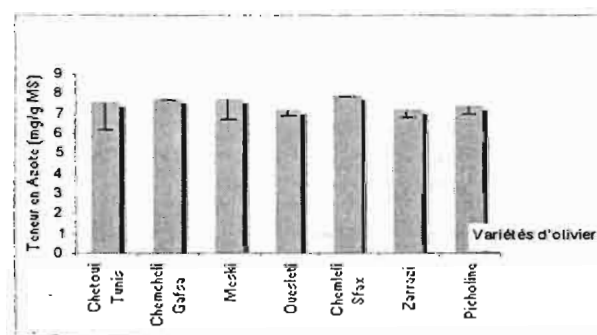


**Figure 9:** Variation de la teneur moyenne en azote en fonction de la variété d'olivier

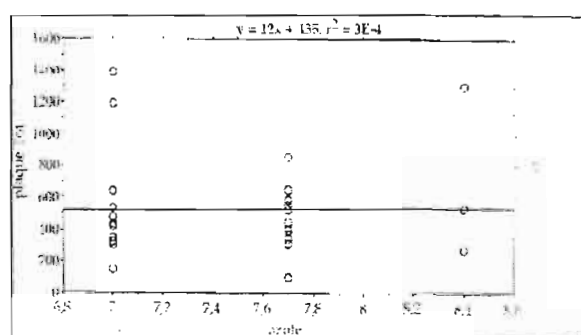


**Figure 10:** Courbe de régression de la teneur en azote des variétés d'olivier en fonction de leur infestation par l'hylésine

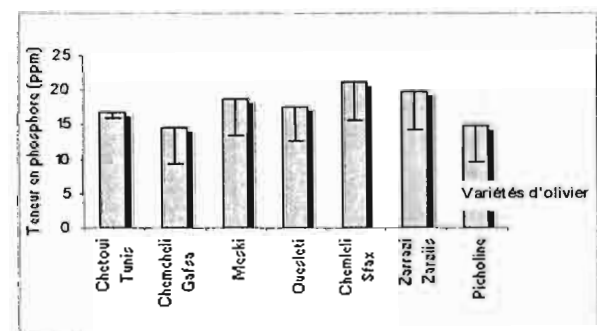


**Figure 11:** Variation de la teneur moyenne en phosphore en fonction de la variété d'olivier

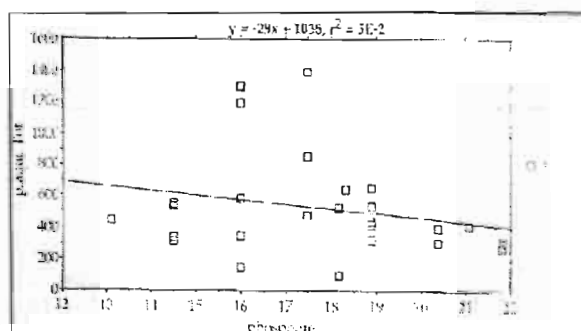


**Figure 12:** Courbe de régression de la teneur en phosphore des variétés d'olivier en fonction de leur infestation par l'hylésine



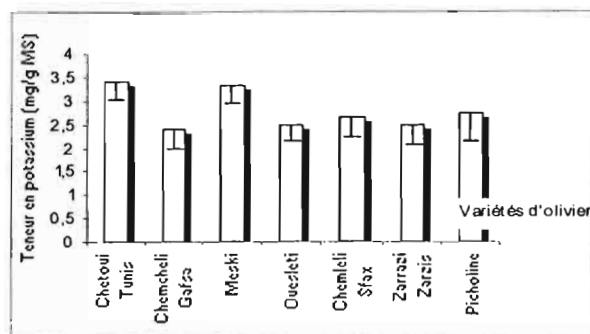


**Figure 13:** Variation de la teneur moyenne en potassium en fonction de la variété d'olivier

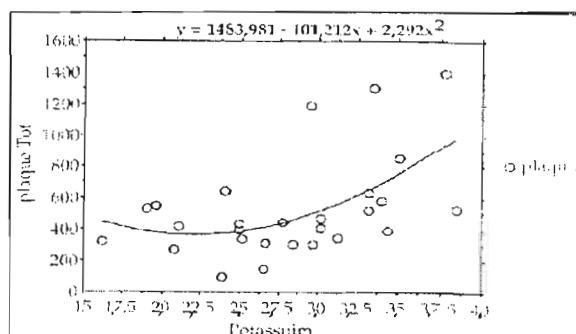


**Figure 14:** Courbe de régression de la teneur en potassium des variétés d'olivier en fonction de leur infestation par l'hylésine

Il ressort des Figures 11, 13 et 15 que comme pour l'épaisseur de l'écorce, les teneurs en N, P et K sont légèrement variables d'une variété à l'autre. Toutefois, cette variation n'explique pas le changement du comportement nutritionnel de l'hylésine vis-à-vis des variétés d'olivier étudiées. Ceci est prouvé par les résultats de l'analyse de la variance par l'application du logiciel Stat View, menée sur les 27 pieds étudiés, qui ne témoignent aucune relation de corrélation entre la concentration de l'écorce en ces éléments minéraux et le niveau d'infestation des oliviers (Figures 10, 12 et 14).

#### - Teneur en composés phénoliques

Les résultats du dosage des composés phénoliques dans l'écorce sont illustrés par la Figure 17.

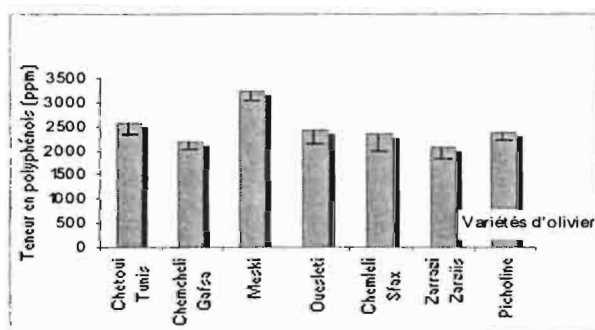


**Figure 17:** Variation de la teneur moyenne en polyphénols en fonction de la variété d'olivier

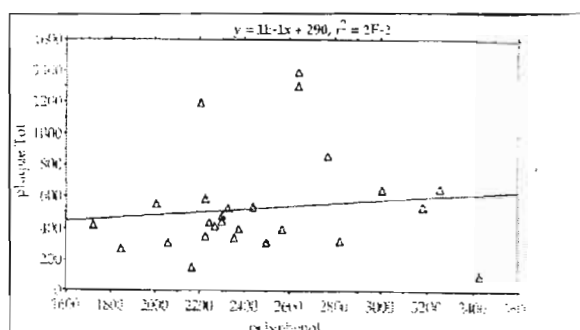


**Figure 18:** Courbe de régression de la teneur en polyphénols des variétés d'olivier en fonction de leur infestation par l'hylésine

Il ressort de la Figure 17 que les teneurs en polyphénols pour les différentes variétés sont approximativement égales excepté pour la variété Meeki dont la teneur moyenne est de 3218,8 ppm. L'analyse de la variance par l'application du logiciel Stat View a confirmé l'absence de corrélation entre les teneurs en composés phénoliques au niveau de l'écorce et le nombre de plaques d'attaque du scolyte (Figure 18).

Ceci est en contradiction avec ce qui a été démontré par Zouiten et al. (2000) sur l'effet répulsif des polyphénols contenus dans les variétés d'olivier résistantes au psylle. De même, Lattanzio et al. (1996) in Zouiten et al. (2000) ont montré qu'une lignée résistante de *Vigna unguiculata* vis-à-vis d'*Aphis craccivora* présente deux fois plus de flavonoïdes (famille des polyphénols) qu'une lignée sensible.

#### 4. Conclusion et perspectives

L'étude du degré d'infestation par l'hylésine de 7 variétés d'olivier à huile et de table et des paramètres physico-chimiques qui en sont responsables, a abouti à plusieurs résultats. Le travail concernant l'interaction Hylésine-Olivier, révèle la présence de deux groupes de variétés bien distincts:

- Groupe des variétés les plus infestées avec Chetoui Tunis
- groupe des variétés les moins infestées avec : Chemcheli Gafsa, Meski, Ouesleti, Chemleli Sfax, Zarrazi Zarzis, la variété Picholine est la moins infestée.

Cette classification n'est plus valable si l'on considère l'attractivité des différentes variétés vis-à-vis des parasitoides dont le niveau reste non tributaire du degré d'infestation.

Par ailleurs les analyses physico-chimiques des portions de l'écorce concernant son épaisseur, sa teneur en eau, sa teneur en polyphénols, sa teneur en azote, sa teneur en phosphore et sa teneur en potassium ont montré que seul la teneur en eau au niveau du tissu cortical est déterminante de la sévérité des attaques du ravageur vis-à-vis de quelques variétés plus que d'autres. Plus la teneur est importante, moins l'infestation est sévère : une teneur inférieure à 42% semble être favorable pour le développement de l'hylésine par contre une teneur supérieure à 44% devient gênante.

Avant de tirer une conclusion définitive sur l'effet de la composition biochimique de la sève au niveau de l'écorce de l'olivier sur les attaques de l'hylésine, il faut poursuivre les analyses chimiques sur une période d'un an afin de s'assurer qu'il n'existe pas de variation des teneurs en éléments minéraux et en composés phénoliques entre les variétés.

Bien que les variétés Chemleli Sfax et Picholine soient peu infestées par l'hylésine, elles ne sont pas parfaitement résistantes à ce phytophage. Pour cela, il faut poursuivre le travail sur ces deux variétés dans le but de déterminer le gène de résistance chez ces deux cultivars et d'en définir un programme d'amélioration génétique.

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**Sub-lethal effects of seven pesticides on development and predation of *Anthocoris nemoralis* (heteroptera, anthocoridae): a predator of *Euphyllura olivina* (homoptera, psyllidae Sub-lethal**

Bam'hamed Touria, Chemseddine Mohamed

**Abstract**

The present study assessed the sub-lethal toxicity of seven chemical pesticides to *Anthocoris nemoralis*. Application of LC20 of Lambda-cyhalothrin, cypermethrin, methomyl, malathion, fenthion, parathion-ethyl and dimethoate on the dorsal side of *A. nemoralis* larvae reduced considerably the speed of their development and altered their capacity of predation. Lambda-cyhalothrin have the most repressive effect on the predation of the five larval stages. Parathion-ethyl reduced the development speed. Results of these laboratory experiments indicated that the most widely used chemical pesticides were toxic to *A. nemoralis* one of the most important biological agent in the olive grooves and alter the two most important potential of this predator (developmental time and predatory activity).

**Key words:** *Anthocoris nemoralis*, sub-lethal, chemical pesticides, development, predation.

## Etude préliminaire de la nématofaune de l'olivier dans quatre localités de la Tunisie

Guesmi I.<sup>1</sup> Horigue-Raouani N.<sup>1</sup> et Kallel S.<sup>2</sup>

### Résumé

Une première étude de la nématofaune de l'olivier a été effectuée dans quatre localités de deux gouvernorats, Sidi Bouzid et Nabeul, de la Tunisie. Des échantillons des racines et du sol ont été prélevés à la limite de la frondaison de l'arbre et ont été traités selon la technique de double centrifugation. Cinq genres de nématodes phytoparasites, *Pratylenchus*, *Meloidogyne*, *Heterodera*, *Rotylenchulus* et *Zygotylenchus* ont été retrouvés dans la rhizosphère de l'olivier. La fréquence de *Pratylenchus* est surtout liée aux oliveraies montrant des symptômes de dépérissement et son effectif le plus important a été observé en présence des cultures en intercalaires avec l'olivier. Deux espèces, *Pratylenchus penetrans* et *P. coffeae*, ont été isolées à partir des racines d'olivier et identifiées selon les critères morphobiométriques. Ce genre était dominant surtout dans les échantillons prélevés des localités de Sidi Bouzid. Des galles et des masses gélatineuses remplies d'œufs de *Meloidogyne* et des femelles d'*Heterodera* ont été observées sur les racines des oliviers issues de Nabeul. Une identification morphologique se basant sur l'examen des figures périnéales des femelles de *Meloidogyne* et d'*Heterodera* d'une part et des critères biométriques d'autre part des juvéniles du second stade d'*Heterodera*, a montré la présence de *M. javanica*, *M. incognita*, *M. arenaria* et d'*H. carotae* sur olivier.

**Mots clés:** Oliviers, Nématodes phytoparasites, *Pratylenchus*, *Meloidogyne*, *Heterodera*.

### Abstract

A first survey of the nematodes associated with olive trees is done in four localities of two governorates, Sidi Bouzid and Nabeul, of Tunisia. Samples of the roots and soil are collected at the edge of the canopy of the tree and are treated according to the technique of double centrifugation. Five genus of plant-parasitic nematodes, *Pratylenchus*, *Meloidogyne*, *Heterodera*, *Rotylenchulus* and *Zygotylenchus* are recovered in association with olive trees. The frequency of *Pratylenchus* is especially bound to the olive groves showing symptoms of fading and its most important population is observed in presence of other cultures with the olive trees. Two species, *Pratylenchus penetrans* and *P. coffeae*, are isolated from the roots of olive trees and are identified according to the morphometric criteria's. This genus especially dominates in the samples collected from the localities of Sidi Bouzid. Galls and gelatinous matrix with eggs of *Meloidogyne* and females of *Heterodera* are observed on the roots of the olive trees collected from Nabeul. A morphological identification being based on the exam of the patterns of the females of *Meloidogyne* and *Heterodera* and the morphobiometric identification of the juvenile of the second stage of *Heterodera*, showed the presence of *M. javanica*, *M. incognita*, *M. arenaria* and *H. carotae* on olive trees.

**Key words:** Olive, plant-parasitic nematodes, *Pratylenchus*, *Meloidogyne*, *Heterodera*.

### 1. Introduction

L'intensification des oliveraies paraît être une source de problèmes phytosanitaires. Les champignons telluriques, comme le *Verticillium*, ont entraîné le dépérissement des oliviers dans certaines régions (Selmi, 2007). Ce champignon peut entrer en interaction avec des nématodes phytoparasites sur d'autres cultures (MacGuidwin et Rouse, 1990). Ceci nous a incité d'étudier la nématofaune associée à l'olivier qui jusqu'à ce temps demeure inconnue en Tunisie.

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## 2. Matériels et méthodes

Une enquête a été réalisée dans deux gouvernorats de la Tunisie : Sidi Bouzid et Nabeul. A Sidi Bouzid, trois parcelles sont prospectées à Ouled Haffouz et trois autres à Regueb. A Nabeul, deux parcelles à Mraissa et une autre à Takelsa sont visitées. Plusieurs variables sont retenues de l'enquête à savoir la variété, la présence ou l'absence des cultures en intercalaires, l'irrigation et la vigueur des oliviers. 31 échantillons de racines, de 20 g chacun, sont prélevés à la limite de la frondaison à partir des arbres à des vigueurs différentes.

Au laboratoire, les nématodes sont extraits à partir des racines par la technique de la double centrifugation (De Grisse, 1969). La détermination des genres des nématodes et leur dénombrement sont effectués sous stériomicroscope. Les genres sont fixés selon la méthode de De Grisse (1969) et montés entre deux lamelles.

Une caractérisation morphobiométrique des espèces est réalisée :

Cas des *Pratylenchus*, des mesures des critères spécifiques des femelles sont prises à l'aide d'un microscope OLYMPUS selon clé de Frederick et Tarjan (1989).

Cas des *Meloidogyne*, une étude morphologique des figures périnéales des femelles adultes, selon la technique de Jepson (1987), est faite.

Cas d'*Heterodera*, une étude morphologique des cônes vulvaires et des figures périnéales des femelles adultes, selon la méthode de Mulvey et Golden (1983), est réalisée.

## 3. Résultats et discussion

Cinq genres de nématodes phytoparasites, *Pratylenchus*, *Meloidogyne*, *Heterodera*, *Rotylenchulus* et *Zygotylenchus* sont retrouvés dans la rhizosphère de l'olivier.

### 3.1. *Pratylenchus* :

Les mesures morphométriques et les critères morphologiques des femelles de *Pratylenchus* (Tableau 1) ont révélé la présence de deux espèces *P. penetrans* (Cobb) Filipjev & Schuurmanns Stekhoven et *P. coffeae* Seinhorst. Ces deux espèces se distinguent respectivement par une spermathèque ronde (figure 1) et ovale (figure 2). *P. penetrans* est rapporté dans d'autres travaux sur oliviers en pépinières au sud de l'Espagne (Nico et al. 2002).

**Tableau 1:** Etude comparée des critères morphométriques des femelles de *Pratylenchus* des deux groupes.

|                                                    | L (µm)           | a             | c             | V             | Stylet (µm)     | Forme de la queue      |
|----------------------------------------------------|------------------|---------------|---------------|---------------|-----------------|------------------------|
| Groupe 1 n=15                                      | 529<br>(470-580) | 23<br>(20-30) | 22<br>(11-40) | 80<br>(75-82) | 14,5<br>(10-17) | SMO, TRC, FNP          |
| <i>P. penetrans</i><br>(Frederick et Tarjan, 1989) | 540<br>(450-620) | 27<br>(22-30) | 20<br>(16-23) | 79<br>(77-83) | 16<br>(15-17)   | HEM-BLP<br>SMO         |
| Groupe 2 n=20                                      | 558<br>(520-640) | 26<br>(20-30) | 19<br>(13-30) | 80<br>(77-83) | 16<br>(10-18)   | SMO-SHM<br>SMO-BLP TRC |
| <i>P. coffeae</i><br>(Frederick et Tarjan, 1989)   | 580<br>(460-630) | 25<br>(21-30) | 21<br>(17-27) | 79<br>(76-82) | 16<br>(15-17)   | HEM-BLP<br>SMO         |

SMO = lisse, TRC = tronquée, FNP = finement pointue, HEM = hémisphérique, BLP = très pointue, SHM = sub-sphérique.

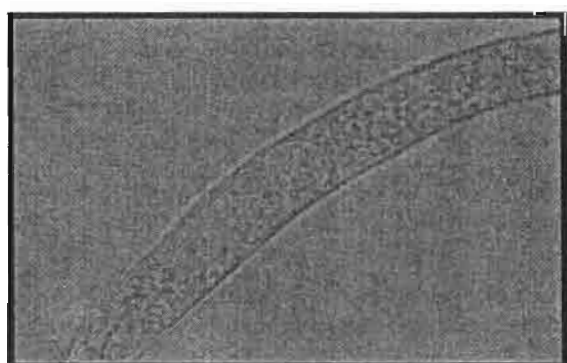


**Figure 1:** Partie médiane du *P. penetrans* (X1000).

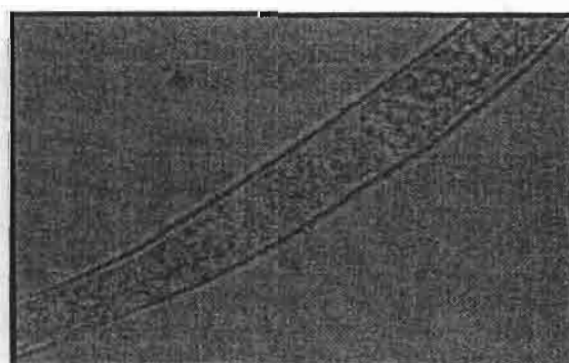


**Figure 2:** Partie médiane du *P. coffeae* (X1000).

Ce genre est trouvé dans les échantillons prélevés des parcelles de Sidi Bouzid. La fréquence de *Pratylenchus* est surtout liée aux oliveraies montrant les symptômes de dépérissement (Fig. 3) et l'effectif le plus important est observé en présence des cultures en intercalaires avec l'olivier (Fig. 4).

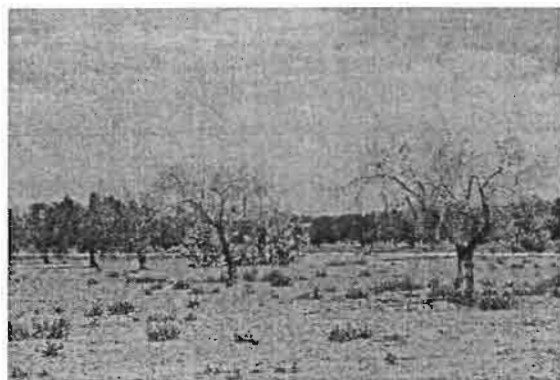


**Figure 3:** Des oliviers montrant les symptômes de dépérissement

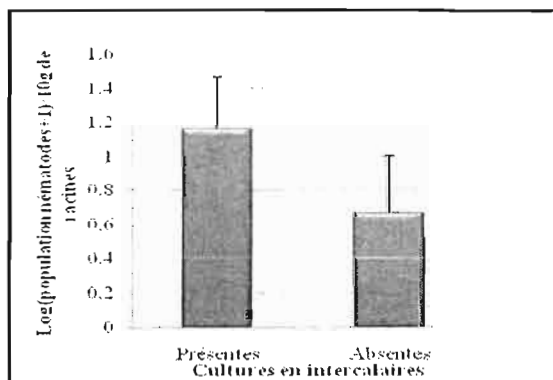


**Figure 4:** Effet des cultures en intercalaires sur les populations de *Pratylenchus* dans les racines de l'olivier (Les barres correspondent à l'Intervalle de confiance au seuil 5%).

### 3.2. Meloidogyne

Le genre *Meloidogyne* est trouvé dans les échantillons de racines des variétés Picholine et Manzanillo cultivées au Cap Bon. L'observation microscopique des figures périnéales des femelles de *Meloidogyne* a montré l'existence de trois espèces *M. javanica* (figure 5), *M. incognita* (figure 6) et *M. arenaria*. Ces espèces sont retrouvées sur oliviers en pépinières en Espagne (Nico et al, 2002). Par contre, les travaux de Castillo et al. (2003) ont montré la présence de *M. baetica*, espèce plus spécifique aux oliviers.

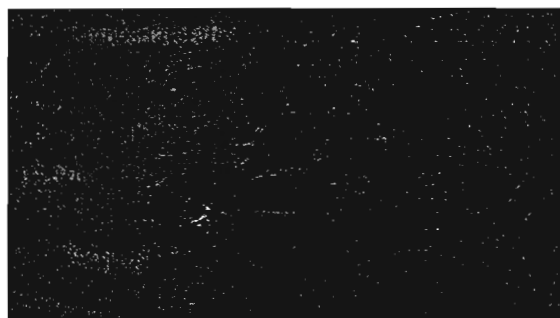


**Figure 5:** Figure périnéale de *M. javanica* (X1000).

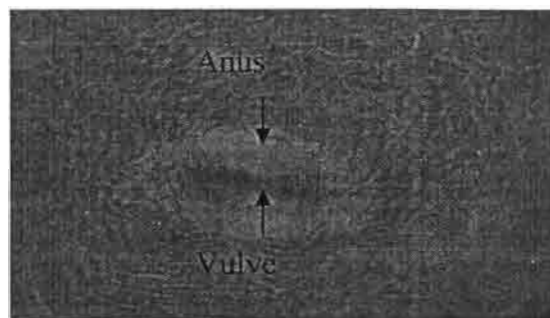


**Figure 6:** Figure périnéale de *M. incognita* (X1000).

### 3.3. Heterodera

Ce genre est trouvé dans les échantillons prélevés des parcelles de Sidi Bouzid et du Cap Bon qui sont équipées d'un système d'irrigation goutte à goutte.

L'observation microscopique du cône vulvaire (Figure 7) et de la figure périnéale (Figure 8) ainsi que la mensuration des critères biométriques des L2 (Tableau 2) ont montré que l'espèce présente est proche d'*Heterodera carotae*. Mais des études plus approfondies sont nécessaires pour caractériser les espèces de ce genre puisqu'en Espagne les travaux de Castillo et al. (1999) ont montré la présence de *H. mediterranea*.

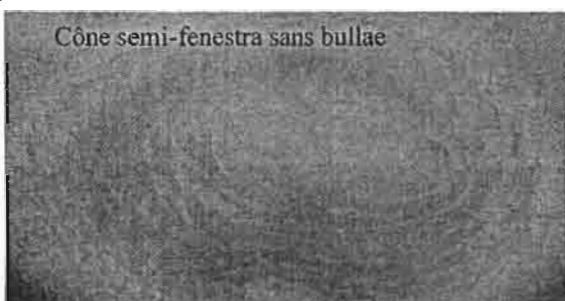


**Figure 7:** Cône vulvaire d'*Heterodera* (X1000).



**Figure 8:** Figure périnéale d'*Heterodera* (X1000).

**Tableau 2 :** Etude comparée des critères morphométriques ( $\mu\text{m}$ ) des L2 d'*Heterodera*

|                                           | L             | L. stylet      | L. queue     | H              |
|-------------------------------------------|---------------|----------------|--------------|----------------|
| <i>Heterodera</i> spp. n=14               | 407 (354-446) | 25 (21-28)     | 44 (32-63)   | 27 (21-34)     |
| <i>H. cruciferae</i> Stone et Rowe (1976) | 431 $\pm$ 19  | 24,1 $\pm$ 1,6 | 50 $\pm$ 2,7 | 25,2 $\pm$ 2,7 |
| <i>H. carotae</i> Mathews (1975)          | 375-452       | 22-25          | 43,5-58,6    | 20,3-31,8      |

### 3.4. Rotylenchulus

Des larves (Planche 1a), des femelles immatures (Planche 1b) ainsi que des femelles matures (Planche 1c) de *Rotylenchulus* sont présentes en faible effectif sur quelques oliviers.

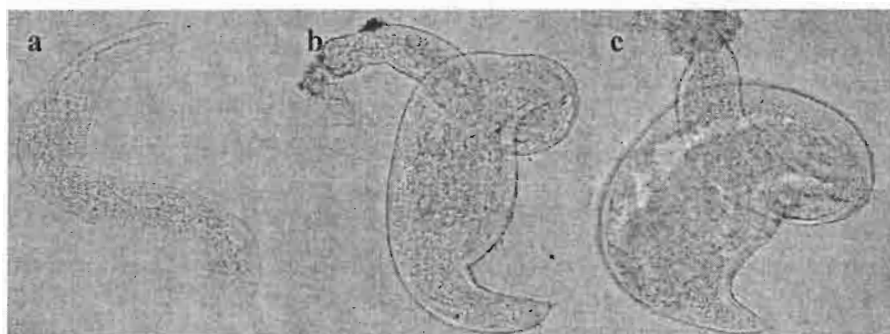


**Planche 1:** *Rotylenchulus* spp. ; a: larve ; b: femelle immature et c: femelle mature (X100)

### 3.5. Zygotylenchus

Des adultes femelles caractéristiques du genre *Zygotylenchus* sont retrouvées sur racines d'oliviers (Planche 2).

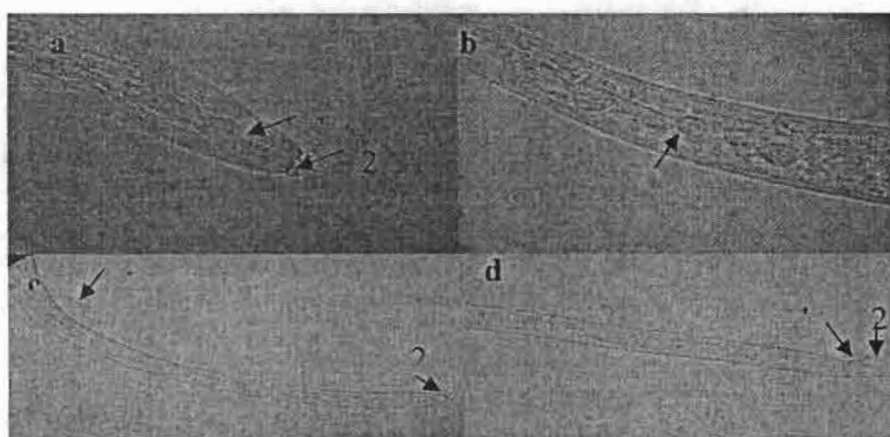


**Planche 2:** *Zygotylenchus* spp. ; a1 : stylet fort à boutons arrondis (X 200) ; a2 : sclérotinisation labiale (X 200) ; b : recouvrement ventral (X 200) ; c1 : vulve médiane chez la femelle (X 80) ; c2 : queue cylindrique chez la femelle (X 80) ; d1 : spicules du mâle (X 80) ; d2 : bursa du mâle (X 80)

### 4. Conclusion

Cinq genres, *Pratylenchus*, *Meloidogyne*, *Heterodera*, *Zygotylenchus* et *Roylenchulus*, sont retrouvés dans la rhizosphère de l'olivier. La nématofaune de l'olivier dans le bassin méditerranéen a été étudiée par plusieurs auteurs et c'est pour la première fois que les genres *Rotylenchulus* et *Zygotylenchus* sont rejoints dans une infestation naturelle en plein champ.

La fréquence des genres identifiés, dans ce travail, est variable sur olivier. De ce fait, une étude plus approfondie tenant compte des relations nématodes-facteurs abiotiques et biotiques serait indispensable pour dévoiler les secrets de ce parasitisme et le rôle des nématodes dans le phénomène de dépérissement de l'olivier.



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## Quorum-sensing et bio-control chez *P. savastanoi* pv. *savastanoi* responsable de la tuberculose de l'olivier et les bactéries associées

Ouzari Hadda-Imene, Khessairi Amel, Imene Fhoula, Noura Raddadi, Daffonchio Daniele and Abdellatif Boudabous

### Résumé

Au niveau de la tuberculose de l'olivier, la formation des tumeurs dépend principalement de la sécrétion par les bactéries responsables de substances agissant comme des hormones de croissance incluant l'acide indole acétique (AIA). Cependant certaines molécules signaux désignées « Acyl-homoserine lactones » (AHLs) ont été identifiées comme responsables de la virulence chez certains phytopathogènes. 43 souches de bactéries ayant montré la production de quantités variables d'AIA, ont été isolées à partir des nodules de différents cultivars de la Tunisie. Les résultats de l'identification des isolats sur la base des profils d'amplification des régions intergéniques 16S-23S et le séquençage du gène ADNr 16S ont montré la présence de *P. savastanoi*, reconnue comme l'agent causal de la tuberculose de l'olivier, au niveau de tous les échantillons analysés. Plusieurs autres espèces ont été isolées, incluant *Pantoea agglomerans*, *Stenotrophomonas maltophilia*, *Pseudomonas putida*, *Burkholderia cepacia* et *Hafnia alvei*. Par ailleurs, des associations stables des bactéries de *P. savastanoi*/*Pantoea* et *P. savastanoi*/*Stenotrophomonas* et/ou *Burkholderia* ont été observées au niveau de toutes les galles, suggérant le rôle potentiel que présentent ces associations dans l'établissement de la maladie (Ouzari et al., 2008). La recherche des molécules signaux d'AHLs chez les différents isolats a montré que seulement les espèces de *Pantoea*, *Stenotrophomonas* et *Burkholderia* en sont productrices. Ce qui montre le rôle de ces espèces qui agiraient en consortium pour l'induction de la maladie. La capacité de certaines bactéries comme *Bacillus thuringiensis* dans le biocontrol et la synthèse de lactonase qui interfère avec le système «Quorum-sensing» d'activation des gènes de virulence chez les bactéries Gram-négatives a été analysée.

**Mots clés:** Tuberculose de l'olivier/ Auxine / *P. savastanoi* / ITS ADNr 16S-23S/ AHLs

### Abstract

In the olive knot disease, the tumour formation depends mainly on the secretion, by the responsible bacteria, of substances acting as growth hormones including the indole acetic acid (IAA). However certain signal molecules indicated «Acyl-homoserine lactones» (AHLs) were identified to be responsible for virulence in some phytopathogenic bacteria. 43 strains having shown the production of variable quantities of AIA, were isolated from the nodules of different cultivars in Tunisia. The results of strains identification, based on the 16S-23S intergenic spacer profiles and the 16S rDNA gene sequence, showed the presence of *P. savastanoi*, recognized as the causal agent of the tuberculosis of the olive tree, in all the analyzed samples. Several other species were isolated, including *Pantoea agglomerans*, *Stenotrophomonas maltophilia*, *Pseudomonas putida*, *Burkholderia cepacia* and *Hafnia alvei*. Besides, a stable associations of *P. savastanoi*/*Pantoea* and *P. savastanoi*/*Stenotrophomonas* and/or *Burkholderia* were observed in all the analysed galls, suggesting the potential role that these associations could present in the disease establishment (Ouzari and al., 2008).

The investigation of signal molecules of AHLs in the various isolates showed that only *Pantoea*, *Stenotrophomonas* and *Burkholderia* species are producer. Showing the potential role that these species could present, by acting as a consortium, for the disease induction. The capacity of some bacteria such as *Bacillus thuringiensis* in the biocontrol and the synthesis of lactonase, which interfere with the "Quorum-sensing" system of virulence gene activation in Gram-négatives bacteria, was analyzed.

## 1. Introduction

Bien que l'olivier se caractérise par son adaptation à tous les types de sols et à différentes conditions environnementales, il est sujet à différents types de dégâts causés en particulier par les maladies fongiques, bactériennes et virales (Boulila, 2001). Ce travail s'intéresse particulièrement à la tuberculose de l'olivier, causée par la bactérie *Pseudomonas savastanoi* pv. *savastanoi*. Cette maladie est répandue dans tous les pays qui cultivent l'olivier (Jiménez Diaz, 1985). Elle est caractérisée par le développement de tumeurs ou galles sur différentes parties aériennes de l'arbre. En Tunisie, des attaques très graves ont été enregistrées dans les régions du centre et du nord et en particulier celles exposées aux chutes des grêles, telles que Kairouan et Kasserine.

Les bactéries de l'espèce *P. savastanoi* ainsi que d'autres espèces phytopathogènes se caractérisent par la synthèse de l'acide indole acétique et les cytokinines qui sont considérés parmi les principaux facteurs de virulence chez ces bactéries. (Glickmann et al., 1998). Selon Surico et al., (1985) et Gardan et al., (1992), les deux phytohormones sont impliquées dans la formation des nodules. Alors que les cytokinines déterminent l'élargissement des nodules, l'acide indole acétique favorise la diminution de la période d'incubation de la maladie.

En dehors de tout traitement externe, l'olivier secrète plusieurs substances naturelles impliquées dans la résistance aux différents types de stress biotiques et abiotiques. On cite l'exemple de l'oleuropéine qui présente une forte activité anti-bactérienne et considérée comme un précurseur des phytoalexines. Ces substances sont des composés antimicrobiens, de faible masse moléculaire, synthétisés et accumulés dans les plantes après attaque par les pathogènes (Bruneteau et Ricci, 1988). Toutefois pour remédier à certaines maladies émergentes, des substances chimiques de prophylaxie sont souvent utilisées. Dans le cas de la tuberculose de l'olivier, ce sont surtout des solutions à base de substances cupriques qui sont utilisées. Néanmoins l'application de ces bactéricides fournit uniquement une réduction provisoire de la population bactérienne, sans la détruire complètement (Teviotdale et Krueger, 2003).

En plus des limites de leur efficacité, les substances chimiques utilisées provoquent la déstabilisation des écosystèmes, en altérant les équilibres de la biocénose et en provoquant des dégâts sur l'environnement et des risques toxicologiques pour l'Homme. La lutte biologique constitue la stratégie de choix la plus encouragée. Parmi les substances proposées, on cite la bactériocine synthétisée par les bactéries *P. syringae ciccaronei* capables d'affecter la croissance des souches de *P. savastanoi*, *P. apii* et *P. persicae* (Lavermicocca et al., 1999 ; 2002).

Une nouvelle catégorie de molécules, appelées « lactonases » a été récemment identifiée et décrite pour son efficacité contre les bactéries phytopathogènes (Dong et al., 2000). Ce sont des molécules qui interfèrent avec le système communication inter-cellulaire nommé « quorum sensing » des bactéries à Gram négatif. En effet ce système est gouverné par la production de molécules dites « homoserine lactones » qui sont synthétisées lorsque la population bactérienne atteint un seuil critique et qui interviennent dans l'activation des gènes de virulence. L'interruption de ce système par des enzymes (lactonases, acylases ou autres) représente une stratégie de lutte prometteuse contre ces bactéries (Carlier et al., 2003).

Dans le présent travail, nous rapportons l'identification des bactéries isolées des galles, l'analyse de la production d'auxine et d'AHL par ces isolats et la sélection de souches de *B. thuringiensis* productrices de lactonases

## 2. Matériel et méthodes

### 2.1. Isolement et culture des souches bactériennes

Les bactéries étudiées, ont été isolées à partir de nodules sur différentes variétés d'oliviers (Cultivars : Chemlali, Chetoui, Nebjmel et Oueslati) et provenant de différentes régions de la Tunisie (Kasserine, Kairouan et Takelsa). Les souches sont isolées sur milieu King B après stérilisation et broyage des nodules. Elles sont ensuite cultivées et conservées sur le bouillon nutritif.

## 2.2. Production d'acide indole acétique

La détection de la production d'AIA a été réalisée selon la méthode de Patten and Glick, (2002). Le taux d'auxine a été mesuré par spectrophotométrie à 535 nm après 72h d'incubation dans un milieu minimum supplémenté de L-tryptophane (100 µg ml<sup>-1</sup>) en utilisant le réactif Salkowski (Gorden et Weber, 1951). La concentration d'AIA dans les cultures a été déterminée par comparaison avec la courbe des standards.

## 2.3. Extraction de l'ADN génomique, amplification de l'ADN et séquençage du gène 16S.

La méthode utilisée est celle décrite par Liop et al. (1999) avec quelques modifications. L'amplification du gène d'ADNr 16S et des régions transcrites 16S-23S, a été réalisée en utilisant les amorces universelles S-D-Bact-0008-a-S-20 et S-D-Bact-1495-a-S-20 et S-D-Bact-1494-a-S-20/S-D-Bact-0035-a-A-15 respectivement (Alm et al., 1996). L'amplification PCR a été accomplie selon les procédures décrites par Ouzari et al., (2006). Le gène *iaaL*, codant pour la IAA-lysine synthétase a été amplifié en utilisant les amorces spécifiques de *P. savastanoi*: IAALF: 5'GGGACCAGCGGCAACATCAA-3' IAALR, 5'-CGCCCTCGGAA-CTGCCATAC-3'. Les séquences issues du gène 16S des différentes espèces isolées ont été identifiées par comparaison avec celles disponibles dans les banques des données en utilisant le programme BLAST dans le Centre National de Biotechnologie et de l'Information (NCBI) (<http://www.ncbi.nlm.nih.gov>).

## 2.4. Mise en évidence de la production des lactones

La méthode utilisée est celle de la diffusion du surnageant de culture à partir des puits. Sur des boîtes de Pétri contenant le milieu LB, 5 ml de gélose molle neutre, préalablement inoculée avec 50 µl de culture jeune de la souche indicatrice *Chromobacterium violaceum*, sont ajoutés en surcouche. Des puits de 10 mm de diamètre sont creusés dans la gélose et leur fond scellé par 10µl de gélose molle liquéfiée. Ces puits sont par la suite remplis par les surnageants de culture des souches à tester. Après incubation de 24 h à 30°C, les diamètres des zones colorées en violet sont mesurés. Ce test est basé essentiellement sur la synergie entre les souches testées et la bactérie *Chromobacterium violaceum* CVO26 qui possède tous les gènes responsables de la production de la violaceine mais présente des mutations au niveau de deux gènes régulateurs de la production d'NAHL. D'où la production de la violaceine sera nécessairement dépendante des molécules NAHL exogènes.

## 3. Résultats et discussion

L'identification des souches isolées des galles de différentes localités a été basée sur les caractères phénotypiques, les profils d'amplification des régions intergéniques 16S-23S et le séquençage du gène ADNr 16S des souches représentatives des groupes ITS. Les résultats ont montré la présence de *P. savastanoi*, reconnue comme l'agent causal de la tuberculose de l'olivier, au niveau de tous les échantillons analysés. Plusieurs autres espèces ont été isolées, incluant *Pantoea agglomerans*, *Stenotrophomonas maltophilia*, *Pseudomonas putida*, *Burkholderia cepacia* et *Hafnia alvei*. Par ailleurs, des associations stables des bactéries de *P. savastanoi*, *Pantoea* et *P. savastanoi*/*Stenotrophomonas* et/ou *Burkholderia* ont été observées au niveau de toutes les galles (Tableau 2), suggérant le rôle potentiel que présentent ces associations dans l'établissement de la maladie (Ouzari et al., 2008).

**Tableau 1:** Caractérisation des bactéries isolées des nodules d'olivier.

| Strains | Knot sample | ITS profile | Auxin production** | iaaL | Closet 16S rDNA sequence            |
|---------|-------------|-------------|--------------------|------|-------------------------------------|
| KnN2    | 1           | 1           | 44.57              | +    | <i>Pseudomonas savastanoi</i>       |
| KnA2    | 1           | 1           | 63.7               | +    |                                     |
| KnE2    | 2           | 1           | 18.74              | +    |                                     |
| KnV2    | 3           | 1           | 14.57              | +    |                                     |
| KnH1    | 4           | 1           | 6.8                | +    |                                     |
| KnK2    | 5           | 1           | 7.12               | +    |                                     |
| KnB1    | 6           | 1           | 10.8               | +    | <i>Pseudomonas sp.</i>              |
| KnW2    | 7           | 1           | 44                 | +    |                                     |
| KnF1    | 8           | 1           | 5.83               | +    |                                     |
| KnM2    | 9           | 1           | 5.83               | +    |                                     |
| Kn48    | 10          | 1           | 3.09               | +    |                                     |
| Kn58    | 11          | 1           | 25                 | +    |                                     |
| Kn59    | 1           | 2           | 4.2                | -    |                                     |
| KnY1    | 2           | 2           | 8.73               | -    |                                     |
| KnT2    | 2           | 2           | 3.45               | -    | <i>Stenotrophomonas maltophilia</i> |
| Kn14    | 4           | 2           | 5.18               | -    |                                     |
| Kn47    | 10          | 2           | 7.62               | -    | <i>Stenotrophomonas maltophilia</i> |
| Kn66    | 10          | 2           | 6.39               | -    |                                     |
| KnR2    | 7           | 3           | 2.65               | -    |                                     |
| Kn42    | 9           | 3           | 10.98              | -    |                                     |
| Kn45    | 9           | 3           | 8.7                | -    | <i>Pantoea agglomerans</i>          |
| Kn52    | 11          | 3           | 13.65              | -    | <i>Pantoea agglomerans</i>          |
| Kn54    | 11          | 3           | 11.37              | -    |                                     |
| Kn55    | 11          | 3           | 10.7               | -    |                                     |
| KnL1    | 3           | 4           | 14.4               | -    | <i>Pantoea oleae</i>                |
| KnU1    | 3           | 4           | 5.7                | -    |                                     |
| KnE1    | 7           | 4           | 8.58               | -    | <i>Pantoea oleae</i>                |
| Kn57    | 1           | 5           | 2.4                | -    |                                     |
| Kn61    | 1           | 5           | 1.89               | -    |                                     |
| KnY2    | 1           | 5           | 7.69               | -    | <i>Pseudomonas sp./ putida</i>      |
| Kn39B   | 4           | 5           | 3.9                | -    |                                     |
| Kn69    | 4           | 5           | 5.84               | -    |                                     |
| KnJ2    | 5           | 5           | 6.48               | -    |                                     |
| KnH2    | 10          | 5           | 4.99               | -    | <i>Pseudomonas sp./ putida</i>      |
| Kn46    | 10          | 5           | 5.48               | -    |                                     |
| Kn39A   | 4           | 6           | 0.82               | -    | <i>Burkholderia cepacia</i>         |
| Kn70    | 4           | 6           | 7.2                | -    |                                     |
| KnX1    | 5           | 6           | 5.18               | -    |                                     |
| KnJ1    | 6           | 6           | 6.48               | -    |                                     |
| KnQ2    | 8           | 6           | 6.15               | -    |                                     |
| KnG2    | 3           | 7           | 2.15               | -    | <i>Hafnia alvei</i>                 |
| KnA1    | 3           | 7           | 7.45               | -    |                                     |
| KnO1    | 7           | 7           | 0.47               | -    |                                     |

\* Les quantités d'auxine ont été mesurées après 72 h de croissance sur milieu minimum avec 0.5 mM de L-tryptophan et sont exprimés en  $\mu\text{g ml}^{-1}$ .

\* Les quantités d'auxine ont été mesurées après 72 h de croissance sur milieu minimum avec 0.5 mM de L-tryptophane et sont exprimés en  $\mu\text{g ml}^{-1}$ .

L'occurrence des bactéries du genre *Pantoea*, séparées du genre *Erwinia* par Gavini et al., (1989) et connues pour leur pouvoir pathogène sur les plantes, a été rapportée par Rojas et al., (2004) comme bactéries isolées et associées à *P. savastanoi* au niveau des galles. Cependant l'association des bactéries du genre *Stenotrophomonas* et *Burkholderia* a été évoquée pour la première fois au niveau du présent travail. Certaines espèces du genre *Burkholderia* sont connues comme des bactéries qui améliorent la croissance des plantes (PGPB) et comme fixateurs d'azote. Toutefois, les souches isolées sont phylogénétiquement plus proches du complexe *B. cepacia* et *B. pyrrocinia* qui représentent les variants cliniques pathogènes pour l'homme. Ces observations témoignent de la capacité d'adaptation des espèces de ce genre dans des niches écologiques aussi différentes.

L'analyse de la production d'AIA a montré que toutes les souches produisent différentes quantités d'auxine après croissance sur milieu minimum contenant du L-tryptophane (Tableau 1). La production de grande quantité d'auxine est connue chez les souches de *P. savastanoi*. Cependant, seulement 3 des souches locales expriment une quantité élevée d'auxine (44 à 63,7  $\mu\text{g ml}^{-1}$ ). Le reste des souches produisent des quantités comparables à celles des souches de *P. savastanoi* rapportées par d'autres auteurs (Glickmann et al., 1998). Des quantités comparables (2,65 à 13,65  $\mu\text{g ml}^{-1}$ ) ont été aussi détectées chez les souches du genre *Pantoea*. A l'exception des souches Kn39A et KnO1 identifiées à *B. cepacia* and *Hafnia alvei* respectivement; et produisant de faibles quantités d'AIA, le reste des souches produit des quantités variant de 1.89 to 8.73  $\mu\text{g ml}^{-1}$  (Tableau 1). La production d'auxine semble être un phénomène commun au niveau des espèces phytopathogènes et des PGPB (Fett et al., 1987; Glickmann et al., 1998; Brandl et Lindow, 1996; Patten et Glick, 2002; Tsavkelova et al., 2007). Cependant, l'expression d'auxine par les souches du genre *Hafnia* n'a pas été citée.

La sécrétion de molécules lactones a été mise en évidence chez 20 souches qui appartiennent seulement aux espèces de *Pantoea*, *Stenotrophomonas* et *Burkholderia*. Le diamètre de diffusion de ces substances est variable d'une souche à une autre (Fig 1A). Ceci pourrait expliquer en partie le rôle de ces espèces qui agiraient en consortium pour l'induction de la maladie.

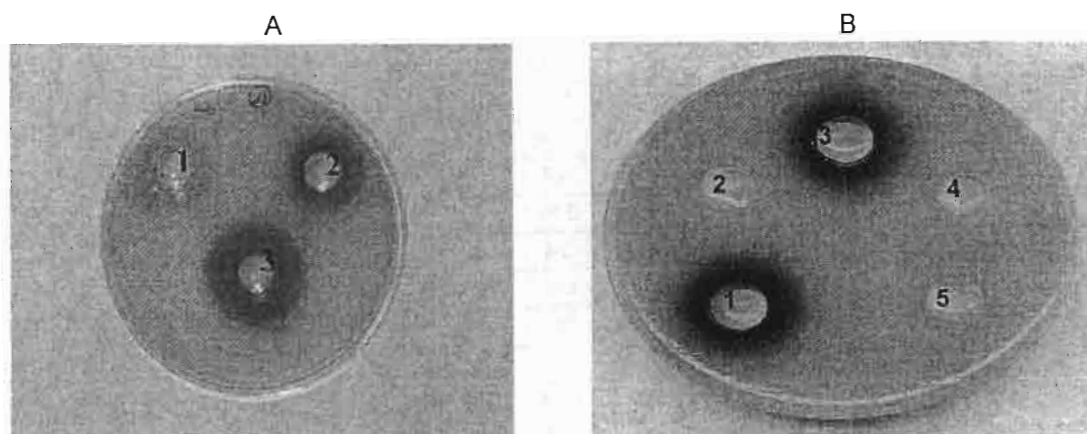


**Figure 1:** A, Production d'NAHL par les souches isolées des galles d'olivier (1 : *P. savastanoi*, 2 : *Burkholderia*, 3 : *Pantoea*); B, Inactivation des molécules de lactones par les souches de *B. thuringiensis* (1 : Témoin positif, 2 : Témoin négatif, 3 : inactivation partielle, 4 et 5 : inactivation totale).

Le rôle potentiel de certaines bactéries telles que *Bacillus thuringiensis* dans le biocontrôle et la promotion de la croissance des plantes a été analysée (Raddadi et al., 2007). Ceci à travers l'évaluation des activités enzymatiques que présentent ces souches incluant la synthèse de phytohormones et de lactonases qui interfèrent avec le système « Quorum-sensing » d'activation des gènes de virulence chez les bactéries Gram-négatives (Fig 1B). En effet, plusieurs souches de *B. thuringiensis* ont montré leur capacité entomopathogène, antibactérienne, PGPB et antifongique, suggérant leur application prometteuse en biotechnologie. La sélection de souches « polyvalentes » a été complétée par l'évaluation de leur innocuité par des tests phénotypiques de cytotoxicité sur les cellules Vero et des réactions PCR ciblant les gènes de virulence de *B. cereus* et *B. anthracis*, tels que ceux de l'hémolysine, *plcR* et de cytolyse (Raddadi et al., 2008).

**Tableau 2:** Associations bactériennes rencontrées au niveau des nodules.

| Knot sample | Locality/ cultivars* | Bacterial association**                                                                    |
|-------------|----------------------|--------------------------------------------------------------------------------------------|
| 1           | Kasserine / Nebjmal  | <i>P. savastanoi</i> (2); <i>Stenotrophomonas</i> ; <i>P. Putida</i> (3)                   |
| 2           | Kairouan / Chemlali  | <i>P. savastanoi</i> ; <i>Stenotrophomonas</i> (2)                                         |
| 3           | Kairouan / Chemlali  | <i>P. savastanoi</i> ; <i>Pantoea</i> (2); <i>Hafnia</i> (2)                               |
| 4           | Kairouan / Oueslati  | <i>P. savastanoi</i> ; <i>Stenotrophomonas</i> ; <i>P. putida</i> (2); <i>Burkholderia</i> |
| 5           | Kairouan / Oueslati  | <i>P. savastanoi</i> ; <i>Burkholderia</i> ; <i>P. putida</i>                              |
| 6           | Kairouan / Oueslati  | <i>P. savastanoi</i> ; <i>Burkholderia</i> ; <i>P. putida</i>                              |
| 7           | Kairouan / Oueslati  | <i>P. savastanoi</i> ; <i>Pantoea</i> (2); <i>Hafnia</i>                                   |
| 8           | Kairouan / Chemlali  | <i>P. savastanoi</i> ; <i>Burkholderia</i>                                                 |
| 9           | Takelsa / Chetoui    | <i>P. savastanoi</i> ; <i>Pantoea</i> (2)                                                  |
| 10          | Takelsa / Chetoui    | <i>P. savastanoi</i> ; <i>Stenotrophomonas</i> (2); <i>P. putida</i>                       |
| 11          | Takelsa / Chetoui    | <i>P. savastanoi</i> ; <i>Pantoea</i> (2)                                                  |

Les souches polyvalentes et sûres ainsi sélectionnées seront testées sur champs pour leur activité inhibitrice via l'interruption des voies du « Quorum Sensing » chez les bactéries à Gram négatif telle que *P. savastanoi*. Ce type de biocontrôle empêche l'établissement de l'infection et diminue le risque d'apparition de résistance puisque l'agent pathogène n'est pas éliminé mais simplement atténué.

En effet, des plantes transgéniques, exprimant les molécules *N*-acyl homosérine lactonase, montrent une résistance significative contre l'infection par *Erwinia carotovora* et sont capables de retarder le développement des symptômes de la maladie (Dong et al., 2002). De ce fait, on note que l'expression du gène *aiiA* codant pour la lactonase chez *B. thuringiensis*, révèle les grandes potentialités de cette espèce dans l'atténuation de la pathogénicité des bactéries à Gram négatif infectant les plantes (Jun et al., 2002). Les travaux de Dong et al., (2004) ont révélé que les souches de *B. thuringiensis* exprimant les AHL-lactonases répriment la virulence QS-dépendante de *Erwinia carotovora* et bloquent la colonisation des cellules du pathogène dans le tissu végétal. Ces données illustrent le potentiel prometteur que peut avoir ce mécanisme d'antagonisme microbien. Interférer dans les signaux de virulence des bactéries pathogènes pourrait en effet prévenir et réprimer les maladies infectieuses sans pour autant se heurter aux problèmes d'apparition de résistances puisque l'agent pathogène n'est pas éliminé mais simplement dépourvu de sa virulence.

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## La modélisation de la variation spatiale de la fertilité du sol dans une oliveraie

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### Résumé

La parcelle d'étude est une olivette dans la ferme de Chaal couvrant 134 ha. L'outil SIG a été utilisé pour superposer les différentes couches d'information et pour établir un modèle d'aide à la décision. Pour ce faire 27 points d'échantillonnage ont été sélectionnés dans la toposéquence. Les résultats des analyses du sol ont montré que ce sol est non salé avec un pH basique stable. Il est très pauvre en matière organique, par contre, il est riche en potassium et phosphore. La modélisation spatiale de la variation de la fertilité du sol a montré que sa fertilité s'améliore en allant du Nord vers le Sud. La partie Sud est plus riche en argile, en matière organique, azote total, potassium et présente de bonnes capacités de rétention en eau. C'est au niveau de cette partie Sud qu'on a noté les productions les plus élevées. La modélisation spatiale permet d'adapter l'application des fertilisants à la variabilité qui existe à l'intérieur de la parcelle. Ainsi, la disponibilité de cartes de distribution des paramètres de fertilité du sol et de la production permet de définir le potentiel productif et les zones d'intervention. Ceci permettra l'application de l'agriculture de précision visant à rendre conforme l'agriculture durable et à réconcilier agriculture et environnement.

**Mots clés:** Fertilité, Sol, Modélisation spatiale, Chaal, agriculture de précision

## Modeling of soil fertility spatial variation in an olive orchard

### Abstract

The olive orchard used for this study is located on a toposequence in central Tunisia (Sfax). The orchard covers 134 Ha and belongs to Chaal farm. The GIS tool was used to overlay the different layers of information and to establish a model for decision support. 27 sampling points were selected along the toposequence. Soil analysis results had shown that this soil is not salted with stable basic pH. It is extremely poor with regards to organic matter with a suitable richness in potassium and phosphorous. Spatial variability of soil fertility showed that fertility is improved when moving from the northern to the southern part. The southern part, where the highest olive yields were recorded, is richer in clay, organic matter, total nitrogen, potassium and has a good water holding capacity. The spatial modeling allows adjusting the application of fertilizers to the variability that exists within the parcel. Thus, the availability of soil fertility parameters distribution maps and productivity one lead to identify the production potential and needs of intervention. This will allows the application of precision agriculture making agriculture sustainable and reconciles agriculture and environment.

**Keywords:** Soil fertility, Soil, Spatial modeling, olive growing, precision agriculture.

### Effect of urban residues on Olive Grove soils.

Sastre-Conde, I. <sup>(2)</sup> Lacasta, C. <sup>(3)</sup> Plaza, A <sup>(1)</sup> , Cabezas, J.G <sup>(1)</sup> and Lobo, M.C. <sup>(1)</sup>

#### Abstract

The application of organic residues induces an increase in organic matter and nutrients soil content, and an improvement in soil physical properties, as porosity, water holding capacity and infiltration capacity, contributing as a result to reduce erosion processes. The aim of this paper is to evaluate in a field experiment the effects of urban residues application (thermal dried sewage sludge and composted urban waste) on soil characteristics and olives growth, as well as determine the optimum dose of these types of residues to improve soil properties. Two years after residues application, some chemical soil parameters such as pH and electric conductivity were significantly modified. A significant increase in phosphorus and potassium contents was observed in amended soils. No significant differences were observed in physical soil properties. The olive production was determined, finding the lower production in the control soils, although the differences in dry weigh were not significant.

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## Etude de l'effet de différentes techniques de l'irrigation déficitaire raisonnée sur la production et la croissance végétative de l'olivier (*Olea europaea*.L) sous le climat semi aride de la Tunisie

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### Résumé

De nos jours, la disponibilité des ressources hydriques est de plus en plus limitée et l'économie de l'eau d'irrigation est nécessaire. Des différentes techniques ont été développées notamment l'irrigation déficitaire. Malgré ses bénéfices, cette technique nécessite la bonne connaissance des stades tolérants au stress hydrique et elle est difficile à appliquer par les agriculteurs. Récemment, une nouvelle technique a été développée, appelée « Dessèchement partiel des racines » (PRD : Partial Root Drying). Elle consiste à alterner l'irrigation de deux côtés de l'arbre. Le présent travail a pour objectif, l'étude de l'effet de différentes techniques sur la production et la croissance végétative de l'olivier (variété Chetoui). En effet, l'application de cinq traitements à savoir : Le pluvial (sans aucune irrigation), 100% PRD et 50%PRD (irrigation avec respectivement 100% et 50% de la dose sur un seul côté de l'arbre avec une alternance de 10 jours), 100%ETC et 50%ETC (irrigation avec respectivement 100% et 50% de la dose sur les deux côtés de l'arbre) a donné des différences significatives. Les résultats ont montré que tous les traitements hydriques déficitaires ont légèrement réduit la production et la longueur moyenne des pousses végétatives par rapport à l'irrigation avec 100% de la dose sur les deux côtés. Concernant le taux d'huile aucune différence significative n'a été notée.

**Mots clés:** Irrigation déficitaire, Olivier, production, huile, végétation

### Effects of different techniques of regulated deficit irrigation on yield and vegetative growth of olive trees (*Olea europaea*. L) under field conditions of the semi arid climate of Tunisia

### Abstract

Water scarcity in the arid zone appears as one of the main factors limiting agricultural development. For this reason it is necessarily to reduce water use for irrigation and thereby increasing crop water use efficiency. This experiment have lead to development of different irrigation practice (partial root drying and regulated deficit irrigation) which aims to study these effects on yield and vegetative growth of olive trees under semi arid climate of Tunisia. Five irrigation treatments were applied: Rainfed (without irrigation), 100%PRD and 50%PRD (Respectively 100% and 50% of dose on one side alternating the wetted and dried sides of the root system every ten days) and 100%ETC and 50%ETC (Respectively 100% and 50% of dose on both sides). Results showed that regulated deficit irrigation and PRD treatments slightly decrease yield compared to fully irrigation except 100%PRD which strongly decreased it. However, irrigation treatments didn't affect the oil content. On shoot length measurements given that all deficit irrigation treatments reduced it compared to fully irrigation.

**Key words:** Deficit irrigation, olive, yield, oil, shoot.

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### **Thème 3**

**Impacts, enjeux, possibilités et limites de l'olivier en hyper-intensif**

## Effect of "topping" pruning on vegetative growth and fruit repartition within the canopy of olive trees conducted under high density planting system

Souha Jomni<sup>2</sup>, Ajmi Larbi<sup>1</sup>, Ali Ben Dhiab<sup>1</sup> and Monji Msallem<sup>1</sup>

### Abstract

In the high density olive orchards, the harvesting is carried out using a harvesting machine. For a good performance of this machine, an adequate olive trees dimension is necessary. As an alternative of the expensive manual pruning, topping the canopy of the trees parallel to the ground by cutting bar provided with circular disc-saws can be used. The main objective of this work is to evaluate the effects of topping Arbequina and Arbosana olive trees on its vegetative, floral and fruiting characteristics. Results obtained here indicate that the topping improve the distribution of fruits within the canopy. Furthermore, oil accumulation in the fruits of the upper levels and its pomological characteristics (size, weight) was significantly higher than those of the lowers levels. Results revealed also that topping induced a vigorous growth mainly at the top part of the canopy.

**Keywords:** Topping pruning, High density planting system, Olive tree.

### Résumé

Le but principal de la conduite d'un verger oléicole en mode hyper intensif est la mécanisation de la récolte. Pour cela, nous devons assurer une uniformité du verger et contrôler la vigueur de l'arbre pour garder la production aux limites de la récolteuse mécanique.

Le « topping » est l'une des techniques permettant le contrôle de la hauteur de l'arbre tout en assurant une meilleure pénétration de la lumière à l'intérieur de la frondaison.

Ce travail, mené sur les deux variétés Arbosana et Arbequina au niveau de deux parcelles conduites en mode hyper intensif à Mornag et à Bir Mchergua, a étudié l'effet de la taille « topping » sur les différents paramètres végétatifs, florifères et fructifères de l'olivier, ainsi que la variation de ces paramètres en fonction de leur position au niveau de la frondaison.

Les résultats obtenus montrent que le « topping » entraîne une meilleure distribution de la production sur l'arbre et une augmentation du rendement en huile, du calibre et du poids des fruits. Cette augmentation est plus importante au niveau des étages les plus élevés de la frondaison.

L'application du « topping » a permis aux arbres d'avoir un développement végétatif plus important notamment au niveau des étages supérieurs de l'arbre.

### 1. Introduction

Since 1994, a new type of olive orchard was developed in Spain, based on the use of high number of trees varying between 1250 and 2500 trees/ha. This new planting system was introduced in Tunisia by private investors in 1999 (Msallem et al., 2004). The inexistence of low vigour varieties and dwarfing root-stock obligated the use of medium vigour varieties such as Arbequina and Arbosana (Leon et al., 2007, De La Rosa et al., 2007). Therefore, the canopy volume and height of trees reached by these varieties after few years from planting caused the apparition of shaded parts into the canopy. León and al. (2007) reported that trees planted in high density suffer from the mutual shading problems by the seventh years after planting. Furthermore, fruit distribution is affected and fruits are mainly located in the upper part of the canopy (Ameur et al., 2008). The mechanical harvest operation will be difficult if the control of the tree vigor was not made. Therefore, in order to optimize light interception by the canopy and to allow the passage of the harvesters over the top of the tree's row it is necessary to control the dimensions of trees. The pruning of the canopy of the trees parallel to the ground by cutting bar provided with circular disc-saws should be used to ensure a good performance of this machine and also to have a better fru distribution by the canopy. The main objective of this work is to evaluate the effects of topping Arbequina and Arbosana olive trees on its vegetative, floral and fruiting characteristics.

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## 2. Material and Methods

This work was carried out in Mornag in a high density planting orchard (1250 trees/ha) planted at 1999 with Arbequina and Arbosana varieties. The pruning trial was carried out in this site in February 2007. The experimental design consisted of two randomized complete blocks with 5 trees each one for every variety. Each tree was divided into three layouts: the bottom one (0-1 m), the medium one (1-2 m) and the top one (up to 2 m). On each layout, five shoots for each quadrant (North, East, West, South and the interior) were selected and labeled in March 2009. Vegetative growth and buds development were determined. Furthermore, each layout was harvested and weighted separately. Fruits samples were taken to determine fruit weight average and oil content on fresh matter basis.

## 3. Results and discussion

Results obtained in this work indicated that vegetative growth of shoots located in the lower part of the canopy (inferior to 1m) have a lower growth speed (0.09 cm per day) in comparison with growth speed of shoots located in the medium and top layout of the canopy (0.19 and 0.35 cm per day, respectively). Topping pruning application improved the vegetative growth of Arbequina and Arbosana varieties except the medium layout of Arbosana variety and the bottom one of Arbequina (Figure 1).

Growth increase was about 32.8% and 81.6% at the bottom and the top layout of Arbosana variety and about 158 and 52.1% at the medium and top layouts of Arbequina. Vossen (2004) reported that application of topping pruning during the winter increased vegetative growth mainly in the upper part of the canopy.

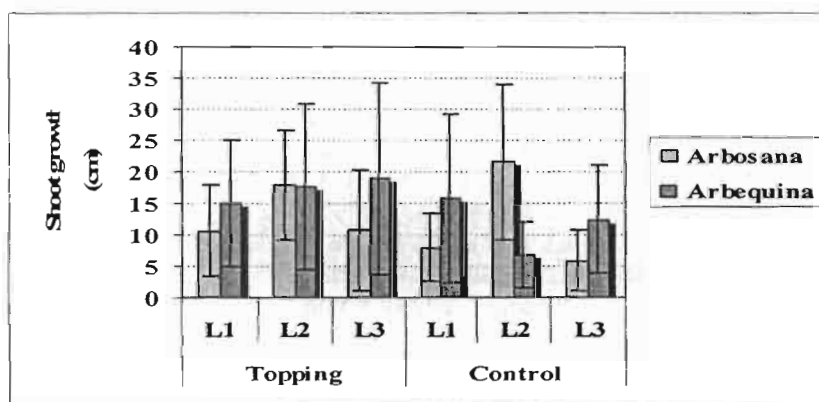


**Figure 1:** Shoot growth for the different layouts of the canopy (L1, L2 and L3) in control and pruned trees of Arbequina and Arbosana varieties

The development of buds into inflorescence or vegetative shoot was recorded for each layout of the canopy. Results obtained here indicated that the medium layout of the canopy of Arbequina variety have the highest floral bud percentage in control and pruned trees. In contrast with the medium part of the canopy, the lower one has the lowest floral buds percentage (Figure 2).

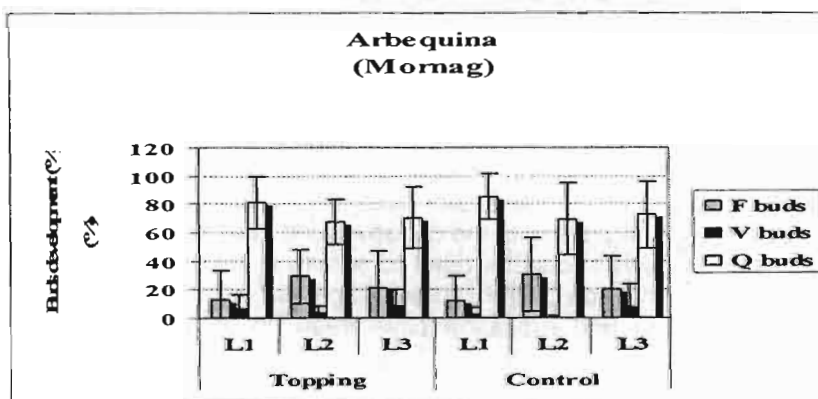


**Figure 2:** Buds development for the different layouts of the canopy (L1, L2 and L3) of pruned and control trees of Arbequina variety.

With respect to Arbosana variety, the highest floral buds percentage was obtained in the upper part of the canopy in pruned and control trees. In deed, floral buds percentage was about 31.6 and 36.8% in pruned and control trees, respectively (Figure 3). However, the lowest one was obtained by the bottom part of the canopy in both pruned and control trees (Figure 3). Our results showed the importance of light interception by the canopy on floral induction. In deed, the highest quiescent buds percentage was obtained by the lower part of the canopy which suffers shading stress. Ameur et al. (2008) indicated that light interception decreased significantly from the upper to the lower part of the canopy of Arbequina and Arbosana trees grown under high density planting system. Several authors reported that the upper part of the canopy has the highest floral buds percentage in comparison with the others parts of the canopy (Acebo et al., 2006; Tombesi et al., 1999 and Pastor et al., 2007). These authors confirm the relationship between flowering and light interception.

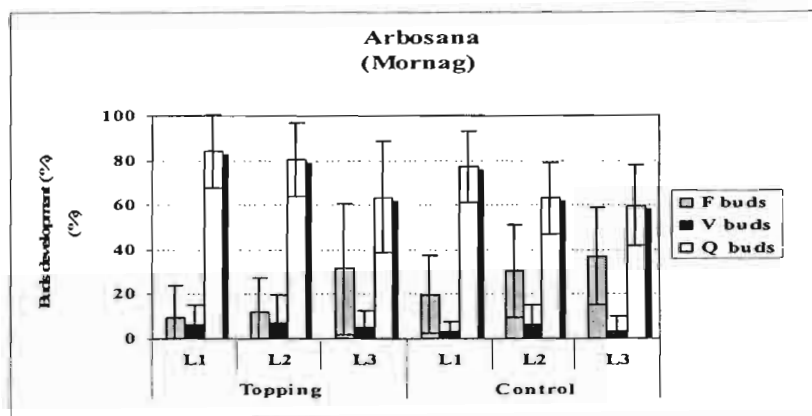


**Figure 3:** Buds development in the different layouts (L1, L2 and L3) of the canopy of pruned and control trees of Arbosana variety.

Fruit distribution within the canopy varied significantly between different layouts. In deed, fruit production of the medium and top layouts of the canopy was significantly superior to that obtained by the lower one in both control and pruned trees (Figure 4). Nevertheless, the topping application led to the increase of fruit production into the medium part of the canopy. Indeed, in control trees, fruits were mainly located in the medium and upper layouts with approximately 39.7 and 40.6%, respectively of the total of the production by tree in Arbosana and 43.8 and 42.5%, respectively for Arbequina. However, for pruned trees, fruits are mainly located in the medium layout of the canopy with 44.3 and 54.6% of the total production, for Arbosana and Arbequina, respectively. Contrary to the medium layout, a decrease of fruit production was obtained in the upper layout of the canopy. Topping application did not affect significantly the production of the lower part of the canopy.

Our results indicated that fruits distribution was significantly affected by its location within the canopy (Figure 4). Indeed, the lowest fructification at the lowers parts of the canopy may be explained by light limitation. Pastor et al. (2007) reported that more than 93% of olive fruits are located at a height superior to 1.5 m.

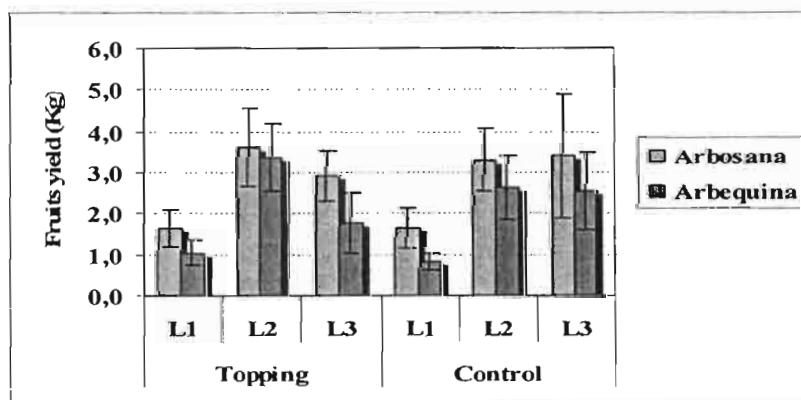


**Figure 4:** Fruits yield in the different layouts (L1, L2 and L3) of the canopy of pruned and control Arbequina and Arbosana varieties.

Similarly to fruits production, the highest oil content on fresh matter basis was obtained in the upper layouts of the canopy in both control and pruned trees (Figure 5). Pastor (2005) reported that the highest oil content for Arbequina variety grown under high density planting system were found at a height superior to 2 m while for the lower part of the canopy comprised between 0 and 0.75 m, the oil content did not outnumber 12.5%. Tombesi (1999) indicated that shading stress decreased oil content. It is important to mention that the topping application did not influence significantly oil content (Figure 5).

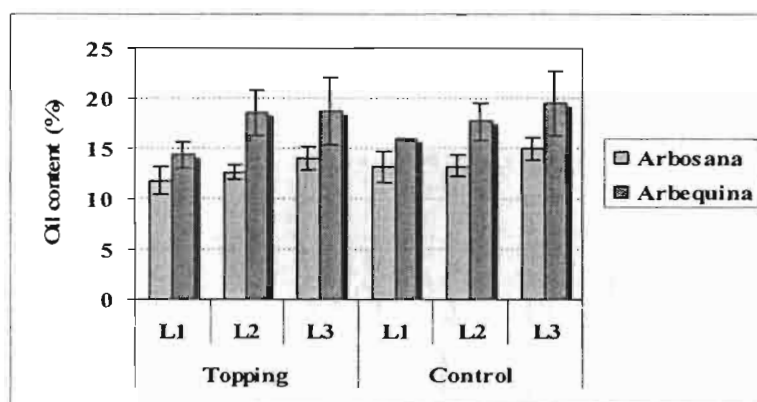


**Figure 5:** Oil content on fresh matter basis for the different layouts (L1, L2 and L3) of the canopy of control and pruned Arbequina and Arbosana varieties.

Finally our results indicated that the topping pruning increased the fruit weight average in the different layouts of the canopy. Increases were about 12.3, 17.4 and 11.7 %, for the lower, medium and the top layout of Arbosana variety (Figure 6). The same tendency was found for Arbequina with an increase about 18.4, 7.9 and 9.25% for the same layouts respectively (Figure 6).

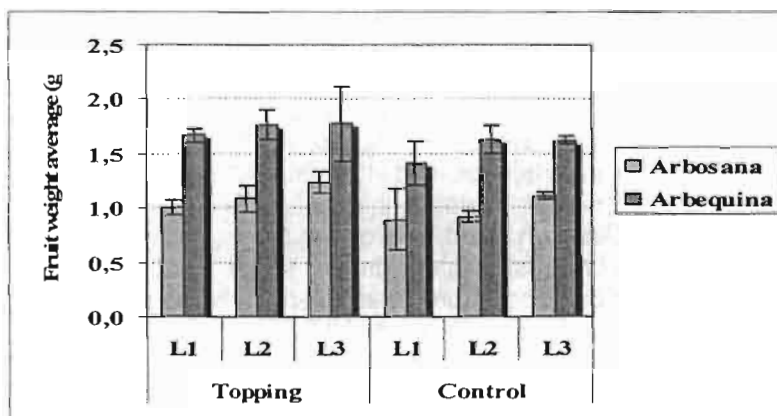


**Figure 6:** Average fruit weight for the different layouts (L1, L2 and L3) of the canopy of pruned and control trees of Arbequina and Arbosana varieties.

In summary, the topping application improved vegetative growth, fruit distribution by the canopy and increased fruit weight average. Furthermore, the flowering percentage was better in pruned trees than in control trees. Therefore, we recommended the application of topping pruning when trees height is superior to 3 m. The topping application may allow a better light interception by the canopy (Ameur et al., 2008) and consequently a better fruit repartition and an adequate trees dimension which facilitate the use of the harvesting machine.

### Acknowledgement

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## **Impacts de l'intensification des plantations oléicoles sur la qualité physico-chimique des sols (station expérimentale de Taous : région de Sfax)**

Narjess Achour<sup>1</sup>, Hafedh Rigane<sup>2</sup>, Boubaker Karray<sup>2</sup>

### **Résumé**

Ce travail a pour but d'étudier l'impact des régimes intensif et hyper intensif sur la qualité physico-chimique des sols dans la station expérimentale de Taous (région de Sfax). L'étude a été effectuée sur quatre parcelles expérimentales : témoin, en pluvial et deux parcelles irriguées. Les résultats obtenus ont montré que les parcelles irriguées en régimes intensif et hyper-intensif marquent des teneurs en conductivité électrique très élevées. Ceci est expliqué par l'enrichissement de l'eau d'irrigation en sels solubles. L'horizon de surface est considéré dans une phase saline. Ces deux parcelles marquent aussi les plus faibles teneurs en matière organique dues au phénomène de minéralisation qui se montre plus accentuée en raison de la richesse de ces sols en microorganismes. En se basant sur le sodium adsorption ratio (SAR), on constate que les deux parcelles témoin et en pluvial sont caractérisées par un fort pH. Par contre, les parcelles irriguées sont classifiées parmi les sols salins.

Mots clés: Sol, régimes intensif et hyper intensif, oliveraie, sol, matière organique.

### **Abstract**

The purpose of this work is to study the impacts of intensive and hyper intensive modes on the physical and chemical quality of the soils in the experimental station of Taous in Sfax region. The study was carried out on four experimental pieces on a control soil, on irrigated parcel with rain water and two irrigated parcels in intensive and hyper-intensive modes. The results obtained showed that the pieces irrigated in intensive and hyper-intensive modes mark a very high electrical conductivity. This is explained by the enrichment of water irrigation in soluble salts. The surface horizon is considered in salt phase. These two pieces mark also the lowest contents of organic matter due to the phenomenon of mineralization which is shown more accentuated because of the richness of these soils in micro-organisms. Besides, basing on sodium adsorption ratio (SAR), we note that the control and irrigated rain pieces are classified as soils with a high pH while the pieces in intensive and hyper intensive modes are classified as saline soils.

**Key words:** soil, intensive and hyper intensive modes, sodization, organic matter.

### **1. Introduction**

La production d'huile d'olive contribue à combler en partie notre déficit en matière d'huiles alimentaires et à réduire, par conséquent, notre dépendance vis à vis du marché extérieur. Toutefois, les rendements, et en dépit des progrès réalisés, restent relativement faibles. L'oléiculture tunisienne est tributaire du climat. Pour cela, un programme national d'intensification de l'oléiculture est mis en place. Il vise l'amélioration de la productivité afin d'avoir un noyau stable de production pour faire face aux besoins nationaux et aux engagements mondiaux.

Depuis 1999, la Tunisie assiste à l'émergence d'une nouvelle oléiculture, qui a bousculé la tradition, inspirée d'un modèle mis au point en Espagne. Cette nouvelle alternative est basée sur un nombre élevé de plantes par hectare. Les objectifs recherchés visent une entrée rapide, des rendements élevés, réguliers et à moindre coût en particulier ce qui concerne la récolte.

En 2000, l'Institut de l'Olivier a mis en place un protocole de suivi, au niveau de la première parcelle installée en Tunisie à Mornag, dont l'objectif est l'élaboration d'un référentiel technique et une évaluation technico-économique de cette opération (Msallem et al, 2004).

Ce travail est une continuation de l'étude précédente mais en étudiant l'aspect environnemental dans la ferme Taous. Il consiste à analyser l'impact de ces régimes de plantation intensif et hyper-intensif sur l'état du sol et ses réserves en eau et en éléments nutritifs.

## 2. Matériel et méthodes

### 2.1. Protocole expérimental

L'étude de cas entamée dans la station expérimentale Taous de l'Institut de l'Olivier s'intéresse à étudier les impacts d'adoption des régimes de plantation intensif et hyper intensif sur la qualité physico chimique des sols et les réserves en éléments nutritifs.

Cette étude a été réalisée moyennant un protocole expérimental tablé sur un prélèvement d'échantillons de sol à partir de quatre parcelles expérimentales (témoin, pluvial, intensif et hyper intensif) dans la station Taous et sur quatre horizons (0-20cm, 20-40cm ; 40-60cm et 60-80 cm) au cours de la période Avril-juillet 2008.

312 échantillons ont été prélevés pour différentes distances et profondeurs. Le mélange des échantillons similaires les a réduits à 112 échantillons aptes pour les analyser.

### 2.2. Méthodes d'analyses

Les méthodes d'analyses employées sont pour la plupart classiques et sont celles utilisées dans le laboratoire de l'Institut de l'Olivier.

#### Les analyses de l'eau d'irrigation:

L'échantillon provient du puits installée au niveau de la station Taous.les paramètres étudiés sont : résidu sec ; conductivité électrique ; pH ; dosage du Sodium, du potassium, du Calcium et magnésium....

#### Les analyses physico- chimiques du sol:

Granulométrie, humidité, conductivité électrique, pH, sodium, potassium, calcium, magnésium, azote total, matière organique, phosphore et chlorures.

## 3. Résultats et discussions

### 3.1. Analyse de l'eau d'irrigation :

L'eau d'irrigation est de qualité marginale titrant un résidu sec de 3,6 g/l. Les différents paramètres analysés sont enregistrés dans le tableau ci-dessous.

**Tableau 1:** Composition chimique de l'eau d'irrigation

| Paramètres | Résidu sec (g/l) | pH   | CE (mS/cm) | K <sup>+</sup> (g/l) | Na <sup>+</sup> (g/l) | Ca <sup>2+</sup> (g/l) | Mg <sup>2+</sup> (g/l) | CO <sub>3</sub> <sup>2-</sup> (g/l) | HCO <sub>3</sub> <sup>2-</sup> (g/l) | Cl <sup>-</sup> (g/l) | SO <sub>4</sub> <sup>2-</sup> (g/l) | NH <sub>4</sub> <sup>+</sup> (g/l) | SAR  |
|------------|------------------|------|------------|----------------------|-----------------------|------------------------|------------------------|-------------------------------------|--------------------------------------|-----------------------|-------------------------------------|------------------------------------|------|
| Valeurs    | 3,6              | 7,81 | 4,69       | 0,35                 | 0,58                  | 0,158                  | 0,081                  | 0                                   | 0,762                                | 7,38                  | 0,01                                | 0,05                               | 5,65 |

### 3.2. Analyses du sol :

#### 3.2.1. Conductivité électrique

Les parcelles en régime hyper-intensif montrent des valeurs largement supérieures par rapport aux autres parcelles. Ceci est essentiellement remarquable au niveau de l'horizon de surface de la

parcelle en régime hyper-intensif de la variété Chemlali pouvant atteindre 9 mS/cm (Figure 1). Cette valeur enregistrée est expliquée par les quantités d'eau d'irrigation importantes enrichies en sels solubles. Pendant cette période, les besoins de l'olivier en eau sont très importants, pour cela des quantités de sels s'accumulent engendrant des concentrations de sels aux alentours des oliviers.

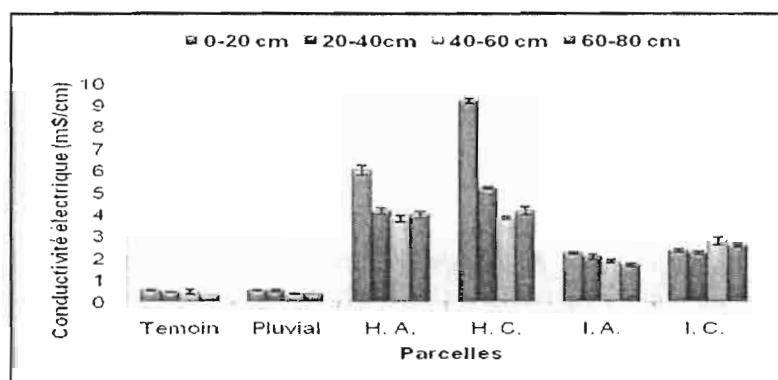


**Figure 1:** Evolution de la conductivité électrique du sol dans les différentes

En se basant sur certaines classifications (Servant, 1975 ; CPCS, 1967 et FAO, 1989), l'horizon de surface est considéré dans une phase saline et il est moyennement salé à salé. Ainsi, une attention particulière doit exister afin de limiter tout risque de salinisation d'horizon de surface qui pourrait influencé l'alimentation des oliviers en eau lorsque le sol est arrosé (Mathieu et Pieltain, 2003).

### 3.2.2. Chlorures

On observe nettement des différences au niveau des teneurs en chlorures (Figure 2). C'est au niveau des parcelles cultivées en régime hyper-intensif qu'on observe de grandes quantités de chlorures pouvant atteindre 40 méq/l en horizon de surface pour la variété Arbequina. En deuxième lieu, se place les parcelles en régime intensif puis celles du régime pluvial et témoin. Sachant que les chlorures peuvent être toxiques en fortes concentrations, il est impératif de remédier cette situation pour éliminer d'éventuels problèmes.

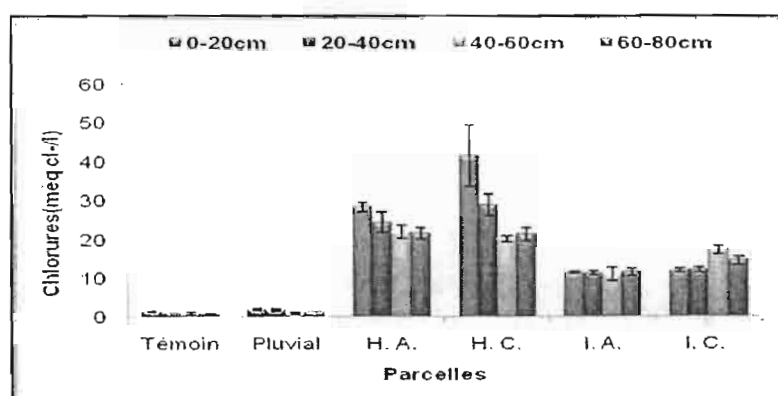


**Figure 2:** Evolution des teneurs en Chlorures dans les différentes parcelles

### 3.2.3. Matière organique

Les faibles teneurs sont marquées dans les parcelles en régimes intensif et hyper-intensif. Donc ces parcelles sont relativement pauvres en M.O. Cette pauvreté est due à la forte minéralisation de cet élément suite à une concentration importante en faune et en flore du sol.

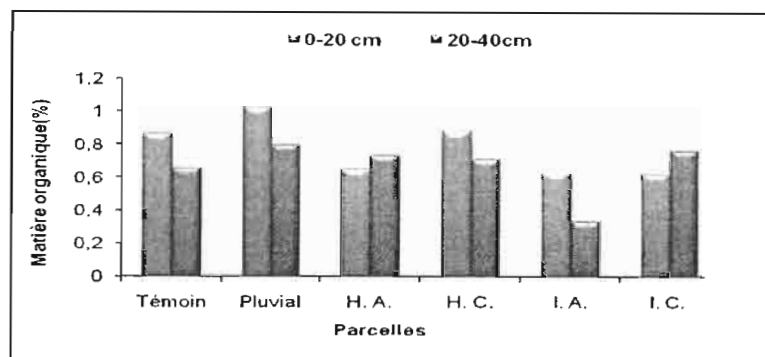


**Figure 3:** Evolution de la matière organique dans toutes les parcelles

#### 4. Conclusion générale

Le diagnostic de l'état du sol après six ans en régime intensif et quatre ans en régime hyper-intensif et en comparant avec des plantations en pluvial âgées de 10 ans et un sol témoin montre une diversité au niveau de sa fertilité.

L'humidité la plus importante se montre au niveau du sol cultivé en mode hyper-intensif dans les différents horizons essentiellement avec la variété Arbequina où les quantités d'eau d'irrigation sont importantes.

Il est à noter que les parcelles en hyper-intensif montrent des teneurs en conductivité électrique largement supérieures par rapport aux autres parcelles. Ceci est expliqué par les quantités d'eau d'irrigation importantes appliquées et qui sont enrichies en sels solubles.

Les teneurs en chlorures présentent des différences très significatives entre les parcelles irriguées (intensive et hyper intensive) et le sol témoin. Le net enrichissement du sol en chlorures est dû à la richesse de cet effluent en sels. Cependant, un risque de toxicité pourrait exister exigeant ainsi une attention particulière.

L'intensification des plantations oléicoles influe sur le pH du sol où on a remarqué des valeurs élevées dans les parcelles irriguées et par conséquent, le sol est touché par un risque de détérioration de la structure prouvé par la mesure du sodium adsorption ratio (SAR) au niveau de ces parcelles. Ces sols sont classifiés parmi les sols salins avec des conditions physiques normales.

Concernant l'évolution de la matière organique, de très faibles teneurs sont marquées au niveau des sols en régime hyper-intensif et surtout intensif. En effet, au niveau de ces deux parcelles, la minéralisation de la matière organique est plus poussée en raison de la concentration de biomasse autour des racines qui pourrait minéraliser le stock humique du sol pour satisfaire les demandes des racines et des plantes.

Les deux parcelles témoin et en pluvial sont les plus pauvres en calcium et en magnésium. Par contre, un enrichissement en ces éléments a été observé au niveau des parcelles en régime intensif et hyper intensif qui est expliqué par les apports d'amendements organiques qui sont riches en Ca, Mg et K.

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## Effect of foliar nutrition (N, P and K) on fruit set and yield of olive trees (CV Arbequina) conducted under a high density planting system.

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### Abstract

The effect of foliar nutrition (N, P and K) using a commercial product (Nutrivant) on fruit set and olive yield has been studied in a commercial olive orchard located in Takelsa (North of Tunisia). Applications (250 mg l<sup>-1</sup>) were made during two years at 4 different dates (March, May, June and July). Application at March was made with pre flowering Nutrivant (10%N, 33% P, 21% K and 1.8% Boron). However the rest of applications were made with post flowering Nutrivant (8%N, 16% P and 39% K). During the first year of the study, a significant increase of fruit set was recorded just after the first application. In deed fruit set increased from 2.53% in control trees to 5.07% in treated trees. On the second year, fruit set increased slightly after the first application from 3.47% in control trees to 4.6% in treated trees. Olive yield increased by 35.7 and 25.3% after the 4 application during the first and the second year of study, respectively. It must be pointed out that trees are damaged by *Spilotea Oleaginae* during the second year of experimentation. Finally, Nutrivant application increased foliar potassium and nitrogen concentration during both years of experimentation. In deed, foliar potassium concentration increased from 0.69 to 0.81% and from 0.75 to 1.13% on the first and the second year, respectively. Also, foliar nitrogen concentration increased from 1.70 to 1.82 and from 1.25 to 1.53 in both years of experimentation. It must be pointed out that leaf sampling was carried out at mid July in both years.

**Keywords:** foliar fertilisation, nitrogen, potassium, olive tree.

### Résumé

L'effet de la fertilisation foliaire (N, P et K) en utilisant un produit commercial (Nutrivant) sur la nouaison et la production a été étudié dans un verger commercial d'olivier situé à Takelsa (Nord de la Tunisie). L'application de 250 mg l<sup>-1</sup> de Nutrivant a été réalisée durant deux années à quatre dates différentes (Mars, Mai, Juin et Juillet). L'application au mois de Mars a été réalisée en utilisant le produit Nutrivant préfloraison contenant (10%N, 33% P, 21% K et 1.8% B). Toutefois, pour le reste des applications nous avons utilisé le produit Nutrivant post-floraison contenant (8%N, 16% P et 39% K).

A la première année de cette étude, nous avons trouvé qu'il se produit une augmentation significative du taux de nouaison suite à la première application réalisée au mois de Mars. En effet, le taux de nouaison a passé de 2,53% chez les arbres témoins à 5,07% chez les arbres traités. A la deuxième année de cette étude, le taux de nouaison a augmenté légèrement chez les arbres traités (4.6%) en comparaison avec les témoins (3.47%).

En ce qui concerne la production, nous avons enregistré une augmentation de 35.7 et 25.3% après les quatre applications durant la première et la deuxième année de l'essai, respectivement. Il est à signaler que les arbres ont été endommagés par la maladie de l'oeil de paon (*Spilotea Oleaginae*) à la deuxième année de notre essai. Finalement, l'application de Nutrivant a engendré une augmentation de la concentration d'azote et de potassium dans les feuilles durant les deux années d'expérimentation. En effet, la concentration de potassium dans les feuilles a augmenté de 0,69% à 0,81% et de 0,75 à 1,13% durant la première et la deuxième année de l'expérimentation. De même, la concentration d'azote dans les feuilles a augmenté de 1,7 à 1,82% et de 1,25 à 1,53% dans les deux années d'expérimentation. Il est à signaler que l'échantillonnage des feuilles a été réalisé vers mi-juillet pendant les deux années d'étude.

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## Effets de certains sprays sur l'efficacité de la récolte mécanique des olives 'arbequina'

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### Resumé

L'objectif de ce travail était d'évaluer l'effet de l'éthéphon, appliqué seul ou en combinaison avec des composés du phosphore (MKP et MAP), sur des arbres adultes d'olivier (*Olea europaea* L.) de la variété 'Arbequina', afin d'améliorer l'efficacité de la récolte mécanique. L'essai a été réalisé en Catalogne (NE Espagne), au cours des campagnes oléicoles 2007 et 2008. Les paramètres étudiés ont été: la force de suppression des fruits (FSF), l'index de maturité, le poids des fruits, la chute naturelle, l'efficacité de vibration (EV), la chute des feuilles (CF), le teneur en huile, et l'intensité de la floraison. Les différents traitements de l'éthéphon n'ont pas affecté d'une manière significative l'index de maturité, le teneur en huile ou l'intensité de la floraison. Par contre, l'éthéphon seul réduisait FSF et augmentait l'efficacité de vibration. P-composés ne semblent pas affecter FSF et CF.

**Mots clés:** 'Arbequina', éthéphon, composés phosphore, force de suppression des fruit, récolte mécanique

## Effect of loosening agent sprays on the efficiency of the mechanical harvesting of 'arbequina' olives

### Abstract

The aim of this work was to evaluate the effect of ethephon, applied either alone or in combination with phosphorus compounds (MKP and MAP), on mature 'Arbequina' olive trees (*Olea europaea* L.) in order to improve the efficiency of mechanical harvesting. The trial was carried out in Catalonia (NE Spain), during the 2007 and 2008 olive crop seasons. Data measured were fruit removal force (FRF), ripeness index, fruit weight, natural drop, shaking efficiency (SE), leaf drop (LD), oil content, and bloom intensity. The trial treatments did not significantly affect ripeness index, oil content or bloom intensity. Ethephon reduced FRF and increased shaking efficiency. P-compounds did not seem to affect FRF and LD.

**Key words:** 'Arbequina' cv., ethephon, phosphorus compounds, fruit removal force, mechanical harvesting.

### 1. Introduction

The hand harvesting of olives (*Olea europaea* L.), in traditional orchards represents more than 50% of their total production cost (Tous and Romero 1992; Barranco et al. 2004). Although continuous mechanical harvesting is quite efficient in the super high density (SHD) orchards, trunk shakers are normally much less efficient in traditional orchards with large trees and in some cultivars, such as 'Arbequina' (Pastor et al. 2001; Tous et al. 2007). This cultivar is the most important in traditional olive Catalan orchards. It is characterized by small fruits and a weeping-open growing habit that reduces the energy transmitted through trunk-vibration to the fruit (Tous and Romero 1992).

The use of ethylene-releasing chemicals, which are used to promote olive fruit abscission, to increase removal rates of mechanical shakers harvesting has been widely studied in many countries and for many years (Lavee and Haskal 1975; Vitagliano 1975; Martin et al. 1981; Tous et al. 1995). Ethephon also causes leaf abscission which can compromise the following year's production if more than 15-20% of the leaves are lost (Martin et al. 1981). Lang and Martin (1989) reported that low ethylene concentration could reduce leaf abscission with an acceptable degree of fruit loosening, but for several cultivars this would not be enough to guarantee efficient and economical mechanical harvesting. It has recently been reported that applying monopotassium phosphate (MKP) alone, or in combination with ethephon, seems to reduce the fruit removal force of olives (Barranco et al. 2004; Burns et al. 2008).

The objective of this study was to evaluate the potential of ethephon (2-chloroethyl phosphoric acid) in combination with P-formulation compounds as an agent for loosening olive fruits and improving the efficiency of the mechanical harvesting of mature 'Arbequina' olive trees under the typical environment conditions found in Catalonia.

## 2. Materials and methods

The study was carried out during the 2007 olive crop season in an olive orchard at IRTA-Mas de Bover, located in Constantí, Tarragona (Spain). The mature 'Arbequina' trees were 80-year-old, growing in rainfed conditions (about 500mm of rainfall).

The trees were sprayed with ethephon formulated as CLOUD (Sapex-Agro, Reus, Spain). The treatments evaluated were untreated control and 6 different treatments (Table 1), and it were applied on November 14<sup>th</sup>.

The force required to remove an olive fruit (FRF) from its pedicel was measured in cent Newton (cN), using a dynamometer (Correx; Bern, Switzerland). Fruits that dropped without being attached to the dynamometer were each assigned an FRF of 0 cN. Fifty fruits per tree were detached just before applying the spray treatment and also 5 and 13 days after application.

Olives that dropped from trees before harvesting (natural drop) were collected from under each tree and weighed (fresh weight). Olive trees were shaken 14 days after spraying. Mechanical harvesting consisted of a 10 sec trunk vibration per olive tree. Fruits not removed by shaking were hand-picked to determine total production. The efficiency of mechanical harvesting (SE) was calculated as a percentage of the fruit removed. In both cases, leaves that dropped (LD) during harvest were also weighed and related to canopy volume of olive tree ( $\text{g m}^{-3}$ ). In May 2008, to assess the possible effects of leaf drop on the following year's bloom, the level of flowering intensity was visually scored on a scale of 0 (no bloom) to 5 (heavy bloom).

## 3: Résultats

The ethephon treatment reduced the 'Arbequina' fruit removal force (FRF). In 2007, five days after application, no significant differences were found between treatments (Fig 1). Thirteen days after ethephon application, FRF values were significantly different for the different treatments. While the reduction in FRF for the control trees was only 25%, the reduction in FRF following ethephon application ranged from 58% to 71%.

The application of ethephon combined with P-compounds clearly improved the efficiency of trunk shaking 14 days after application (Table 1). Shaking efficiency (SE) reached levels of 68% to 76% for treated olive trees, as opposed to 57% for the control trees.

On the other hand, ethephon also caused leaf drop (LD) (Table 2). Total LD significantly varied between treatments, with values for the control being significantly different from the others. The concentration of  $500 \text{ mg}\cdot\text{L}^{-1}$  of ethephon induced more total LD than that of  $300 \text{ mg}\cdot\text{L}^{-1}$ . The application of P-compounds had no incidence on total LD. The highest LD was found at  $500 \text{ mg}\cdot\text{L}^{-1}$ , in combination with any dose of P-compound.

In spring of 2008, although not statistically significant, olive trees treated with larger concentrations of ethephon had lower indexes of bloom induction (Table 2).

## 4. Discussion

Spraying olive trees with low concentrations of ethephon, either alone or in combination with P-compounds, was sufficient to reduce the FRF of the 'Arbequina' olives. The efficiency of shaker-mechanical harvesting in this cultivar increased when ethephon spraying was applied.

The results obtained from this study into SE using a mechanical shaker produced higher dropped fruit values than previous trials conducted under similar conditions (Tous et al. 1995). Spraying reduced doses of ethephon in combination with P-fertilizers increased SE by almost 20%.



The LD observed in 2007 did not affect either the following blooming intensity or the following crop yield (2008).

Although MAP is richer in phosphorous than MKP, the treatments with MKP fertilizers showed a statistically greater reduction in FRF 13 days after spraying with ethephon. But this reduction in FRF did not improve the efficiency of mechanical harvesting regarding the other treatments with ethephon.

As Burns et al. (2008) observed, spraying MKP at 4% onto olive trees had no effect on FRF or on LD. Our results suggest that applying P-compounds either alone or in combination with ethephon as an olive fruit loosening agent has no effect in terms of improving shaking efficiency.

Despite the fact that many studies have already been done to investigate ways of improving fruit removal by mechanical harvesting with trunk shaker, more work is still required if we are to prevent the erratic results obtained when ethephon sprays are applied to mature 'Arbequina' olive trees. To the best of our results, no previous field trials have reported such large reductions in FRF associated with the application of these low concentrations of ethephon. Small applications of ethephon ( $300 \text{ mg}\cdot\text{L}^{-1}$ ) caused sufficient fruit loosening to improve shaking efficiency with minimum leaf drop in the 'Arbequina' cultivar growing in the environmental conditions of Catalonia (NE of Spain).

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### **Thème 4**

**Changement climatique, gestion de l'eau et développement durable  
de l'olivier**

## Effet de trois doses d'irrigation sur le statut hydrique, les échanges gazeux et la fluorescence chlorophyllienne de l' *Olea europaea* cv. Meski

Haouari Afef Amani Bchir

### Résumé

L'effet de trois doses d'irrigation sur le statut hydrique, les échanges gazeux et la fluorescence chlorophyllienne a été étudié sur des Oliviers de table variété Meski cultivés en plein champ dans la région d'Enfidha durant le mois d'Avril, Mai et Juin. Les arbres irrigués par la dose la plus réduite T1 (100% de flux de sève) montrent des valeurs plus faibles de paramètres caractérisant l'état hydrique de la plante ( $C.R.E$ ,  $\Psi_{Hb}$ ,  $\Psi_{HM}$  et  $\Psi_{HX}$ ) par comparaison aux arbres irrigués par la dose agriculteur T0 (témoin). Par contre, aucune différence n'a été observée entre les arbres témoins et ceux irrigués par la dose 60%  $ET_0$  (T2). Les traitements T0 et T2 les plus irrigués ont conduit à une amélioration des niveaux des échanges gazeux ( $A_{nette}$ ,  $g_s$  et  $E$ ) et de la fluorescence chlorophyllienne ( $F_v/F_m$ ,  $F_v/F_m$ ,  $J_T$  et  $qP$ ), alors que le traitement T1 montre les niveaux les plus faibles de ces paramètres.

**Mots clés:** *Olea europaea*; Olivier; statut hydrique foliaire, échanges gazeux, fluorescence chlorophyllienne.

## The effect of different irrigation regimes on leaf water status, gas exchange and chlorophyll fluorescence of *Olea europaea* (cv. Meski)

### Abstract

The effect of three doses of irrigation on water statute, gas exchange and chlorophyll fluorescence has been studied in Table Olive tree Meski variety grown in field in region d'Enfidha during three month (April, Mai and June). The trees irrigated by the smallest dose T1 (100% sap flow) shows lower values of water status parameters ( $CRE$ ,  $\Psi_{Hb}$ ,  $\Psi_{HM}$  and  $\Psi_{HX}$ ) compared to trees irrigated by the agriculture dose T0 (control). No difference was detected between control trees and trees irrigated by the dose 60%  $ET_0$  (T2). T0 and T2 the most irrigated treatments led to an improvement in gas exchange ( $A_{nette}$ ,  $g_s$  and  $E$ ) and chlorophyll fluorescence ( $F_v/F_m$ ,  $F_v/F_m$ ,  $J_T$  and  $qP$ ) parameters, while T1 treatment show the lowest levels of these parameters.

**Key words:** *Olea europaea*; olive tree; irrigation; gas exchange parameters; chlorophyll fluorescence

### 1. Introduction

Le sous secteur de l'Olivier de table en Tunisie a été considéré comme secondaire face à celui d'Olivier à huile. Les oliveraies à olives de table n'occupent actuellement que 25 000 ha (3,25 millions d'oliviers) dont 12 000 ha (C.O.I, 2007) sont conduits en régime irrigué.

Le rendement du secteur des olives de table reste encore assez faible et ne dépasse pas actuellement en moyenne 0,6T/ha. Ce rendement jugé très faible est dû en partie essentiellement à la mauvaise gestion des apports hydriques et nutritionnels.

L'analyse de la composition minérale des feuilles permet de dresser le statut nutritionnel de l'arbre. La teneur en éléments minéraux des feuilles est influencée par plusieurs facteurs y compris la fertilisation et l'irrigation

L'objectif principal de ce travail est de déterminer l'effet de trois régimes d'irrigation sur la nutrition minérale de la variété d'Olivier de table Meski.

## 2. Matériels et méthodes

### 2.1. Site expérimental

La parcelle d'étude est une oliveraie de table variété Meski, Picholine et Manzanille. Elle est située au périmètre irrigué de l'Office des Terres Domaniales d' Enfidha (OTD).

### 2.2. Matériel végétal

L'étude est réalisée sur une seule variété d'Olivier de table "Meski". Les Oliviers, objet de ce travail, sont âgés de 23 ans, ils sont issus de boutures herbacées, avec un écartement en carré de 7m x 7m.

### 2.3. Dispositif expérimental

Le dispositif expérimental retenu pour cet essai est Complètement Aléatoire (CA).  
Les traitements appliqués sont :

T0 : irrigation adoptée par l'agro-Combinat d'Enfidha (dose agriculteur).

T1: irrigation par 100% des apports hydriques estimés par le flux de sève xylémique.

T2: irrigation par 60% de l'évapotranspiration de référence ( $ET_0$ ).

### 2.4. L'analyse minérale

La méthode utilisée pour le dosage de l'azote total est celle de kjeldahl

$N (\%) = (V \times 0,1 \times 14) / (P \times 10)$  Avec:

N (%): teneur d'azote en % de la matière sèche;

V: volume de la solution de titrage (ml) ;

P : prise d'essai en gramme

Le dosage du phosphore est effectué par spectrophotométrie à 460 nm (Martin-Prével et al.1984).

$P\% = (Lecture \mu g P/ml \times 25 \times V) / (10^5 \times P)$

Avec : P = prise d'essai en gramme ;

V = volume de la solution déminéralisée.

Le dosage du potassium et du calcium est réalisé par photométrie à flamme;

$K \text{ et } Ca\%MS = (N \times D \times V) / (10^4 \times P)$  Avec P : poids de prise d'essai en g ;

N : la valeur trouvée en  $\mu g / ml$  ;

V : le volume de la solution des cendres en ml ;

D : la dilution de la solution à photométrie

### 2.5. Analyse statistique

Les analyses de variances sont effectuées grâce au logiciel statistique SPSS, version 16. Les moyennes ont été séparées par le test de Duncan au seuil de 5 %. Les résultats sont représentés par les moyennes d'au moins 3  $\pm$  les écarts types (SE, standard error).

## 3. Résultats et Discussion

### 3.1. Evolution des doses d'irrigation appliquées durant la période expérimentale

Durant la période expérimentale (Avril, Mai et Juin) la dose d'irrigation appliquée par l'agriculteur (T0) est de l'ordre de 474 L/arbre/semaine, celle estimée par la méthode climatique (60%  $ET_0$ ) et selon les données de flux de sève xylémique (100% de flux de sève) montre une variation au cours de la période de l'essai. (fig. 1).

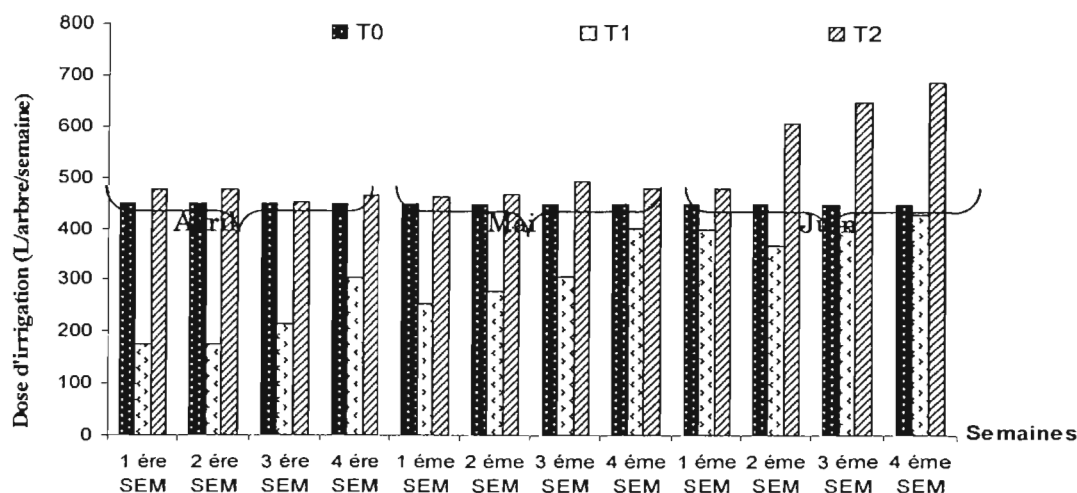


**Figure 1:** Evolution de doses d'irrigation appliquées (L/arbre/semaine) pour les trois traitements durant la période expérimentale (Avril- Mai - Juin).

## 3.2. Effet des doses d'irrigation sur le statut minéral foliaire

### 3.2.1. L'azote foliaire

Les teneurs en azote foliaire chez la variété Meski enregistrées durant la période expérimentale sont présentées par la figure 1.

Les valeurs les plus élevées en azote foliaire sont enregistrées durant le mois d'Avril, cette période coïncide généralement avec l'activité physiologique importante chez l'Olivier (phase de floraison).

Les valeurs les faibles en azote foliaire sont enregistrées durant le mois de Juin et sont de l'ordre de 1,63 ; 1,47 et 1,54%MS respectivement chez les traitements hydriques T0, T1 et T2.

L'analyse de la variance ne montre pas de différences significatives ( $P > 0,05$ ) entre les trois traitements. La dose d'irrigation n'a pas d'effet sur la teneur foliaire en azote.

L'évolution de la teneur en azote des feuilles chez la variété Meski pour les trois traitements semble être en relation avec les stades physiologiques de l'arbre. Par contre, Braham (1997) a montré chez l'Olivier var. Chemlali une relation étroite entre l'alimentation hydrique et la teneur foliaire en azote.

La diminution des taux de l'azote foliaire enregistrée durant la période expérimentale s'explique par la chute estivale de la teneur de feuilles des Oliviers en azote correspond à la période qui suit la sclérification du noyau, d'où une bonne partie des réserves en azote a été utilisée dans le processus du grossissement des fruits (Sebastiani et al., 2003).

D'après Garcia et al. (1992), la consommation en azote montre une augmentation suivant deux pics de forte demande, le premier en automne et la seconde au printemps.

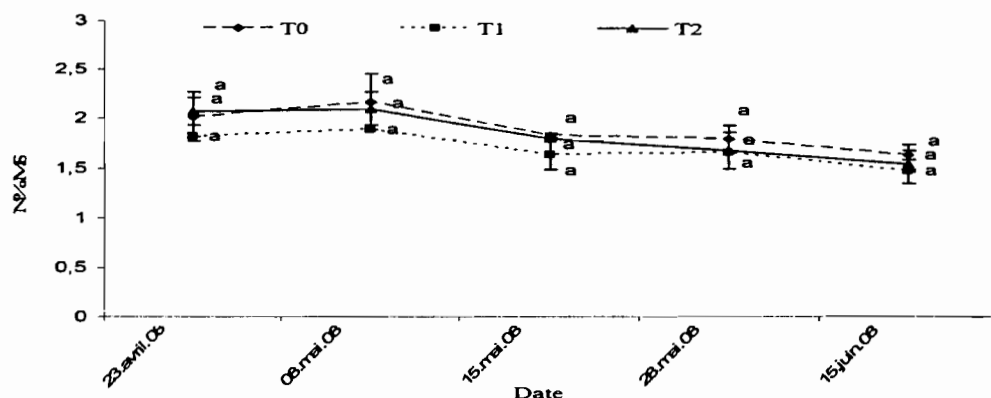


**Figure 2:** Evolution de la teneur foliaire en azote chez la variété Meski sous trois régimes hydriques. Les moyennes de chaque point de la courbe suivies par la même lettre, ne diffèrent pas statistiquement entre elles au seuil de 5%.

### 3.2.2. Le phosphore

L'évolution de la teneur foliaire en phosphore de l'Olivier var. Meski recevant trois régimes hydriques durant la période expérimentale (mois d'Avril, Mai et Juin) est très comparable à celle obtenue pour l'azote (Fig. 3).

Durant le mois d'Avril, les teneurs en phosphore enregistrées sont de 0,14; 0,12 et 0,13% MS respectivement chez les Oliviers irrigués par la dose agriculteur (T0), 100% de flux (T1) et 60% ET<sub>0</sub> (T2). Au cours du mois de Juin, ces teneurs deviennent 0,08 ; 0,07 et 0,08%MS respectivement pour les traitements T0, T1 et T2.

L'analyse de la variance montre que les traitements hydriques n'ont pas d'effet significatif sur la teneur foliaire en phosphore.

La diminution de la teneur foliaire en phosphore à partir du mois d'Avril jusqu'au mois du Juin correspond à la diminution caractérisant la période de floraison et de sclérification. Cette diminution est liée au besoin des fruits en phosphore (Fernandez-Escobar et al., 1999 ; Sebastiani et al., 2003).

La chute des teneurs en phosphore est accentuée par le manque d'eau durant la période estivale. En effet, Bouaziz (1995) signale que le minimum est d'autant plus bas que la dose d'eau est plus faible, ceci peut être expliqué par le fait que le phosphore est fortement lié à l'utilisation de l'amidon et des sucres ainsi que l'activité photosynthétique déployée pour la fixation du carbone (Loussert et Brousse, 1978).

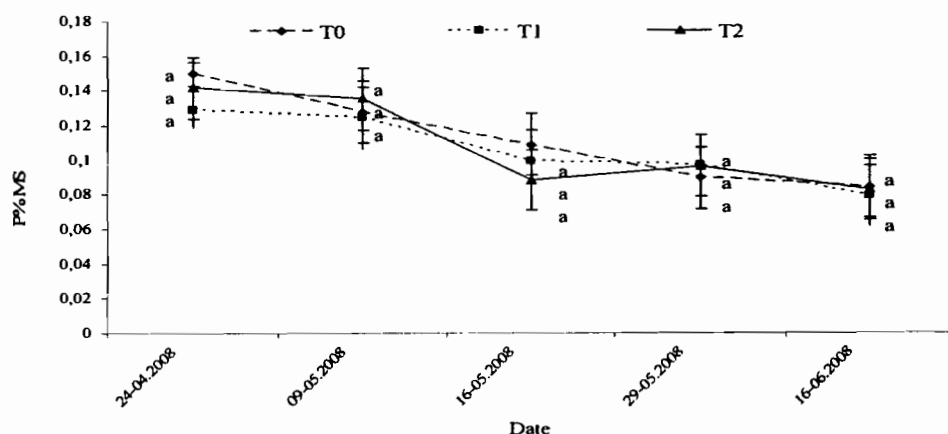


**Figure 3:** Evolution de la teneur en phosphore chez la variété Meski sous trois régimes hydriques. Les moyennes de chaque point de la courbe suivies par la même lettre, ne diffèrent pas statistiquement entre elles au seuil de 5%.

### 3.2.3. Le potassium

Les teneurs en potassium dans les feuilles de l'Olivier var. Meski pour les trois traitements (T0, T1 et T2) sont présentées par la Figure 4.

Les valeurs enregistrées de la teneur foliaire en potassium montrent une diminution régulière à partir du mois d'Avril (1,023 ; 1,01 et 1,088% MS respectivement pour T0, T1 et T2) jusqu'au mois de Juin (0,8; 0,76 et 0,84% MS respectivement pour T0, T1 et T2).

L'évolution des teneurs en potassium dans les feuilles au cours de l'expérimentation présente la même cinétique que celle trouvée pour l'azote.

Bouaziz (1995) a démontré que les taux du potassium augmentent avec la dose d'irrigation. Par contre, chez l'espèce « *Rosmarinus officinalis* » l'application de trois doses d'irrigation ne montre pas d'effet sur la teneur foliaire en cet élément (Sardans et al., 2005).

Les teneurs foliaires élevées en potassium durant la période de floraison est en relation directe avec une régulation convenable du métabolisme hydrique de l'olivier qui permettra un bon déroulement des réactions organo-minérales au cours de cette période critique du cycle biologique de l'Olivier (Braham, 1983).

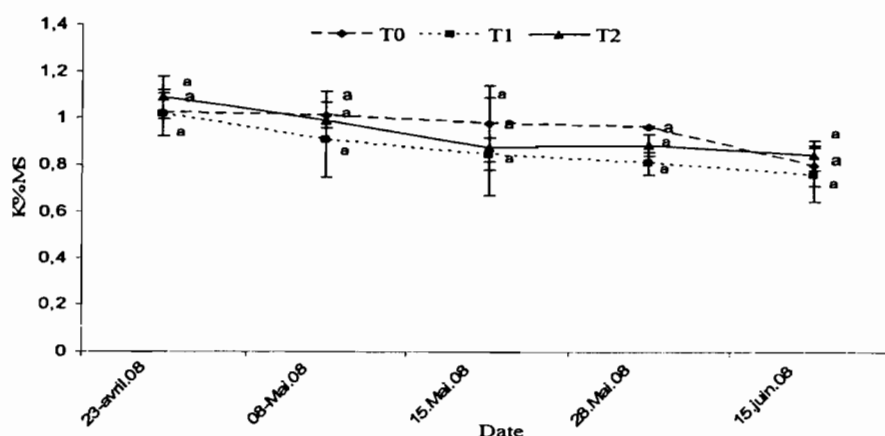


**Figure 4:** Evolution de la teneur en potassium chez la variété Meski sous trois régimes hydriques. Les moyennes de chaque point de la courbe suivies par la même lettre, ne diffèrent pas statistiquement entre elles au seuil de 5%.

### 3.2.4. Le calcium

La variation des teneurs en calcium enregistrée chez la variété Meski sous trois régimes hydriques est présentée par la figure 5.

Les valeurs minimales sont observées à la fin du mois d'Avril (1,18% ; 1,16% et 1,18%MS respectivement pour T0 ; T1 et T2) puis, ces teneurs augmentent régulièrement jusqu'au mois de Juin (2,20 ; 2 et 2,37 % MS respectivement pour T0 ; T1 et T2).

L'analyse de la variance au seuil de 5% ne montre pas des différences significatives entre les traitements hydriques.

L'évolution de la teneur en calcium durant la période expérimentale (Avril, Mai et Juin) est similaire à celle trouvée par Fernandez-Escobaret al. (1999) qui ont montré l'augmentation de taux du calcium dans les feuilles de l'olivier durant les phases de floraison et de nouaison.

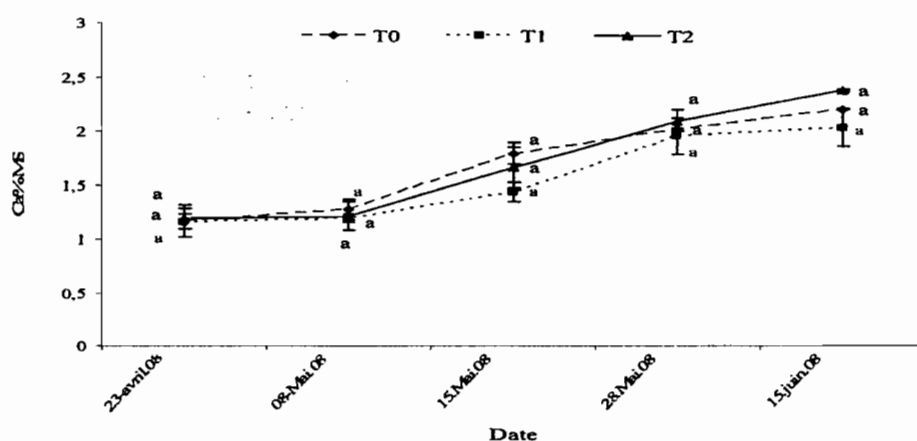


**Figure 5:** Evolution de la teneur en calcium chez la variété Meski sous trois régimes hydriques. Les moyennes de chaque point de la courbe suivies par la même lettre, ne diffèrent pas statistiquement entre elles au seuil de 5%.

## 4. Conclusion

L'étude de l'effet de trois doses d'irrigation sur le statut minéral chez l'Olivier de table cv. Meski conduit en intensif nous a permis de conclure que la variation de la concentration des principaux



éléments minéraux (azote, phosphore, potassium et calcium) dans les feuilles ne semble pas en relation avec les doses d'irrigation appliquées. L'évolution de la concentration foliaire en ces éléments semble être liée plutôt au cycle biologique de l'Olivier qu'aux apports hydriques.

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## Effet de trois doses d'irrigation sur le statut minéral de l'Olivier (*Olea europaea*) variété Meski

Haouari Afef Amani BCHIR

### Résumé

Le diagnostic foliaire est principalement utilisé pour suivre l'effet des engrais et tester l'effet des conditions extérieures sur la nutrition de l'arbre. L'olivier est sensible aux apports hydriques et ceci se reflète dans la composition de la feuille. L'effet de trois doses d'irrigation: la dose agriculteur T0 (témoin), T1 (100% de flux de sève) et 60% ET<sub>0</sub> (T2), sur la teneur foliaire en principaux éléments minéraux a été étudié sur des oliviers de table variété Meski cultivés en plein champ dans la région d'Enfidha. L'analyse de l'état nutritionnel de l'Olivier montre que les teneurs foliaires en azote(N), phosphore(P), potassium(K) et calcium(Ca) pour les trois traitements hydrique sont comparables avec des différences non significatives ( $P>0,05$ ).

**Mots clés:** *Olea europaea* ; Olivier ; irrigation ; diagnostic foliaire; nutrition minérale

## The effect of different irrigation regimes on nutritional status of *Olea europaea* (cv. Meski)

### Abstract

The foliar diagnosis has an utility mainly to follow the effect of fertilizers and to test the effect of external conditions on the tree nutrition. Olive tree is sensitive to the water conditions and this is reflected on the composition of the leaves. The effect of three doses of irrigation: the agriculture dose T0 (control), T1 (100% sap flow) and 60% ET<sub>0</sub> (T2), on the foliar content of principal mineral nutrients was studied on table olive cultivars 'Meski in olive orchard located in Enfidha. The nutritional status analysis of the Olive tree shows that the levels of Nitrogen (N), phosphorus (P), potassium (K) and calcium (Ca) for the three water treatments were similar with no significant differences ( $P>0.05$ ).

**Key Words:** *Olea europaea*; olive tree; irrigation; leaf analysis; mineral nutrition.

### 1. Introduction

La culture de l'Olivier occupe une place de choix sur le plan écologique, agronomique et sociologique, mais le secteur de l'Olivier de table en Tunisie a été considéré comme secondaire face à celui d'Olivier à huile. Ce sous secteur souffre d'une multitude de problèmes à savoir le mauvais choix variétale et une gestion des ressources hydriques et nutritionnelles à la parcelle marginaliser. L'amélioration de la productivité de ce secteur est liée aux plusieurs facteurs y compris la satisfaction de leurs exigences biotiques essentiellement hydrique, d'où l'orientation vers une gestion raisonnée des eaux d'irrigation disponibles permettant de combler le déficit pluvial, de réduire les pertes d'eau et surtout d'améliorer la productivité de ce secteur en assurant des rendements élevés et réguliers.

L'assimilation photosynthétique dans les cellules mésophylliennes au niveau des pigments chlorophylliens semble être le facteur primordial d'une part, affectée par les disponibilités hydriques et nutritionnelles, et d'autre part le facteur déterminant de la productivité photosynthétique et agrobiologique

L'objectif du présent travail est d'étude l'effet des différentes doses d'irrigation appliquées sur le comportement de l'Olivier « cv. Meski » dans le semi-aride (OTD de l'Enfidha) afin de déterminer les stratégies éco physiologiques développées par l'Olivier sous les conditions de restriction hydrique.

## **2. Matériels et méthodes**

### **2.1. Site expérimental et matériel végétal**

L'essai a été menée dans une parcelle d'Olivier de table, situer au périmètre irrigué des Terres Domaniales de Enfidha (OTD). L'étude est réalisée sur la variété Meski, les oliviers sont âgés de 23ans avec un écartement de 7m\*7m, la densité est de 207 arbre/ha.

### **2.2. Dispositif expérimental**

Le dispositif expérimental retenu pour cet essai est Complètement Aléatoire (CA).

Les traitements appliqués sont :

T0 : irrigation adoptée par l'agro-Combinat d'Enfidha (Témoin).

T1: irrigation par 100% des apports hydriques estimés par le flux de sève xylémique.

T2: irrigation par 60% de l'évapotranspiration de référence ( $ET_0$ ).

### **2.3. Paramètres mesurés**

#### **2.3.1. Le Contenu relatif en eau**

Le contenu relatif en eau (C.R.E) est calculé selon la technique de Barrs et Weatherley (1962) cité par Boujnah et al. (2004).

$$RWC = [(P_f - P_s) / (P_t - P_s)] \times 100 \text{ avec : } P_f : \text{ poids frais, } P_{sat} : \text{ poids saturé et } P_s : \text{ poids sec}$$

#### **2.3.2. Potentiel hydrique foliaire**

La détermination du potentiel hydrique foliaire se fait au moyen d'une chambre à pression (Modèle PMS 1000) (Scholander et al., 1965).

#### **2.3.3. Echanges gazeux foliaires**

La photosynthèse nette ( $A_{nette}$ ), la transpiration ( $E$ ) et la conductance stomatique ( $g_s$ ) sont mesurées sur des feuilles du côté Sud de trois arbres de chaque traitement entre 11 :00 h et 13 :00 h, à l'aide d'un analyseur à gaz infrarouge (IRGA) portable (LICOR-6400-O2 LED).

#### **2.3.4. Fluorescence chlorophyllienne**

La mesure de la fluorescence chlorophyllienne est réalisée par un fluorimètre licor-6400

### **2.4. Analyse statistique**

Les analyses de variances sont effectuées grâce au logiciel statistique SPSS, version 16. Les moyennes ont été séparées par le test de Duncan au seuil de 5 %. Les résultats sont représentés par les moyennes d'au moins 3  $\pm$  les écarts types (SE, standard error).

## **3. Résultats et Discussion**

### **3.1. Le contenu relatif en eau des feuilles (C.R.E)**

Chez les arbres irrigués par la dose 100% de flux de sève(T1), le contenu relatif en eau foliaire augmente progressivement de 71,82% (Avril) à 80,87% (Juin) avec un taux d'augmentation de 11,2 %. A la fin de l'essai, les traitements T0 (dose agriculteur) et T2 (60%  $ET_0$ ) montrent un taux d'augmentation plus important par rapport au traitement T1. Il est respectivement de l'ordre 19,25% et 19,8%.

L'effet positif de l'apport hydrique sur le contenu relatif en eau est récemment observé chez la variété d'olive à l'huile « Chamlali » (Ben Ahmed et al., 2007). Alors que, le manque d'eau dans le sol

induit le stress hydrique chez la plante et par la suite une réduction de ce paramètre qui diminue différemment selon le degré du stress (Braham, 1997 ; Galmés et al., 2007).

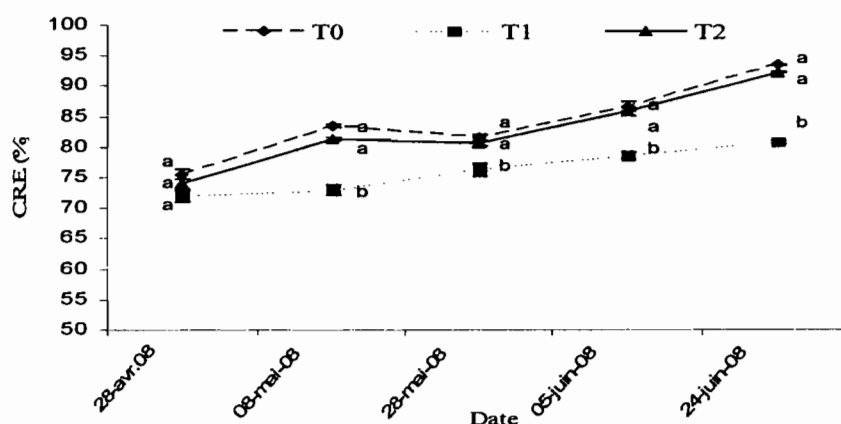


**Figure 1:** Evolution du contenu relatif en eau des feuilles chez la variété Meski sous trois régimes hydriques. Les moyennes de chaque point de la courbe suivies par la même lettre, ne diffèrent pas statistiquement entre elles au seuil de 5%.

### 3.2. Le potentiel hydrique foliaire

Durant la période expérimentale, Pour les trois traitements hydriques, le potentiel hydrique foliaire mesuré avant le lever du soleil (05 :00 h) chez la variété Meski, montre une augmentation régulière dès le début de l'essai vers la fin de la période expérimentale. Il passe de -0,55 à -0,27MPa chez T0, de -0,58 à -0,34MPa chez T1 et de -0,57 à -0,29MPa chez T2. Le traitement T1 présente le taux d'augmentation du potentiel hydrique le plus faible (41,4%). Ceux des traitements T0 et T2 sont respectivement de 50,8% et 49 % (Figure 2).

Les potentiels hydriques foliaires mesurés au midi solaire (13 :00 h) dès l'installation de l'essai chez les Oliviers irrigués par les doses agriculteur, 100% de flux de sève et 60% ET<sub>0</sub>, sont respectivement de -2,3 MPa ; -2,7 MPa et -2,2 MPa.

La variation du potentiel hydrique foliaire de base reflète l'évolution des réserves hydriques dans le sol (Tognetti et al., 2006). Dans ce sens, katerji et al. (1990) rapportent que ce potentiel est plus sensible au changement du régime hydrique que le potentiel minimum.

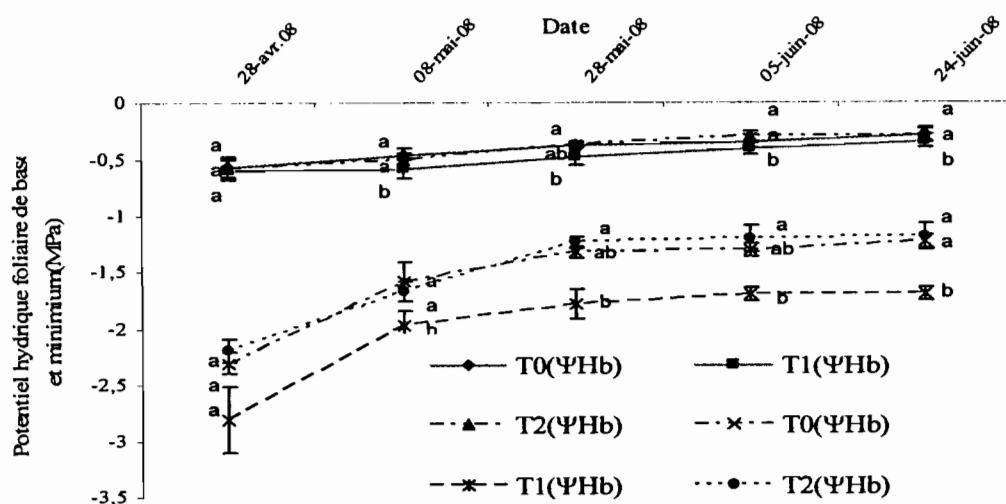


**Figure 2:** Evolution du potentiel hydrique foliaire de base et minimum chez la variété Meski sous trois régimes hydriques. Les moyennes de chaque point de la courbe suivies par la même lettre, ne diffèrent pas statistiquement entre elles au seuil de 5%.

### 3.3. Effet des doses d'irrigation sur les échanges gazeux

#### 3.3.1. La photosynthèse nette ( $A_{\text{nette}}$ )

Chez le traitement T1, tout au long de la période de mesure, la photosynthèse nette varie de 9 à  $12,66 \mu\text{mol m}^{-2}\text{s}^{-1}$ . Une amélioration plus importante de l'assimilation photosynthétique a été enregistrée chez les traitements les plus irrigués T0 et T2, leurs valeurs varient respectivement de 9 à  $17,75 \mu\text{mol m}^{-2}\text{s}^{-1}$  et de 9,7 à  $17,5 \mu\text{mol m}^{-2}\text{s}^{-1}$ .

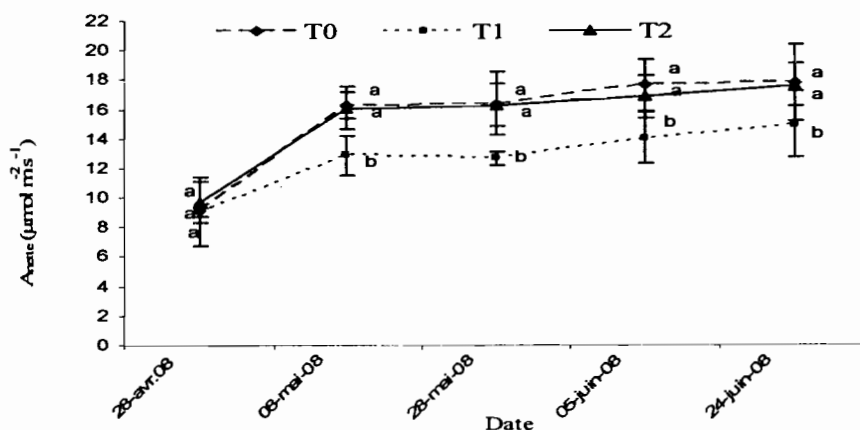


**Figure 3:** Evolution de la photosynthèse nette chez la variété Meski sous trois régimes hydriques.

Les moyennes de chaque point de la courbe suivies par la même lettre, ne diffèrent pas statistiquement entre elles au seuil de 5%.

#### 3.3.2. La conductance stomatique ( $g_s$ )

L'observation des résultats relatifs de la régulation des pertes hydriques par les stomates évaluées par la mesure de la conductance stomatique montre que les régimes d'irrigation T0 et T2 ont présenté l'effet le plus bénéfique sur la conductance stomatique.

Chez l'Olivier de table variété Meski et Picholine, Chehab et al. (2002) ont montré que la dose d'irrigation la plus élevée agit en augmentant la conductance stomatique. Mais, selon Cecelia Rousseaux et al. (2007), ce paramètre a été lié à d'autres facteurs que l'apport hydrique, comme le type de la culture et les conditions climatiques.

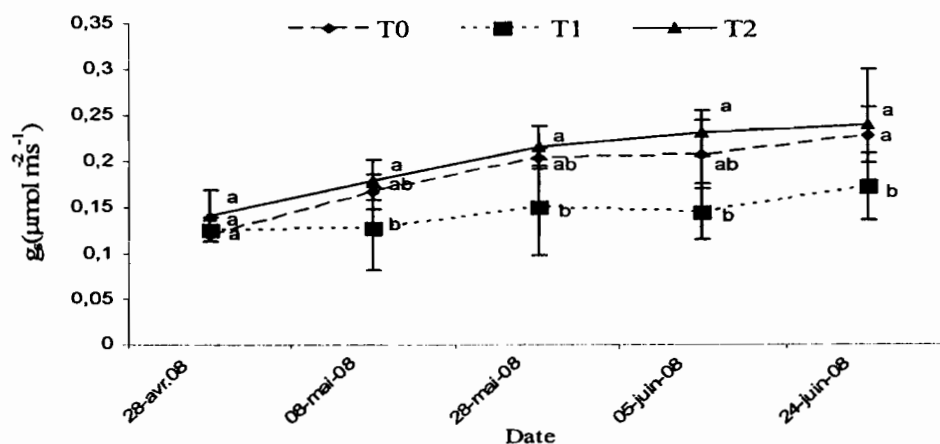


**Figure 3:** Evolution de la conductance stomatique chez la variété Meski sous trois régimes hydriques.

Les moyennes de chaque point de la courbe suivies par la même lettre, ne diffèrent pas statistiquement entre elles au seuil de 5%.

### 3.3.3. La transpiration (E)

Chez les Oliviers irrigués par 100% de flux de sève (T1), les valeurs de la transpiration enregistrées sont plus faibles par comparaison à celles des arbres irrigués par la dose agriculteur (T0). Ce paramètre varie de 2,89 à 6,3 mmol m<sup>-2</sup>s<sup>-1</sup> et de 3,47 à 5,31 mmol m<sup>-2</sup>s<sup>-1</sup> respectivement chez les traitements T0 et T1 à partir du mois d'Avril jusqu'à la fin du mois de Juin. Chez les arbres irrigués par 60% ET<sub>0</sub>, les valeurs de E sont comparables à celles des arbres irrigués par la dose agriculteur. Elles varient de 3,65 à 6,1 mmol m<sup>-2</sup>s<sup>-1</sup>.

Les résultats de la transpiration obtenus en réponse aux traitements hydriques appliqués montrent que ce paramètre augmente avec l'accroissement des doses d'eau appliquées.

Ces résultats sont similaires à ceux rapportés par Cecelia Rousseaux et al. (2007), par Chehab et al. (2002) et par Boujnah (1997), qui ont noté que l'apport d'eau d'irrigation augmente la transpiration chez l'Olivier.

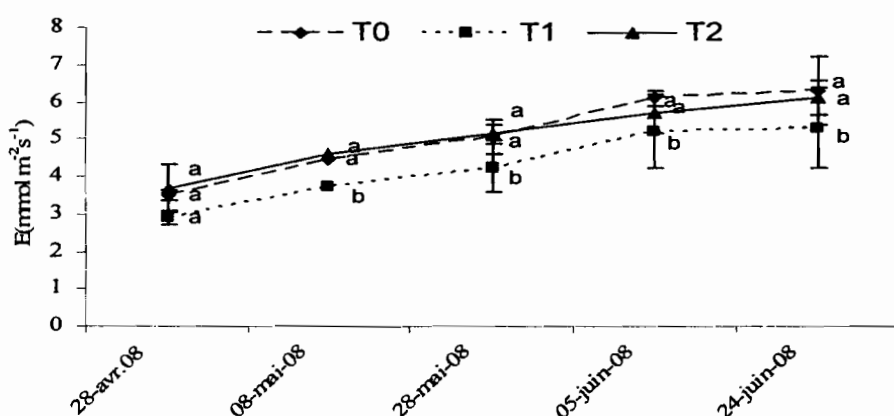


**Figure 4:** Evolution de la transpiration chez la variété Meski sous trois régimes hydriques.

Les moyennes de chaque point de la courbe suivies par la même lettre, ne diffèrent pas statistiquement entre elles au seuil de 5%.

**Tableau :** Comparaison des paramètres de la fluorescence chlorophyllienne chez la variété Meski sous différents régimes hydriques (T0, T1, T2) avec F<sub>v</sub>/F<sub>m</sub> : rendement quantique du PSII, F<sub>v</sub>/F<sub>m</sub> : rendement max du PSII, qP : quenching photochimique, J<sub>T</sub> : taux de transfert des électrons.

| Traitements | F <sub>v</sub> /F <sub>m</sub> | F <sub>v</sub> /F <sub>m</sub> | qP (μmol m <sup>-2</sup> s <sup>-1</sup> ) | J <sub>T</sub>           |
|-------------|--------------------------------|--------------------------------|--------------------------------------------|--------------------------|
| 23/04/2008  | T0 0,341 ±0,02 <sup>a</sup>    | 0,754±0,013 <sup>a</sup>       | 0,43±0,01 <sup>ab</sup>                    | 59,953±0,08 <sup>a</sup> |
|             | T1 0,30±0,01 <sup>b</sup>      | 0,706±0,016 <sup>b</sup>       | 0,45±0,003 <sup>a</sup>                    | 51±0,98 <sup>b</sup>     |
|             | T2 0,363±0,025 <sup>a</sup>    | 0,75±0,037 <sup>a</sup>        | 0,396±0,008 <sup>b</sup>                   | 60,418±0,12 <sup>a</sup> |
| 15/05/2008  | T0 0,366±0,02 <sup>ab</sup>    | 0,787±0,05 <sup>a</sup>        | 0,319±0,015 <sup>b</sup>                   | 64,729±0,45 <sup>a</sup> |
|             | T1 0,336±0,01 <sup>b</sup>     | 0,718±0,03 <sup>b</sup>        | 0,36±0,03 <sup>a</sup>                     | 57±0,6 <sup>a</sup>      |
|             | T2 0,378±0,02 <sup>a</sup>     | 0,771±0,018 <sup>a</sup>       | 0,29±0,02 <sup>b</sup>                     | 66±0,765 <sup>b</sup>    |
| 11/06/2008  | T0 0,462±0,017 <sup>a</sup>    | 0,793±0,05 <sup>a</sup>        | 0,28±0,01 <sup>b</sup>                     | 79±0,34 <sup>a</sup>     |
|             | T1 0,342±0,005 <sup>b</sup>    | 0,746±0,05 <sup>b</sup>        | 0,33±0,001 <sup>a</sup>                    | 62,701±0,64 <sup>b</sup> |
|             | T2 0,445±0,01 <sup>a</sup>     | 0,786±0,018 <sup>a</sup>       | 0,27±0,01 <sup>b</sup>                     | 75,379±0,56 <sup>a</sup> |

Les moyennes de la même colonne suivies par la même lettre, ne diffèrent pas statistiquement entre elles au seuil de 5%.

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## Effets de l'irrigation à l'eau saline sur la composition chimique et la qualité de l'huile de l'olivier Cv. Chemlali de plein champ

Ben Ahmed Chedlia B. Ben Rouina, S. Sensoy, M. Boukhris & F. Ben Abdallah

### Résumé

D'une culture traditionnellement pluviale, l'oléiculture irriguée connaît ces dernières décennies une extension notoire, malgré l'insuffisance des quantités d'eaux de qualité, sous les climats semi aride et aride de la Tunisie. Cette extension des oliveraies irriguées est basée essentiellement sur l'utilisation des ressources non conventionnelles d'eaux telles que celles salines. Au cours de cette étude réalisée durant deux saisons (2004 / 2006), le développement des fruits, la production d'olives et la qualité de l'huile produite sont examinés. Deux qualités d'eau sont utilisées pour l'irrigation de l'olivier Chemlali, âgés de 14 ans : FW désigne la parcelle irriguée à l'eau douce ( $C. E = 1,2 \text{ dS / m}$ ) ; alors que SS correspond à celle irriguée à l'eau saline ( $C. E = 7,5 \text{ dS / m}$ ). L'irrigation à l'eau saline a induit une régression du poids moyen d'une olive, de la charge totale de l'arbre en fruits et de la teneur en huile. Les productions moyennes d'olives ont enregistré une régression de 36 % pour passer de 42,5 Kg / arbre / an, chez le traitement FW à seulement 27 Kg / arbre / an chez celui SS. Pour ces deux traitements, le contenu des fruits en huile a respectivement baissé de d'une moyenne de 27,85 % à 25,7 %. Cependant, la concentration des composés phénoliques majeurs (tyrosol, hydroxytyrosol, acide vanillique, euleropeine, acide syringique et o-coumarique) ainsi que celle des phénols totaux se trouvent améliorées par l'irrigation à l'eau saline. Durant la saison 2005/2006, la concentration des phénols totaux dans l'huile de la parcelle SS s'est accrue de 11 % ; puisque égale à 223 mg / Kg en comparaison à 198 mg / Kg chez le témoin (FW). De surcroît, l'huile produite par les arbres SS présente des teneurs en acides oléique, linoléique et heptadécanoïque plus élevées en comparaison à celle des arbres FW. L'huile produite par les arbres des deux traitements est classée dans la catégorie « extra vierge ».

**Mots clés:** irrigation, qualité d'huile, composés phénoliques, eau saline, production d'olives.

## Saline water irrigation effects on oil composition and quality of field grown olive cv. Chemlali

### Abstract

Traditionally, olive was cultivated under rain-fed conditions. In recent decades, the olive plantation has been extended to irrigated lands. However, in arid and semi arid regions as those in Tunisia, the limited water availability and the increased need for good water quality for urban and industrial sector use have led to the use of large quantities of marginal water, as saline water, for olive tree irrigation. Fruit development characteristics, yield and oil quality were investigated during two successive crop seasons (2004 / 2006) on fourteen - year - old -Chemlali Sfax olive trees subjected to the following treatments: FW and SS corresponding, respectively to the irrigation with fresh water ( $EC = 1.2 \text{ dS m}^{-1}$ ) and saline water ( $EC = 7.5 \text{ dS m}^{-1}$ ). The irrigation with saline water has led to the decrease of fruit weight, olive yield and oil content. The mean olive yield was of 42.5 and 27 Kg /tree /year, respectively in FW and SS treated plants with a relative reduction of 36% in salt stressed plants. The oil content was of 27.85 and 25.7 % Fw during 2005/2006 crop season in FW and SS irrigated plants, respectively. However, major phenolic compounds (tyrosol, hydroxytyrosol, vanillic, oleuropein, syringic, o-coumaric) and total phenols have increased under saline water irrigation regime. During 2005/2006 crop season, total phenol content was of 198 and 223 mg /Kg in FW and SS treatments, respectively with an increment of 11% under greater salinity conditions. Furthermore, SS treated plants showed higher contents of oleic, linoleic, linolenic and heptadecanoic acids than FW ones. The oil samples of both treatments were classified as "extra virgin". Despite the fact of the decrease of fruit weight and oil content in SS plants, the improvement of their phenolic compounds and total phenols contents assumes that the use of such water quality for olive cultivation could be enhanced, at least in the case of the Chemlali cultivar grown under described experimental conditions.

**Key words:** Irrigation, oil quality, phenolic compounds, saline water, olive yield.



## 1. Introduction

Traditionally, olive tree was cultivated under rain-fed conditions. In recent decades, the olive plantation has been extended to irrigated lands. However, in arid and semi arid regions as those in Tunisia, the limited water availability and the increased need for good water quality for urban use restrict the use of fresh water for irrigation. So, large quantities of marginal water, as saline water, are used for olive tree irrigation (33% of irrigated lands are saline water irrigated (16000 ha)).

Most papers dealing with the assessment of olive water needs reported that olive tree is characterized by its limited water requirements and its tolerance to salinity [1, 2, 3]. Existing data on the effects of salinity conditions on yield, quality and phenolic composition of virgin olive oil are few and sometimes contradictory. In 1994, [4] have signalled that salinity alters pollen germination and fruit set, and that it causes the increase of both aliphatic and triterpenic alcohol contents in oil and the percentage of linoleic acid. According to [5], the olive irrigation with saline water ( $EC_e = 6.5 \text{ dSm}^{-1}$ ) increased the fruit dry weight and oil percentage. The same authors stated that no effects were recorded when irrigation was made with water less than  $4.5 \text{ dSm}^{-1}$ . Similarly, [6] did not record any effect of irrigation with brackish water (up to  $4 \text{ g/l}$  of solid residue) on yield and oil percentage of some olive varieties grown intensively in the central part of Tunisia.

Besides, it has been shown that olive yield response to salinity is planting density dependent [8]. The effects of salt treatment at  $EC_e$  of  $4.5 \text{ dSm}^{-1}$  were a 12% increase in oil yield for the 830 trees/ha planting density, and an 18% decrease for the 410 trees/ha one. However, at high salinity ( $EC_e = 7.5 \text{ dSm}^{-1}$ ), the decrease of oil yield was at 89 and 74% of the control in the high and low planting density, respectively. Concerning the oil quality, many studies [8, 9, 10, 11] showed that olive oil produced under high water salinity level has higher amounts of total phenols. In addition, the ratio of unsaturated/saturated fatty acids decreased significantly at moderate and high salinity levels.

Nowadays, the controlled use of marginal water (saline water, treated waste water) to improve the qualitative characteristics of horticultural products is becoming more and more important [8, 9, 10, 11], particularly under actual conditions of limited water resources and rainfall scarcity in arid regions.

The objectives of this study were to determine the effects of saline water used for irrigation on fruit development and the quantitative and qualitative parameters of VOO obtained from trees of the cv. Chemlali grown at a high density orchard. In particular, we are interested in the fatty acid composition and concentrations of phenols of VOO and the evolution of soil moisture and salinity with soil depth and around the irrigation source. Our experimental approach allows us to improve the understanding of the qualitative response (oil quality) of field-grown Chemlali olive tree to saline water irrigation in arid region in Tunisia.

## 2. Materials and Methods

### 2.1. Plant Material and Treatments

Olive trees (*Olea europaea* L. cv. Chemlali), planted in 1992 in a sandy soil at a density of 625 trees  $\text{ha}^{-1}$  ( $4 \times 4 \text{ m}$  layout) at Sfax, Tunisia ( $34^{\circ}43\text{N}$ ,  $10^{\circ}41\text{E}$ ), were used in 2004 and 2005. The sandy soil of the experimental orchard (90.5% sand, 4.5% clay and 5% silt) was characterized by an organic matter of 1.1%, 13.4%  $\text{CaCO}_3$ , 1.3% N, pH of 7.6, a field capacity (measured at 33 KPa) of 11.8% and a wilting point (measured at 1500 KPa) of 5.9%.

In 2004, twenty trees with four replications of 5 trees each were selected to be similar in potential yield and canopy. The Chemlali olive trees were subjected to the following treatments: irrigation with fresh water,  $1.2 \text{ dS m}^{-1}$   $EC_e$  (FW); and saline water,  $7.5 \text{ dS m}^{-1}$   $EC_e$  (salt stress, SS). The water used was either that supplied by the Tunisian National Water Carrier (FW), or saline water (SS) from the local reservoir situated in the area of the Olive Tree Institute in Sfax. The fresh and saline water used with amount of  $4000 \text{ m}^3 / \text{ha} / \text{year}$  were characterized by 145 and  $600 \text{ mg/l Na}^+$ , 326 and  $1169 \text{ mg/l Cl}^-$ , 280 and  $520 \text{ mg/l K}^+$ , 94 and  $261 \text{ mg/l Ca}^{2+}$ , 57 and  $102 \text{ mg/l Mg}^{2+}$ , respectively [1]. The irrigation was delivered using a drip system with 4 drip nozzles (two per side), of  $4 \text{ l h}^{-1}$  per tree set in a line along the rows (at 0.5 m from the trunk). The plants were subjected to the same olive cultivation practices in the area.

## 2.2. Fruit Growth Characteristics and Yield

Control of fruit weight (FrW), fruit diameter (FD), fruit volume (FV) and fruit water content (FWC) of harvested olives were made, during both crop seasons, four times per month from June to December. In every measurement, 60 olives from four plants per treatment (15 olives per plant) were collected for characterization (fresh weight, water content, fruit diameter and volume). For the oil analyses, three samples of 4 Kg of fruits each were harvested for each treatment at maturation. For olive yield determination, 10 trees per treatment were chosen and the harvest was made in mid-December of each year manually to guarantee accuracy.

## 2.3. Oil Quality Indices

Extinction coefficients  $K_{232}$  and  $K_{270}$  were measured at 232 and 270 nm, respectively. Free acidity and peroxide value expressed as milliequivalents of active oxygen per kilogram of oil (meq  $O_2$  / kg) were measured following the analytical method described in the European Regulation EEC 2568/91 [12]. Fatty acid composition was determined based on the European Regulations EEC 2586/91 method. The chlorophyll fraction at 670 nm and the carotenoid fraction at 470 nm were evaluated from the absorption spectrum of each virgin olive oil sample (7.5 g) dissolved in cyclohexane. Oxidative stability is evaluated using a 679 Rancimat apparatus (Metrohm, Switzerland) at 120 °C and 20  $lh^{-1}$  air flow. The concentration of total polyphenols was estimated with Folin-Ciocalteu reagent at 725 nm. The different phenolic compounds analysed were determined by HPLC system. The standards used in the quantification of phenolic substances are: Tyrosol, hydroxytyrosol, vanillic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid, o-coumaric acid, oleuropein, glycoside oleuropein and ferulic acid.

## 2.4. Statistical Analysis

Statistical analyses were performed using the SPSS 10. Windows and treatment means were compared using least significant difference (LSD) test at  $p < 0.05$ . At least three replicates were used for each laboratory test.

## 3. Results and discussions

### 3.1. Soil Salinity

The soil salinity variation was greater in soil irrigated with saline water than in that irrigated with fresh water. For both treatments, there is a slight decrease in E<sub>Ce</sub> through the soil depth. Furthermore, the higher salt accumulation was registered at soil layer of 0-0.3 m. In all soil depths, the salts were more accumulated during summer season (July) and salt distribution through the soil depth was affected by autumn-winter rainfall leaching (December). The seasonal variation of soil salinity in the 1.2 m depth showed that it was lower during autumn-spring period than that of the summer one (Table 1).

**Table 1:** Distribution of soil salinity (E. C dS  $m^{-1}$ ) measured at 0.6 m from nozzle at Different Layers in FW and SS treatments during 2004 and 2005 crop Seasons.

| Treatmt<br>Period            | FW   |      |      |      | SS  |     |     |     |
|------------------------------|------|------|------|------|-----|-----|-----|-----|
|                              | H1   | H2   | H3   | H4   | H1  | H2  | H3  | H4  |
| Soil salinity (dS $m^{-1}$ ) |      |      |      |      |     |     |     |     |
| July 05                      | 1.9  | 1.75 | 1.52 | 1.4  | 7.7 | 6.9 | 6.5 | 6.1 |
| December05                   | 1.30 | 1.25 | 1.23 | 1.10 | 6.7 | 6.0 | 5.8 | 5.5 |

H1, H2, H3 and H4 represent the different soil depths from the surface (30, 60, 90 and 120 cm, respectively). Values are the means of three soil samples measurements ( $n = 3$ ).

### 3.2. Fruit Characteristics and Yield

During both crop seasons, salinity has altered fruit diameter (FD), fruit volume (FV) and fruit weight (FrW). For both salt treatments, FrW averages increased markedly with time. In June 2004, their values were of 0.38 and 0.28 g in FW and SS treated plants, respectively; and they reached 1.33 and 1.05 g, respectively in December. In 2005, these values were of 0.44 and 0.36 g in FW and SS treated plants and reached 1.38 and 1.22 g, respectively for the respective periods. These results showed that

the increase was more important under irrigation with fresh water. Furthermore, the fruit fresh weight in SS plants was statistically lower than that in FW ones ( $p = 0.0056$ ). In 2004, FW plants showed higher fruit water content values than the SS ones (53.39 and 50.88% in FW and SS, respectively). However, in 2005, the two irrigated treatments showed almost similar values (51.43 and 52.27%, respectively) for which differences were not statistically significant. The non significant differences in FWC values between the two crop seasons for both treatments revealed the role of the active root zone of Chemlali olive in the upholding of a suitable hydration level for its tissues.

**Table 2:** Olive Yield ( $\text{Kg tree}^{-1}$ ) of olive trees (cv. Chemlali) grown under FW and SS irrigation.

| treatment | FW                         | SS                         | Relative reduction in SS (%) |
|-----------|----------------------------|----------------------------|------------------------------|
| 2004      | $38 \pm 2.9^{\text{a, w}}$ | $22 \pm 2.2^{\text{b, w}}$ | -42,1                        |
| 2005      | $14 \pm 2.5^{\text{a, x}}$ | $9 \pm 2.8^{\text{b, x}}$  | -35                          |
| Mean      | $27^{\text{a}}$            | $15.5^{\text{b}}$          | -42.6                        |

Values represent the means of ten samples  $\pm$  standard deviations. Different letters (a, b) indicate significant differences ( $P < 0.05$ ) between saline water irrigation treatments within each year. Different letters (w, x) indicate significant differences ( $P < 0.05$ ) between crop seasons within each treatment.

The average olive production of SS plants during the experimental period ( $15.5 \text{ kg tree}^{-1}$ ) was much lower (42%) than that of FW ones ( $27 \text{ kg tree}^{-1}$ ). For both treatments, the first crop season was marked by higher olive yield (Table 2). The yield variation in both treatments could be due to the alternate bearing phenomenon characterizing the olive tree production and/or to the effects of climatic conditions characterizing the experimental site.

### 3.3. Oil Quality Indices

During both crop seasons, the irrigation treatments did not affect the oil accumulation in the Chemlali olive tree as no statistically significant differences were observed between total oil content of the two treatments ( $p > 0.05$ ). It was of 27.8 and 30.5 % Fw in FW and of 25.7 and 28.3 % Fw in SS during 2004 and 2005 crop seasons, respectively (Table 3).

**Table 3:** Total oil content, Free Acidity, Peroxide Value, Extinctions Coefficients of total chlorophyll and carotenoids contents of Virgin Olive Oils from Olive Trees (Cv. Chemlali) grown under FW and SS Irrigation.

| Treat-ment | Total oil content (% Fw) | Free acidity (%) | Peroxide value (meq $\text{O}_2$ / kg) | $K_{232}$       | $K_{270}$        | Total chlorophyll (mg / kg) | Carotenoid s (mg / kg) |
|------------|--------------------------|------------------|----------------------------------------|-----------------|------------------|-----------------------------|------------------------|
| 2004       |                          |                  |                                        |                 |                  |                             |                        |
| FW         | $27.85 \pm 2.6$          | $0.25 \pm 0.02$  | $3.2 \pm 0.31$                         | $1.06 \pm 0.02$ | $0.05 \pm 0.01$  | $9.5 \pm 0.07$              | $0.38 \pm 0.07$        |
| SS         | $25.7 \pm 2.05$          | $0.24 \pm 0.02$  | $2.9 \pm 0.12$                         | $1.05 \pm 0.04$ | $0.05 \pm 0.02$  | $9.2 \pm 0.09$              | $0.41 \pm 0.04$        |
| 2005       |                          |                  |                                        |                 |                  |                             |                        |
| FW         | $30.56 \pm 3.0$          | $0.34 \pm 0.04$  | $4.6 \pm 0.34$                         | $1.62 \pm 0.06$ | $0.11 \pm 0.02$  | $10.19 \pm 0.05$            | $0.42 \pm 0.06$        |
| SS         | $28.32 \pm 2.2$          | $0.32 \pm 0.05$  | $4.4 \pm 0.28$                         | $1.76 \pm 0.06$ | $0.16 \pm 0.018$ | $10.22 \pm 0.04$            | $0.44 \pm 0.075$       |

Values are the means of three different VOO samples ( $n = 3$ )  $\pm$  standard deviations.

In contrast with previous reports [5, 7, 8, 9, 10, 11], the high saline treatment SS we applied to Chemlali olive tended to decrease oil content relative to FW treated plants (though not significantly so). Furthermore, the non statistically significant differences in oil content between both saline water treatments, in comparison to olive yield, is certainly an advantage for the use of saline water for Chemlali olive cultivation, being extended to saline irrigated lands in arid regions in Tunisia. Chlorophyll concentrations in the virgin oils ranged from 9.5 to 10.2 and from 9.2 to 10.2 mg / kg in FW and SS, respectively (Table 3). Differences between the treatments were not significant ( $p > 0.05$ ) in either season. As well for the chlorophylls contents, during both crop seasons, the carotenoid contents were not influenced by saline irrigation regimes.

During 2004 crop season, the free acidity ranging from 0.24 to 0.25 % and peroxide value, ranging from 2.9 to 3.2 meq O<sub>2</sub> kg<sup>-1</sup> of the different olive oils samples, respectively in SS and FW, were considerably lower than the upper limit of 0.8 % as oleic acid and 20 meq O<sub>2</sub> kg<sup>-1</sup> as the peroxide value established by the EU legislation for extra virgin olive oil. Moreover, these two quality indices were not influenced by water quality treatment, since no statistically significant differences were observed between both treatments ( $p > 0.05$ ). In the second crop season, the free acidity and peroxide value have increased in both treatments, if compared to those recorded during the first one (Table 4). The free fatty acid level of both olive oil samples we analysed was lower than those recorded in Barnea olive oil subjected to similar salinity treatments [7], and differences between FW and SS treated plants were not significant.

**Table 4:** Fatty Acid Composition (%) of Virgin Olive Oils from Olive Trees (Cv. Chemlali) Grown Under Fresh Water Irrigation (FW) and Saline Water Irrigation (SS) in 2004 and 2005.

| Fatty Acid                | 2004                      |                           | 2005                      |                           |
|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
|                           | FW                        | SS                        | FW                        | SS                        |
| palmitic acid             | 19.82±0.25 <sup>a,w</sup> | 16.1±0.35 <sup>b,w</sup>  | 16.7±0.32 <sup>a,x</sup>  | 15.51±0.42 <sup>b,w</sup> |
| palmitoleic acid          | 2.57±0.15 <sup>a,w</sup>  | 2.1±0.17 <sup>a,w</sup>   | 1.76±0.12 <sup>a,x</sup>  | 1.65±0.11 <sup>a,x</sup>  |
| heptadecanoic acid        | 0.12±0.01 <sup>a,w</sup>  | 0.11±0.02 <sup>a,w</sup>  | 0.14±0.03 <sup>a,w</sup>  | 0.13±0.03 <sup>a,w</sup>  |
| heptadecenoic acid        | 0.24±0.02 <sup>a,w</sup>  | 0.22±0.03 <sup>a,w</sup>  | 0.27±0.05 <sup>a,w</sup>  | 0.26±0.08 <sup>a,w</sup>  |
| stearic acid              | 2.19±0.015 <sup>a,w</sup> | 2.01±0.016 <sup>a,w</sup> | 2.83±0.016 <sup>a,x</sup> | 2.25±0.025 <sup>a,x</sup> |
| oleic acid                | 55.58±1.05 <sup>a,w</sup> | 59.3±2.03 <sup>b,w</sup>  | 60.73±2.45 <sup>a,x</sup> | 64.59±2.56 <sup>b,x</sup> |
| linoleic acid             | 16.14±0.65 <sup>a,w</sup> | 17.96±0.52 <sup>a,w</sup> | 16.52±1.01 <sup>a,w</sup> | 17.26±0.96 <sup>a,w</sup> |
| linolenic eicosanoic acid | 0.56±0.08 <sup>a,w</sup>  | 0.63±0.078 <sup>b,w</sup> | 0.56±0.08 <sup>a,w</sup>  | 0.55±0.09 <sup>a,x</sup>  |
| eicosanoic acid           | 0.34±0.03 <sup>a,w</sup>  | 0.41±0.08 <sup>b,w</sup>  | 0.4±0.06 <sup>a,x</sup>   | 0.43±0.07 <sup>a,w</sup>  |
| eicosenoic acid           | 0.16±0.02 <sup>a,w</sup>  | 0.14±0.04 <sup>a,w</sup>  | 0.18±0.05 <sup>a,w</sup>  | 0.16±0.07 <sup>a,w</sup>  |
| Uns/ Sat ratio            | 3.34 <sup>a,w</sup>       | 4.31 <sup>b,w</sup>       | 3.98 <sup>a,w</sup>       | 4.61 <sup>b,w</sup>       |
| Mono/ Poly ratio          | 3.5 <sup>a,w</sup>        | 3.32 <sup>a,w</sup>       | 3.68 <sup>a,w</sup>       | 3.74 <sup>a,w</sup>       |

Values are the means of three different VOO samples ( $n = 3$ ) ± standard deviations. Different letters (a, b) indicate significant differences ( $P < 0.05$ ) between saline water irrigation treatments within each year. Different letters (w, x) indicate significant differences ( $P < 0.05$ ) between crop seasons within each treatment.

The comparison of spectrophotometric absorption characteristics in the UV region at 232 and 270 nm between oils from the two saline irrigated treatments did not show significant differences ( $p = 0.433$ ). Taking into account the values of free acidity, peroxide value and  $K_{232}$  and  $K_{270}$ , the oil samples obtained from both treatments met the European Union requirements for the extra virgin olive oil category. The distribution of fatty acid composition of the oil samples of both saline water treatments covers the normal range expected for virgin olive oil (Table 4). For both treatments, the most abundant acid was the oleic one with values recorded in oil obtained from SS treated plants were statistically higher ( $p = 0.0024$ ) than those in oil of FW ones. However, the oil obtained from high saline water irrigated plants would be nutritionally better than that obtained in the case of fresh water irrigated ones.

Table 5 reports the concentrations of the major phenolic, total phenols and oxidative stability of VOO samples in both treatments. Total phenols contents of VOO were significantly influenced by the salinity treatments. During 2004 crop season, the total phenols contents were of 181 and 214 mg / kg, respectively in FW and SS treatments. During the second crop, these values reached 198 and 223 mg / kg, respectively. The use of saline water at 7.5 dS m<sup>-1</sup> has reinforced the phenols accumulation and did not altered the oil quality. The three phenolic compounds in highest concentrations in both oil samples are hydroxytyrosol, tyrosol and glycoside oleuropein.

**Table 5:** Phenolic Composition Concentrations (mg / Kg), Total Phenols Contents (mg/kg of oil) and Oxidative Stability (h) of Olive Oils from Olive Trees (Cv. Chemlali) Grown Under Fresh Water Irrigation (FW) and Saline Water Irrigation (SS) in 2004 and 2005.

| Phénols                 | 2004                          |                               | 2005                          |                               |
|-------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
|                         | FW                            | SS                            | FW                            | SS                            |
| Tyrosol                 | 42.5 ± 2.36 <sup>a,w</sup>    | 66.4 ± 3.21 <sup>b,w</sup>    | 45 ± 2.56 <sup>a,w</sup>      | 72.5 ± 2.36 <sup>b,x</sup>    |
| Hydroxytyrosol          | 84.5 ± 3.56 <sup>a,w</sup>    | 96.7 ± 2.06 <sup>b,w</sup>    | 88.5 ± 2.47 <sup>a,w</sup>    | 105.4 ± 3.56 <sup>b,x</sup>   |
| Oleuropein              | 10.5 ± 2.84 <sup>a,w</sup>    | 12.7 ± 2.56 <sup>a,w</sup>    | 12.5 ± 3.3 <sup>a,w</sup>     | 16.4 ± 2.84 <sup>b,x</sup>    |
| glycoside oleuropein    | 23.4 ± 2.56 <sup>a,w</sup>    | 34.6 ± 2.47 <sup>b,w</sup>    | 25.5 ± 2.5 <sup>a,w</sup>     | 37.1 ± 2.56 <sup>b,w</sup>    |
| Vanillic                | 13.2 ± 1.56 <sup>a,w</sup>    | 15.6 ± 1.04 <sup>a,w</sup>    | 16.4 ± 2.3 <sup>a,x</sup>     | 17.6 ± 2.45 <sup>a,w</sup>    |
| Caffeic                 | 10.5 ± 1.24 <sup>a,w</sup>    | 13.6 ± 1.03 <sup>b,w</sup>    | 13.2 ± 2.45 <sup>a,x</sup>    | 16.9 ± 2.12 <sup>b,x</sup>    |
| Syringic                | 8.6 ± 1.2 <sup>a,w</sup>      | 10.7 ± 1.24 <sup>b,w</sup>    | 10.7 ± 1.24 <sup>a,x</sup>    | 12.4 ± 1.24 <sup>b,x</sup>    |
| p-coumaric              | 4.2 ± 1.32 <sup>a,w</sup>     | 5.39 ± 1.42 <sup>a,w</sup>    | 6.6 ± 1.2 <sup>a,w</sup>      | 8.2 ± 1.2 <sup>a,x</sup>      |
| o-coumaric              | 3.2 ± 1.02 <sup>a,w</sup>     | 4.6 ± 1.25 <sup>a,w</sup>     | 5.3 ± 1.32 <sup>a,w</sup>     | 7.1 ± 1.32 <sup>b,x</sup>     |
| Ferulic                 | 2.96 ± 1.43 <sup>a,w</sup>    | 4.5 ± 1.65 <sup>b,w</sup>     | 4.25 ± 1.02 <sup>a,x</sup>    | 6.8 ± 1.22 <sup>b,x</sup>     |
| Total phenols (mg/kg)   | 181.46 ± 2.35 <sup>a, w</sup> | 214.17 ± 2.45 <sup>b, w</sup> | 198.08 ± 4.56 <sup>a, x</sup> | 223.67 ± 3.47 <sup>b, x</sup> |
| Oxidative stability (h) | 16.02 ± 1.02 <sup>a, w</sup>  | 18.84 ± 1.45 <sup>b, w</sup>  | 16.43 ± 2.03 <sup>a, w</sup>  | 21.73 ± 2.14 <sup>b, x</sup>  |

Abbreviations used: VOO, virgin olive oil; FW, fresh water treatment; SS, saline water treatment; Fw, fresh weight; ECe, electrical conductivity; FrW, fruit weight; FD, fruit diameter; FV, fruit volume; FWC, fruit water content; MI, maturation index; LSD, least significant differences; PAL, phenylalanine ammonia-lyase.

#### 4. Conclusion

In conclusion, saline water ( $EC = 7,5 \text{ dSm}^{-1}$ ) used for olive tree (cv. Chemlali) irrigation appears to be beneficial not only for water resources management, but also have a direct effects on both quantitative and qualitative characteristics of virgin olive oil in the case of Chemlali olive cultivar tested in this experiment. The changes in VOO fatty acid and phenolic compositions induced perhaps by water deficit, resulting from saline water irrigation as has been confirmed. Recent researches confirmed the protective role of phenols, as natural antioxidants, against cardiovascular diseases and colon, breast and skin cancers. The higher levels of phenolic compounds found in Tunisian Chemlali olive oil would be with more benefits for people. Furthermore, severe studies report different pharmacological activities of olive oil phenols, other than antioxidant potential.

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S4-P5

## Etude comparative de la réponse de l'olivier au stress salin durant les périodes d'intense végétation et de repos : De la physiologie aux pratiques de conduite

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### Résumé

L'olivier, arbre verdoyant caractérisant le paysage méditerranéen est connu par sa tolérance moyenne à la salinité. Ce phénomène est tributaire des stades biologiques de l'arbre. L'objectif de cette étude est de comparer la croissance végétative, les performances photosynthétiques et le statut hydrique de l'olivier Chemlali soumis à l'irrigation avec l'eau saline (C. E = 7,5 dS / m), durant différentes phases critiques de son cycle végétatif (croissance intense : printemps et hiver et repos végétatif : été et hiver), durant années successives. Les mesures relatives à l'élongation des rameaux, le statut hydrique foliaire, l'activité assimilatrice du CO<sub>2</sub>, la conductance stomatique et la transpiration ont été réalisées sur des olivier de la variété Chemlali, âgés de 14 ans conduits en plein champs et irrigués à l'eau saline (CE = 7,5 dS / m) en comparaison aux arbres irrigués à l'eau douce (CE = 1,2 dS / m). En comparaison à l'eau douce, l'irrigation à l'eau saline induit une réduction significative des performances photosynthétiques et de la croissance des pousses végétatives. Cependant, cette réduction varie en fonction de la saison biologique de l'arbre, liée à son tour aux conditions climatiques. La réduction relative du potentiel minimum ( $\Psi_{min}$ ) durant l'été se situe entre 44 et 56 % respectivement, la 1<sup>ère</sup> et 2<sup>ème</sup> année. L'assimilation nette du CO<sub>2</sub> a régressé de 55 % et 76 % au cours du printemps et de 32 et 57 % durant l'automne, respectivement durant les deux années. Cette chute de la photosynthèse a induit une réduction de l'élongation des rameaux située entre 19 et 15 % la première année et entre 43 et 16 % la deuxième. De ces résultats, il semble que l'olivier Chemlali est plus sensible à l'excès de sels durant les phases d'intense croissance que durant celles du repos végétatif.

### Comparative responses of saline water irrigated olive tree during the intense vegetative growth and plant rest phases: from physiology to management practices

### Abstract

The evergreen olive tree, characterizing the Mediterranean landscape, is known by its moderate tolerance to salinity conditions. Nevertheless, olive salt tolerance is cultivar and plant growth phase dependent. The aim of this study was to compare growth, water relations and photosynthetic performances of saline water irrigated olive tree (*Olea europaea* L. cv Chemlali) during most critical vegetative growth phases: intense vegetative growth phase (spring and autumn) and plant rest phases (summer and winter). Shoot elongation rates, leaf water relations, photosynthetic activity, stomatal conductance and transpiration rates were investigated during two successive crop seasons (2004 - 2006) on fourteen - year - old -Chemlali Sfax olive trees, grown under field-conditions and irrigated with fresh water (CF, EC = 1.2 dS m<sup>-1</sup>) and saline water (SW; ECe = 7.5 dS m<sup>-1</sup>). The irrigation with saline water has led to a significant reduction of shoot growth and photosynthetic performances activity, if compared to FW-treated plants. Nevertheless, the extent of this reduction varied with the vegetative growth phase of the olive tree and the surroundings climatic conditions. The relative reduction of leaf midday water potential of SW plants, in comparison to FW plants, during summer season was of 44 and 56%, respectively during the first and the second crop season. The reduction of CO<sub>2</sub> assimilation activity in SW plants during spring season was of 55 and 76% in the first and the second crop season, respectively. During autumn, this reduction was of 32 and 57%, respectively. The decline of shoot elongation rates in SW plants, if compared to FW treated ones, during 2004/2005 crop was of 19 and 15%, respectively during spring and autumn season. During the second crop season, this reduction was of 43 and 16%, respectively. From these results, the Chemlali olive tree seems to be more sensitive to water salinity during the intense vegetative growth phase than during the rest phase. Such tendency allows us suggest that, if two water resources are available, it would be more practical to preserve good water quality for olive irrigation during the intense vegetative growth phase, and that of low quality during the rest phase.

**Key words:** Irrigation, *Olea europaea*, photosynthetic performances, saline water, salt tolerance, vegetative growth phase.

## 1. Introduction

Due to its socio – economic importance and its limited water requirements, the olive tree is the most cultivated crop in arid region in Tunisia. Since irrigation is known to increase the yield (Patumi et al., 2002; Moreno et al., 2006; Ben Ahmed et al., 2007a, Ben Rouina et al., 2007), olive tree cultivation is being extended to irrigated lands (Ben Rouina et al., 2006). In Tunisia, the limited water resources on the one hand, and the increased need for good water quality for urban and industrial sectors use, on the other hand restrict the use of fresh water for irrigation. As well olive is considered as moderately tolerant to salinity (Therios and Misopolinos, 1988; Rugini and Fedeli, 1990, Ben Ahmed et al., 2006), large quantities of saline water are used for olive tree irrigation. However, olive salt tolerance is cultivar dependent (Tattini et al., 1995; Chartzoulakis et al., 2002).

Salt stress inhibits photosynthesis and impairs growth. Most of salt stresses are due to  $\text{Na}^+$  salts particularly NaCl. Gucci et al. (1997) suggested that salt stress has double effects on olive tree. Firstly, it limits water availability to plant tissues leading to water deficit and secondly, it induces the accumulation of salt ions in root zone and in aerial parts. Chartzoulakis (2005) and Chartzoulakis et al. (2002) showed that salt accumulation in the root zone of olive tree causes the development of an osmotic stress and disrupts cell ion homeostasis by inducing an inhibition in the uptake of essential nutrients as has been reported in wheat (Raza et al., 2007) and in sunflower (Akram et al., 2007).

In salt stressed olive plants, Chartzoulakis (2005) reported the inhibition of plant growth by moderate and high salinity. However, Wiesman et al. (2004) pointed out that long term irrigation with intermediate saline water ( $4.2 \text{ dS m}^{-1}$ ), in comparison to the control treatment ( $1.2 \text{ dS m}^{-1}$ ), did not affect significantly vegetative growth of Barnea olive tree. Moreover, Tattini et al. (1995) demonstrated that the inhibitory effects of salinization on growth of olive cuttings of the salt tolerant "Frontoio" grown at  $100 \text{ mMol NaCl}$ , fully recovered reaching values similar to the control, once salinization was relieved.

## 2. Material and Methods

### 2.1. Plant Material and Experimental Design

Trials were conducted at the experimental site of the Olive Tree Institute in Sfax, Tunisia ( $34^\circ 43 \text{ N}$ ,  $10^\circ 41 \text{ E}$ ), on own – rooted cutting of one – year – old *Olea europaea* L. plants cvs Chemlali, Meski and Picholine measuring  $80 - 100 \text{ cm}$  in height. The olive plants were transplanted in 10-L pots filled with a sand perlite mixture (3:1, v/v). The plots were kept in environmental conditions with natural sunlight and temperature and covered with plastic film and aluminum foil to avoid evaporation from the soil surface and to minimize temperature increase inside the containers. At the beginning of each month during the experimental period, all the plants were weighed at early morning and in evening in order to determine the water lost by transpiration by weight difference. During 6 months before the imposition of different salt stress levels, plants were irrigated daily with Half Strength Hoagland Solution. When the plants had developed shoots of  $15 - 20 \text{ cm}$  in length, the salt stress treatments were applied.

The experiment comprised 9 treatments of three cultivars x 3 salinity levels in a factorial design (each treatment consisted of nine plants divided into three blocks of 3 plants each). NaCl was added at 0 (control plants, CP),  $100 \text{ mMol}$  (moderate salt stress, MSS) and  $200 \text{ mMol}$  (high salt stress, HSS) to Half Strength Hoagland Solution in increment of  $25 \text{ mMol}$  per day to avoid salt shock. The amount of water used for irrigation during the whole experimental period was equal to the amount lost by transpiration (Table 1). The irrigation was applied four times per week. The experimental period started on 10<sup>th</sup> January and ended on 15<sup>th</sup> June 2007.

**Table 1:** Water use for different olive trees irrigation during the experimental period.

| Month          | Aug | Sep | Oct | Nov | Dec | Jan | Feb | Mar | Apr | May | Jun |
|----------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Water use (ml) | 920 | 758 | 545 | 365 | 130 | 120 | 190 | 320 | 470 | 550 | 760 |



## 2.2. Leaf water relations, shoot growth and gas exchange measurements

At the end of the experiment, concomitant measurements of leaf relative water content (RWC) were calculated. Gas exchange measurements were taken from 10:00 to 11:00 am on well exposed and fully expanded leaves, from the median part of the shoot from 3 plants per treatment, using a portable gas exchange system (Li-Cor Inc. 6200). We measured net photosynthesis ( $P_n$ ,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and stomatal conductance ( $G_s$ ,  $\text{mmol m}^{-2} \text{s}^{-1}$ ). These measurements were made at this time because under arid climate in Tunisia, the water limitations effects in olive plants are greatest at this time coinciding with the intense plant activity than in midday coinciding with the rest phase adapted by the plants to avoid damaging its survival mechanism under severe environmental conditions (Ben Ahmed et al., 2007a).

## 2.3. Mineral contents, proline and soluble sugars content determinations

At the end of the experiment, leaves (from the median part of shoots) and roots were harvested, washed with distilled water to eliminate the dust, oven dried at 70°C for 72 h and then ground to a fine powder. One gram of the ground tissue material was placed in an oven at 250°C for 3 hours and then transferred to 100 ml of dilute nitric acid. Cations concentrations ( $\text{Na}^+$  and  $\text{K}^+$ ) were determined from digested material using a flame photometer (Jenway, PEP-7) and the chloride ( $\text{Cl}^-$ ) using a chloride meter (Jenway, PCLM-3).

Leaf and root samples served for proline content determination were immediately frozen in liquid nitrogen. Free proline was determined according to Bates et al. (1973) method. A 0.5g of frozen powder was mixed with 5.0 ml aliquot of 3% (w/v) sulfosalicylic acid in glass tubes covered at the top and boiled in a water bath at 100 °C. The mixture was centrifuged at 2000 g for 5 min at 25 °C. A 200  $\mu\text{l}$  aliquot of the extract was mixed with 400  $\mu\text{l}$  distilled water and 20 ml of the reagent mixture (30 ml glacial acetic acid, 20 ml distilled water and 0.5 g of ninhydrin) and boiled at 100 °C for 1h. After cooling the mixture, we added 6.0 ml of toluene. The chromophore containing toluene was separated and absorption at 520 nm was read, using toluene as a blank. Proline concentration was calculated using L-proline for the standard curve.

Soluble sugars contents were determined by Robyt and White (1987) method. A 0.3 g of fresh tissue (leaf + root) were mixed with 5.0ml aliquot of methanol (80%) in glass tubes covered at the top and boiled at 70 °C for 30 min. After cooling the mixture, a 1ml aliquot of the extract was mixed with 1.0 ml phenol and 5.0 ml of concentrated sulphuric acid. After agitation and cooling of the reagent mixture,  $A_{640}$  was read using methanol as a blank. Soluble sugars concentration was calculated using glucose solution as a standard curve. All the analyses (for mineral nutrients, proline and sugars) were carried also on young and old leaves separately.

## 2.4. Data Analysis

Statistical analyses were performed using the SPSS-10. Windows. Significant differences between values of all parameters were determined at  $p \leq 0.05$  according to Duncan's Multiple Range Tests. At least three replicates were made for each field and laboratory measurements.

## 3. Results

### 3.1. Leaf water content and shoot growth

For the three olive cultivars, NaCl stress caused a significant decrease in RWC (Table 2). In general, RWC decreased gradually with the increase of external NaCl concentrations with statistically significant differences were found among cultivars ( $p < 0.05$ ). Under both salt stress treatments, Picholine plants showed the highest relative reduction of RWC (20 and 33 %, respectively in SS1 and SS2) and SER characteristics (23 and 41 %, respectively). The Chemlali cultivar was the less affected one. For all cultivars, the relative reduction of RWC was higher in SS2 than in SS1 conditions as well for SER.

The differences in relative water content among cultivars induced a similar pattern for shoot elongation rates. The cultivar with lowest level in RWC experienced the highest reduction in shoot elongation rates and thus the lower plant growth. The shoot growth is also dependent on the concentration of

NaCl at which plants were exposed. Regardless of the cultivar, the relative reduction of SER was higher in SS2 than in SS1 treatments. These changes in leaf water status and shoot growth among cultivars and treatments will certainly be more revealed via photosynthetic performances patterns.

**Table 2:** Effects of different salt stress treatments on relative water content and shoot elongation rate of all olive cultivars.

|     | RWC                    |                          |                          | SER                     |                         |                          |
|-----|------------------------|--------------------------|--------------------------|-------------------------|-------------------------|--------------------------|
|     | Chemlali               | Meski                    | Picholine                | Chemlali                | Meski                   | Picholine                |
| CP  | 93±3,19 <sup>a,v</sup> | 88±2,05 <sup>ab,w</sup>  | 85±2,33 <sup>b,w</sup>   | 6,2±0,66 <sup>a,v</sup> | 5,4±0,63 <sup>b,w</sup> | 4,8±0,65 <sup>b,w</sup>  |
| SS1 | 85±2,89 <sup>aw</sup>  | 72,3±2,46 <sup>b,x</sup> | 68,1±2,04 <sup>b,y</sup> | 5,1±0,71 <sup>a,w</sup> | 4,2±0,54 <sup>b,x</sup> | 3,7±0,41 <sup>c,y</sup>  |
| SS2 | 72±2,78 <sup>a,x</sup> | 65,1±2,44 <sup>b,y</sup> | 57,2±2,01 <sup>b,z</sup> | 4,1±0,61 <sup>a,x</sup> | 3,5±0,42 <sup>b,y</sup> | 2,86±0,44 <sup>c,z</sup> |

Values represent means ± S.E. of three replications per treatment. Column with different letters from v to z (v – z) indicate significant differences ( $p < 0.05$ ) with respect to irrigation treatment regardless of the cultivar, and column separated by different letters from a to c indicate significant differences with respect to the cultivar regardless of the salt water treatment. RWC: relative water content (%); SER: shoot elongation rate ( $\text{cm month}^{-1}$ ).

**Table3:** Effects of different salt stress treatments on net photosynthesis, stomatal conductance and transpiration rate of all olive cultivars.

|     | Pn                       |                          |                           | Gs                        |                           |                           |
|-----|--------------------------|--------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
|     | Chemlali                 | Meski                    | Picholine                 | Chemlali                  | Meski                     | Picholine                 |
| CP  | 25,6±2,89 <sup>a,v</sup> | 18,7±2,55 <sup>b,w</sup> | 16,3±2,01 <sup>b,w</sup>  | 163,4±3,26 <sup>a,v</sup> | 121,6±4,15 <sup>b,w</sup> | 118,7±3,98 <sup>b,w</sup> |
| SS1 | 15,7±2,01 <sup>a,w</sup> | 12,2±1,95 <sup>b,x</sup> | 10,5±1,26 <sup>bx,y</sup> | 120,4±3,96 <sup>a,w</sup> | 95,4±4,21 <sup>b,x</sup>  | 82,4±3,87 <sup>b,y</sup>  |
| SS2 | 12,3±1,24 <sup>a,x</sup> | 9,5±1,04 <sup>b,y</sup>  | 8,5±0,98 <sup>b,y</sup>   | 115,6±4,21 <sup>a,w</sup> | 90,3±3,89 <sup>b,x</sup>  | 75±3,55 <sup>b,z</sup>    |

Values represent means ± S.E. of nine replications per treatment. Column with different letters from v to z (v – z) indicate significant differences ( $p < 0,05$ ) with respect to irrigation treatment regardless of the cultivar, and column separated by different letters from a to c indicate significant differences with respect to the cultivar regardless of the salt stress treatment. Pn: Net photosynthesis ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), Gs: Stomatal conductance ( $\text{mmol m}^{-2} \text{s}^{-1}$ ); and E: Transpiration rate ( $\text{mmol m}^{-2} \text{s}^{-1}$ ).

### 3.2. Gas Exchange Characteristics

Net photosynthesis (Pn), stomatal conductance (Gs) and transpiration rates (E rates) of all cultivars, conducted under different salinity levels, were depicted in table 3. Significant differences were found among cultivars and treatments. Regardless of the cultivar, Pn decreased with increasing NaCl concentrations in the external solution. The olive cultivar with high RWC (Chemlali) had also the highest photosynthetic performances as well in SS1 as in SS2 treatments. In both cultivars (Picholine and Meski), Pn and Gs were reduced with a similar relative reduction under SS1 conditions. However, Meski plants maintained their Pn at higher level than that of Picholine. Among all treated plants, the cultivar with lowest reduction of Pn (Picholine) showed also the lowest levels of Pn under both salinity treatments. Under control conditions, Chemlali had higher Pn, Gs and E rates than the other two olive cultivars. The highest reduction of Pn, Gs and E rates was observed in cultivar with high photosynthetic performances as well for RWC and SER characteristics. The Picholine olive was the most affected cultivar by different salinity levels.

Despite the higher relative reduction of Pn, Gs in the Chemlali olive plant, it maintained better photosynthetic activity and thus shoot growth than the Picholine and Meski. The different patterns of Pn in all cultivars justified the shoot growth pattern. In fact, the cultivar with higher photosynthetic activity, even under control or stressed conditions, experienced also better shoot elongation rates and in so doing better plant growth.

Figure 1 depicted relative water content in young and old leaves of all cultivars. Under both salinity treatments, in all cultivars young leaves showed higher RWC than the old ones. The better levels of leaf water status were maintained in Chemlali leaves. It showed values of 74.5 and 69.1% in young and old leaves, respectively under irrigation with water containing 200 mMol NaCl. These values explained well the better shoot growth registered in Chemlali than in other cultivars.

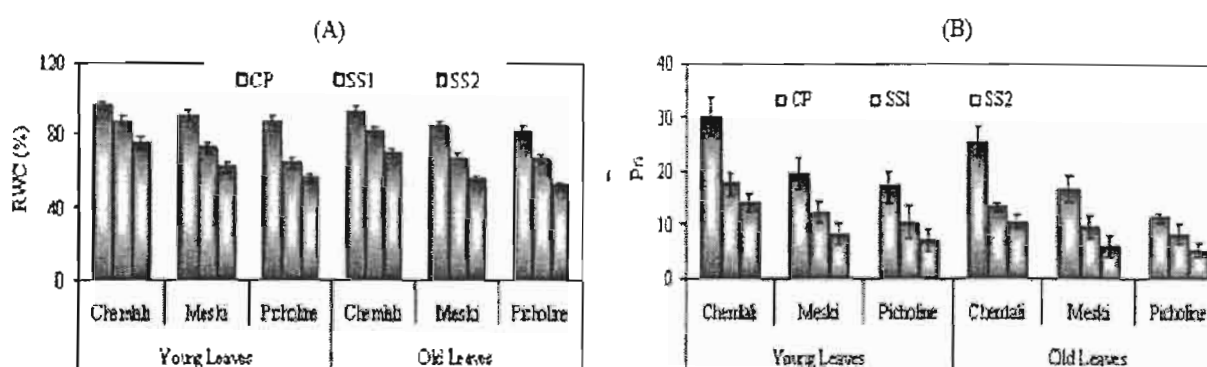


**Figure 1:** Relative water content (RWC, A) and net photosynthesis (Pn, B) in young and old leaves of all cultivars under different salt stress treatments. Values represent means of 3 and 9 replications for RWC and Pn measurements, respectively  $\pm$  S.E.

Photosynthesis activity was measured in young and old leaves, separately in all cultivars under control and salinity treatments and results were depicted in Figure 1 (B). Under both salinity treatments, leaves with lower RWC had experienced also the lowest levels of Pn. Young and old leaves of Chemlali plant were more photosynthetically active than those of Meski and Picholine. Regardless of the cultivar, in both leaves tissues, Pn was significantly reduced by different salinity treatments.

### 3.3. Mineral Contents

For all olive cultivars,  $\text{Na}^+$  content increased, as well in leaves as in roots, with increasing external NaCl concentrations (Table 4). Amounts recorded in roots exceeded those in leaves. A more pronounced increase was observed in the Picholine cultivar. The increase in  $\text{Na}^+$  content was associated with that of  $\text{Cl}^-$  content in both plant tissues (leaves + roots). As well for  $\text{Na}^+$  concentration, in all cultivars, the  $\text{Cl}^-$  content increased with increasing salinity levels in the external solution. Under SS1 treatment, the level of  $\text{Na}^+$  content in leaves of Picholine was 1.5 fold higher than that in Chemlali. However, in SS2 conditions, it was at 2 fold higher. Under intermediate salinity level, leaf  $\text{Na}^+$  content was similar in both Picholine and Meski. However, under SS2 treatment, Picholine accumulated  $\text{Na}^+$  at higher level than the Meski cultivar.

Regardless of the cultivar, the accumulation of  $\text{Na}^+$  and  $\text{Cl}^-$  ions in both plant tissues was accompanied with the decrease of  $\text{K}^+$  content. The  $\text{K}^+$  content decreased with increasing salinity level with amounts recorded in leaves were higher than those in roots. The plant with higher  $\text{Na}^+$  and  $\text{Cl}^-$  contents has the lowest level of  $\text{K}^+$  content in both plant tissues. The cultivar with better photosynthetic activity and leaf relative water content (Chemlali) showed the lowest  $\text{Na}^+$  and or  $\text{Cl}^-$  contents and higher content of  $\text{K}^+$  in both leaves and roots. For all treated plants, roots showed higher  $\text{Na}^+$  and  $\text{Cl}^-$  contents than the leaves.

**Table 4:**  $\text{Na}^+$ ,  $\text{Cl}^-$  and  $\text{K}^+$  contents in leaves and roots of all cultivars under different treatments.

| Element           | organ  | Chemlali |      |      | Meski |      |      | PICHOLINE |      |      |
|-------------------|--------|----------|------|------|-------|------|------|-----------|------|------|
|                   |        | CP       | SS1  | SS2  | CP    | SS1  | SS2  | CP        | SS1  | SS2  |
| $\text{Na}^+$ (%) | Leaves | 0.05     | 0.12 | 0.18 | 0.10  | 0.22 | 0.31 | 0.11      | 0.23 | 0.38 |
|                   | Roots  | 0.09     | 0.35 | 0.54 | 0.12  | 0.46 | 0.65 | 0.15      | 0.57 | 0.72 |
| $\text{Cl}^-$ (%) | Leaves | 0.07     | 0.11 | 0.15 | 0.08  | 0.15 | 0.26 | 0.12      | 0.21 | 0.36 |
|                   | Roots  | 0.09     | 0.45 | 0.61 | 0.16  | 0.48 | 0.75 | 0.24      | 0.60 | 0.84 |
| $\text{K}^+$ (%)  | Leaves | 2.54     | 1.55 | 1.27 | 1.82  | 1.49 | 1.15 | 1.75      | 1.42 | 0.85 |
|                   | Roots  | 2.00     | 1.27 | 0.85 | 1.63  | 1.15 | 1.10 | 1.25      | 1.05 | 0.74 |

### 3.4. Proline and total sugars contents

The proline contents in leaves and roots of all cultivars conducted under different salinity levels are shown in Table 5. Significant differences ( $p < 0.05$ ) were recorded among cultivars and treatments with some exceptions. Regardless of the cultivar, as well in leaves as in roots, the more severe the salt stress was, the higher the proline accumulation was. Under both salinity treatments, the Chemlali plants showed higher proline contents in both plant tissues (leaves + roots) than other olive cultivars. Under SS2 treatment, the proline content in leaves increased at 4.3, 3.08 and 2.7 times, respectively in Chemlali, Meski and Picholine cultivars if compared to their respective CP.

As well for proline accumulation, in all olive cultivars, the sugars contents (Tab. 5) increased with increasing external NaCl concentrations. In contrast to proline accumulation behaviour, soluble sugars were more accumulated in Picholine than in Meski and Chemlali. However, differences between cultivars treated with SS2 NaCl level were not statistically significant. The olive cultivar with higher photosynthetic activity (Chemlali) showed the lowest levels of sugars contents in both plant tissues (leaves + roots). As well for proline accumulation, leaves of all stressed plants accumulated soluble sugars at higher amounts than the roots.

**Table 5:** Effects of different treatments on proline and total sugars contents in leaves and roots of all olive cultivars under different salt stress treatments

|               | Proline ( $\mu\text{mol mg}^{-1}$ Fw) |                          |                        | Soluble Sugars ( $\mu\text{mol mg}^{-1}$ Fw) |                          |                          |
|---------------|---------------------------------------|--------------------------|------------------------|----------------------------------------------|--------------------------|--------------------------|
|               | Chemlali                              | Meski                    | Picholine              | Chemlali                                     | Meski                    | Picholine                |
| <b>Leaves</b> |                                       |                          |                        |                                              |                          |                          |
| CP            | 0,63±0,04 <sup>a</sup>                | 0,72±0,03 <sup>a,e</sup> | 0,81±0,03 <sup>e</sup> | 0,05±0,01 <sup>a</sup>                       | 0,08±0,01 <sup>a,d</sup> | 0,09±0,02 <sup>d</sup>   |
| SS1           | 1,96±0,04 <sup>b</sup>                | 1,72±0,03 <sup>b</sup>   | 1,81±0,03 <sup>b</sup> | 0,11±0,01 <sup>b</sup>                       | 0,14±0,01 <sup>b</sup>   | 0,15±0,02 <sup>b,c</sup> |
| SS2           | 2,56±0,05 <sup>c</sup>                | 2,21±0,03 <sup>d</sup>   | 2,11±0,05 <sup>d</sup> | 0,15±0,018 <sup>b,c</sup>                    | 0,19±0,009 <sup>c</sup>  | 0,2±0,01 <sup>e,c</sup>  |
| <b>Roots</b>  |                                       |                          |                        |                                              |                          |                          |
| CP            | 0,34±0,04 <sup>a</sup>                | 0,38±0,03 <sup>a</sup>   | 0,4±0,05 <sup>a</sup>  | 0,02±0,01a                                   | 0,05±0,02a,c             | 0,06±0,03a               |
| SS1           | 0,5±0,03 <sup>b</sup>                 | 0,5±0,03 <sup>b</sup>    | 0,51±0,04 <sup>b</sup> | 0,07±0,02b,c                                 | 0,09±0,01b               | 0,11±0,02b,d             |
| SS2           | 0,72±0,04 <sup>c</sup>                | 0,66±0,05 <sup>c,d</sup> | 0,6±0,06 <sup>d</sup>  | 0,11±0,006b,d                                | 0,11±0,01b,d             | 0,14±0,018d              |

Values represent means  $\pm$  S.E. of three replications per treatment. Means within each column separated by different letters are significantly different at  $p < 0,05$ . Fw fresh weight.

### 4. Discussion

Through the analysis of leaf water relations characteristics of all olive cultivars, we observed a common response to salinity. The earliest response of the different cultivars to salinity is the decrease of RWC. The decrease in RWC was a result of the high salt concentrations in the external solution and the accumulation of salt ions in both leaves and roots. Indeed, high salt concentration caused osmotic stress and dehydration at the cellular level (Munns, 2002). The decrease in RWC in salt stressed olive plants was a response in order to activate water uptake and retention by actively growing tissues. This process seemed to work more efficiently in Chemlali than in Meski and Picholine olive cultivars. Regardless of the cultivar, the highest RWC level registered in leaves could be due to the higher accumulation of  $K^+$  and lower contents of  $Na^+$  and  $Cl^-$  to maintained better photosynthetic activity. This behaviour justified well the role played by high  $K^+$  content in the maintenance of turgor process.

Photosynthesis was reduced by salt stress but to a variable extent in the different cultivars tested. In general, the highest relative reduction was observed in the cultivar which had inherently high photosynthetic performances (Chemlali). A higher uptake and accumulation of salts in leaves of cultivars with low photosynthesis could explain their stress sensitivity. Olive cultivars with high photosynthetic performances can perform well under limited water availability caused by salt stress conditions. The shedding of old leaves and leaf necrosis observed in Picholine and Meski cultivars, respectively at 100 and 200 mM NaCl (data not shown) could result from high salt accumulation in leaves. The same behavior has been observed by Ben Ahmed et al. (2006, 2007b) and Ben Rouina et al. (2006) in adult Chemlali olive trees irrigated for 12 years with saline water ( $EC = 7.5 \text{ dS m}^{-1}$ ). Besides, large genotypic differences are detected regarding  $Na^+$  accumulation in the roots and its transport to aerial growing parts. The salt exclusion mechanism seems to work more effectively in the Chemlali cultivar than in Meski and Picholine. Indeed, the moderately salt tolerant Chemlali plants have a more efficient mechanism regulating salt translocation to the growing tissues. The lower  $Na^+$

content in young leaves of the Chemlali cultivar, even at 200 mM NaCl, indicated the existence of an inhibition mechanism of Na<sup>+</sup> transport to leaves. This mechanism has allowed young leaves to maintain better RWC and Pn levels than the old ones, and justified the important role played by old leaves to prevent excessive salt accumulation in apical leaves to accomplish their photosynthetic activity.

Face to the accumulation of salt ions at the whole plant level, stressed olive trees tend to accumulate osmolytes compounds such as proline and soluble sugars in their tissues in order to activate water uptake to actively growing tissues. For all olive cultivars tested, the proline accumulation was more important than the sugars. This behaviour let us suggest that proline is more involved in salt tolerance mechanism than the sugars. However, the sugars compounds were more accumulated in salt sensitive plants which displayed lower photosynthetic activity.

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## Effet d'une pollution fluorée sur l'assimilation photosynthétique de l'olivier « *Olea europaea* »

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### Résumé

Dans la nature, les végétaux se trouvent fréquemment confrontés à divers types d'agressions d'origines abiotique et biotique tels que la sécheresse, la salinité, l'infection par des phytophages et la pollution atmosphérique. Ces contraintes affectent la croissance et le développement de la plante qui répond le plus souvent par des modifications morphologiques et physiologiques lui permettant de mieux s'adapter et résister dans ces conditions contraignantes. Le présent travail a fait l'objet d'une étude d'évaluation de l'effet d'une pollution fluorée, issue de la société industrielle d'acide phosphorique et d'engrais à Sfax, sur l'assimilation photosynthétique de l'olivier. L'exposition des oliviers à ces fumées riches en fluorures a entraîné une diminution de la photosynthèse. Cette diminution peut être due soit à une fermeture stomatique partielle entraînant une baisse de la conductance stomatique, soit à une réduction de la surface assimilatrice faisant suite à des nécroses foliaires. Les mesures des paramètres photosynthétiques et hydriques en plus de la densité et la longueur des stomates, montrent que l'origine de la réduction de l'assimilation photosynthétique, sous l'effet des fluorures, semble due à une diminution de la conductance stomatique.

Mots clés: Evaluation, Pollution, fluorures, photosynthèse, conductance stomatique.

## Effect of fluoride pollution on the photosynthetic assimilation of the olive tree « *Olea europaea* »

### Abstract

In nature, plants are frequently faced to various biotic and abiotic aggressions such as drought, salinity, infection by pests and air pollution. These constraints affect the growth and development of plants which usually respond by morphological and physiological changes allowing them to survive in such stringent conditions. The aim of the present work is to study the effect of fluoride pollution, coming from a phosphate fertilisers producing factory in the region of Sfax, on the photosynthetic activity of olive trees. These trees exposed to the fumes given off by the chimney have shown a decrease in photosynthesis appearing to be due either to a partial closure of stomata or to a decrease of assimilative surfaces in response to leaf necrosis. Measurements of water and photosynthetic parameters in addition to the density and length of stomata, show that the reduction in photosynthetic activity, under the effect of fluoride, is mainly due to a decrease in stomatal conductance.

**Key words:** Evaluation, Pollution, fluorides, photosynthesis, stomatal conductance

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## **Adaptabilité à l'irrigation déficitaire de trois cultivars d'olivier (*Olea europaea* L.) : Chetoui , Picholine et Manzanille**

**Mahjoub Imen Ben Mechlia Nétij**

### **Résumé**

L'adaptabilité à l'irrigation déficitaire des trois cultivars d'olivier, Chetoui, Picholine et Manzanille, a été étudiée. Un travail expérimental a été conduit pendant quatre ans dans la région de Tunis. Deux niveaux d'irrigation déficitaire ont été adoptés, I1=100mm et I2=200mm. Un suivi de la croissance des rameaux et de la production a été effectué. Le rendement cumulé pour le traitement I1 est de 20% inférieur à I2, pour les cultivars Chetoui et Picholine. Pour Manzanille cette différence est de 15%. La croissance végétative n'a pas montré de différences claires entre les traitements hydriques. Elle est plutôt affectée par la charge en fruits des arbres. Les interactions entre la charge en fruit et les apports hydriques seront discutées dans la communication.

**Mots clés:** Olivier \ stress hydrique \ Production \ Charge en fruits \ Croissance végétative.

## **Adaptability to deficit irrigation of three olive cultivars (*Olea europaea* L.): Chetoui Picholine and Manzanille**

### **Abstract**

The adaptability to deficit irrigation of three olive cultivars, Chetoui, Picholine and Manzanille, was investigated. A four years experiment was carried out in the region of Tunis. Two deficit irrigation levels were adopted, I1=100mm and I2=200mm. Shoot growth and production were monitored. For both Chetoui and Picholine cvs, cumulated yield over four years was about 20% lower for I1 than for I2. For Manzanille cv. this difference was 15%. The Vegetative growth did not show clear differences between the adopted irrigation levels. It was rather affected by tree fruit load. The interactions between fruit load and water supply levels will be discussed in the communication.

**Key words:** Olive tree \ Water stress \ Production \ Fruit load \ Vegetative growth.



## Influence of irrigation regimes and growing area location on Chétoui olive oil quality

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### Abstract

The ultimate goal of the present study was to attest the influence of irrigation strategies on Chétoui olive oil quality planted in the north and the center of the country. Our results showed that the different irrigation regimes don't affect the amount of oil for both growing area. However, the percentage of the damage fruit affects the acidity of the oil. Under the same applied water, the fatty acid composition of Chétoui north olive oil was not affected, in contrast the percentage of oleic acid of Chétoui center olive oil exhibit a decrease until 56%. The amount of supplied water decrease the total phenol content in the oil extracted only from Chétoui of the center. In contrast, phenols from Chétoui north olive oil were not affected by irrigation. In both growing area location, the volatile compounds most affected by the water status of olive tree were hexanal, *E*-2-hexenal, hexenyl acetate and hexyl acetate. In conclusion, results of this study indicated that Chétoui north olive oil quality is positively affected by irrigation. In contrast, the high amount of water was detrimental on the Chétoui center olive oil quality.

**Keywords:** Irrigation strategies, growing area, Chétoui olive oil, aroma composition.

### Résumé

L'objectif de la présente étude vise à étudier l'influence de différents régimes de l'irrigation sur la qualité de l'huile de la variété Chétoui planté dans deux sites géographiques différents.

Les résultats obtenus démontrent que les différentes quantités d'eaux ajoutées n'ont aucune influence sur le pourcentage d'huile obtenu indépendamment du site géographique. Sous la même quantité d'eau appliquée, le profil en acide gras est stable dans l'huile obtenu du fruit de Chétoui du Nord, tandis qu'il décroît jusqu'à 56% au niveau de l'huile de la variété Chétoui du Centre. La quantité d'eau appliquée décroît la concentration en phénols, ce qui diminue par conséquent l'amertume au niveau de l'huile de la variété Chétoui du Nord. La matrice en composés volatiles, dans les deux régions, la plus influencé par le régime hydrique: hexanal, *E*-2-hexenal, acétate d'hexenyl et acétate d'hexyl.

En somme, la qualité de l'huile de la variété Chétoui du Nord n'est pas affectée par l'irrigation, en revanche, celle du Chétoui du Centre est négativement affectée par l'irrigation et par le pourcentage d'eau appliqué à l'arbre.

**Mots clés:** régimes d'irrigation, composition en acide gras, phénols, amertume, volatiles

## 1. Introduction

In Tunisia, the olive tree is generally grown under rain-fed conditions, mainly in a region with limited water resources. Nevertheless, since irrigation increases the yield of the olive orchard, even with allow amount of water, there is increasing interest in irrigated agriculture. The main goal of this study was to attest the influence of irrigation regimes of Chétoui olive oil quality grown in the North and the Centre of the Tunisian country.

## 2. Materials and Methods

The study was carried out on monovarietal virgin olive oils from the second main Tunisian cultivar, Chétoui. Olive samples were hand picked, in triplicate, from good quality fresh and healthy fruits. Chétoui variety was planted in the North and the Centre of the country. In both olive orchard, water was delivered three time each week (from June to September) using a localized irrigation system with four drip nozzles of 8 l/h each per tree (two per side), set in a line along the rows at a distance of 0.5 m from

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the trunk. Olive Chétoui cultivars were tested in a factorial combination with three irrigation levels [three plots of 36m<sup>2</sup> (6m x 6m) each were designed for each irrigation]: well irrigated (T3) (T2) and stressed (T1) receiving a seasonal water irrigation amount equivalent to 100, 75 and 50% of crop evapotranspiration (ET<sub>c</sub>) calculated using the Penman–Monteith–FAO method (Allen et al., 1998) with a single estimated crop coefficient (K<sub>c</sub> = 0.6) and a coverage coefficient (K<sub>r</sub> = 0.5) (D'Andria et al., 2004).

## **2.1. Oil Isolation**

Oil isolation was carried out using an Abencor analyzer. The fresh olives (2.0–3.0 kg) were crushed with a hammer mill and were then slowly mixed for 30 min at 25 °C. The resulting paste was centrifuged at 3500 rpm for 3 min. The oil was separated by decanting. All samples were subsequently filtered over anhydrous Na<sub>2</sub>SO<sub>4</sub>, placed in amber glass bottles and stored in the dark at a temperature of 4°C.

## **2.2. Analytical Indices**

Free acidity and peroxide value were determined according to the European Communities official methods (EEC Reg. 2568, 1991).

## **2.3. Fatty acid composition**

Fatty acid methyl esters (FAMES) from the oil samples were carried as described by Issaoui et al (2008).

## **2.4. Phenols**

The total phenols were determined calorimetrically at 765 nm using the Folin Ciocalteu reagent and are expressed as 3, 4-Dihydroxyphenyl ethanol (3, 4-DHPEA) on the methanolic extract of virgin olive oil (Montedoro, Servili, Baldioli & Miniati, 1992).

## **2.5. Evaluation of the intensity of bitterness**

Evaluation of the intensity of bitterness was carried with the procedure described by Beltrán et al (2007).

## **2.6. Rancimat assay**

Oxidation stability was evaluated by the Rancimat apparatus (Mod. 743, Metrohm Ω, Switzerland) using an oil sample of 3 g warmed to 120°C and an air flow of 20L/h. Stability was expressed as induction time (hours).

## **2.7. Volatile compound analyses**

### **2.7.1. Extraction**

Solid phase micro extraction was used as a technique for headspace sampling of virgin olive oils. Supelco SPME devices coated with polydimethylsiloxane (PDMS, 100 µm) were used to sample the headspace of 2ml of virgin olive oil inserted into a 5ml glass vial and allowed to equilibrate for 30 min. after the equilibration time; the fiber was exposed to the headspace for 50 min at room temperature. Once sampling was finished, the fiber was withdrawn into the needle and transferred to the injection port of the GC and GC-MS system (Campeol, Flamini, Chericoni, Catalano, & Cremonini, 2001).

### **2.7.2. Identification**

GC analyses were accomplished with an HP-5890 series II instrument equipped with a HP-5 capillary column (30 m x 0.25 mm, 0.25 µm film thickness), working with the following temperature programme: 60°C for 10 min, ramp of 5°C/min to 220°C; injector and detector temperatures, 250°C; carrier gas, nitrogen (2ml/min); detector FID; split ratio, 1:30; injection, 0.5µl.

GC-EIMS analyses were performed with a Varian CP 3800 gas-chromatograph equipped with a DB-5 Capillary column (30m x 0.25 mm; coating thickness= 0.25µm) and a Varian Saturn 2000 ion trap mass

detector. Identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their linear retention indices relative to the series of *n*-hydrocarbons, and on computer matching against commercial (NIST 98 and ADAMS) and home made library mass spectra built from pure substances and components of known oils and MS literature data (Stenhagen et al., 1974; Massada, 1976; Jennings & Shibamoto, 1980; Swigar & Silverstein, 1981; Davies, 1990; Adams, 1995). Moreover, the molecular weights of all the identification substances were confirmed by GC-CIMS, using MeOH as CI ionizing gas (Flamini et al., 2003).

## 2.8. Statistical analysis

All parameters were determined in triplicate for each sample. Variance (ANOVA) was processed by SPSS statistical package (Version 12.00 for Window, SPSS Inc. Chicago, Illinois, 2003).

## 3. Results and discussion

### 3.1. Fatty acid profiles

With regard to the effect produced in the fatty acid composition by the irrigation practices applied in both olive orchards, these were very weak and moreover did not show similar trends in the obtained oils. The irrigation regime barely affected the Chétoui north oil fatty acid composition (Table I). Also, the MUFA/PUFA and O/L ratio, around 5.0, did not appear to be influenced by irrigation, and therefore the intrinsic oxidative stability of the oil remained unchanged. In contrast, when the same amount of water was applied on the orchard of Chétoui Centre, the oils exhibited a decrease in the oleic acid levels (from 60.0% to 56%) and an increase in the linoleic acid percentage (from 23.0% to 28.5%).

**Table I:** influence of irrigation regimes on Chétoui olive oil quality parameters and fatty acid composition

|                  | Chétoui olive oil  |                    |                    |                   |                   |                   |
|------------------|--------------------|--------------------|--------------------|-------------------|-------------------|-------------------|
|                  | Chétoui North      |                    |                    | Chétoui Centre    |                   |                   |
| Maturation state | 2.2 <sup>b</sup>   | 2.3 <sup>ab</sup>  | 2.3 <sup>ab</sup>  | 2.3 <sup>ab</sup> | 2.9 <sup>a</sup>  | 2.7 <sup>a</sup>  |
| Acidity (%)      | 0.4 <sup>c</sup>   | 0.4 <sup>c</sup>   | 0.5 <sup>c</sup>   | 0.7 <sup>b</sup>  | 1.1 <sup>a</sup>  | 1.2 <sup>a</sup>  |
| Peroxide value   | 13.3 <sup>b</sup>  | 13.3 <sup>b</sup>  | 13.3 <sup>b</sup>  | 14.6 <sup>a</sup> | 12.0 <sup>b</sup> | 14.6 <sup>a</sup> |
| Grade            | Extra Virgin       | Extra Virgin       | Extra Virgin       | Extra Virgin      | Virgin            | Virgin            |
| C16:0(%)         | 10.7 <sup>c</sup>  | 10.7 <sup>c</sup>  | 10.7 <sup>c</sup>  | 12.6 <sup>b</sup> | 13.2 <sup>a</sup> | 11.5 <sup>b</sup> |
| C16:1            | 0.4 <sup>b</sup>   | 0.4 <sup>b</sup>   | 0.4 <sup>b</sup>   | 0.5 <sup>a</sup>  | 0.5 <sup>a</sup>  | 0.3 <sup>c</sup>  |
| C18:0            | 2.6 <sup>a</sup>   | 2.5 <sup>a</sup>   | 2.6 <sup>a</sup>   | 2.7 <sup>a</sup>  | 2.7 <sup>a</sup>  | 2.7 <sup>a</sup>  |
| C18:1            | 71.1 <sup>bc</sup> | 71.1 <sup>bc</sup> | 71.1 <sup>bc</sup> | 60.0 <sup>d</sup> | 56.0 <sup>e</sup> | 56.0 <sup>e</sup> |
| C18:2            | 14.4 <sup>d</sup>  | 14.3 <sup>d</sup>  | 14.4 <sup>d</sup>  | 23.0 <sup>c</sup> | 26.5 <sup>b</sup> | 28.5 <sup>a</sup> |
| C18:3            | 0.8 <sup>b</sup>   | 0.8 <sup>b</sup>   | 0.8 <sup>b</sup>   | 0.9 <sup>b</sup>  | 1.1 <sup>a</sup>  | 1.1 <sup>a</sup>  |
| SFA              | 13.3 <sup>b</sup>  | 13.3 <sup>b</sup>  | 13.3 <sup>b</sup>  | 15.3 <sup>a</sup> | 16.0 <sup>a</sup> | 14.1 <sup>b</sup> |
| MUFA             | 72.0 <sup>b</sup>  | 72.0 <sup>b</sup>  | 72.0 <sup>b</sup>  | 59.3 <sup>d</sup> | 56.3 <sup>e</sup> | 56.3 <sup>e</sup> |
| PUFA             | 15.1 <sup>d</sup>  | 15.1 <sup>d</sup>  | 15.1 <sup>d</sup>  | 24.6 <sup>c</sup> | 27.7 <sup>b</sup> | 29.6 <sup>a</sup> |
| O/L              | 5.0 <sup>a</sup>   | 5.0 <sup>a</sup>   | 5.0 <sup>a</sup>   | 2.6 <sup>b</sup>  | 2.1 <sup>b</sup>  | 2.0 <sup>b</sup>  |

The potential oxidative susceptibility (lower oxidative stability) is therefore much higher in Chétoui Centre VOO (1222.0 to 1448.6) than Chétoui North VOO (Fig.1.).

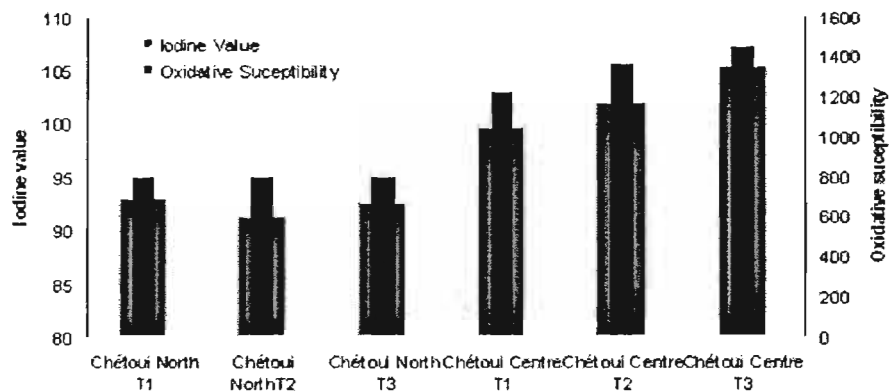


**Figure 1:** Influence of irrigation regimes on Chétoui olive oil oxidative susceptibility B. Total phenol content and bitterness.

A study by our research group confirmed that there is a decrease in the amount of total phenols in the Chétoui Centre olive oil. Hence, the decrease is about the half with the increase of water supplementation (Fig.2.). Therefore, olive fruits grown under water deficit in the south of the country (tree submitted under rain fed conditions) should contain a higher level of phenolic. However, there is no significant modification in the amount of total phenols when we duplicate the amount of water for the Chétoui north olive tree (Fig.2.).

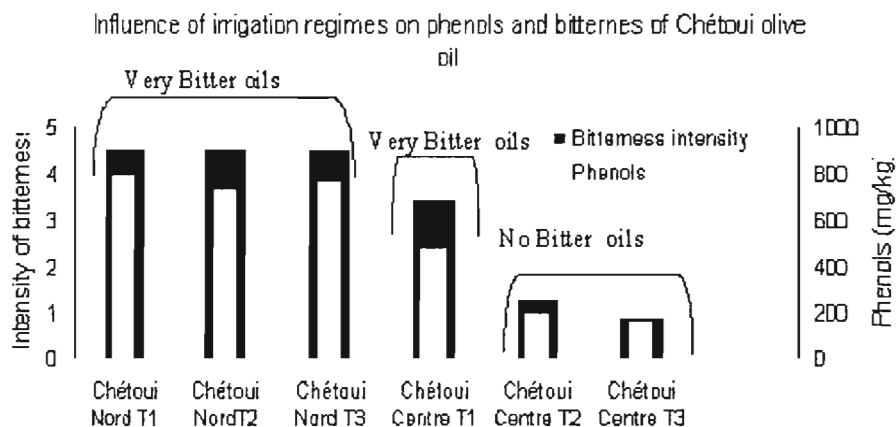


**Figure 2:** Influence of irrigation regimes on phenols and bitterness intensity on Chétoui olive oils.

In fact, a sharp decrease in the bitterness index was observed in oils obtained from Chétoui south when the irrigated strategies were applied. Hence, oils from no irrigated and irrigated Chétoui south at 50% were very bitter (bitterness index were 4.5 and 3.4 respectively). In contrast, when we increase the amount of water until 75 and 100%, the bitterness index decrease until 1.3 and 0.9 respectively, and oils belong to the non bitter category. However, in oils obtained from Chétoui north olive variety no statistically significant differences were found (Table Fig.2.).

### 3.2. Shelf life prediction of Chétoui olive oils

As described in phenols, the shelf life prediction of Chétoui Centre olive oil was negatively affected by the irrigation regimes and by the high percentage of water added to the tree before harvesting. In contrast, Chétoui North olive oil shelf life appears not to be affected by both the irrigation and the amount of water (Fig.3.).

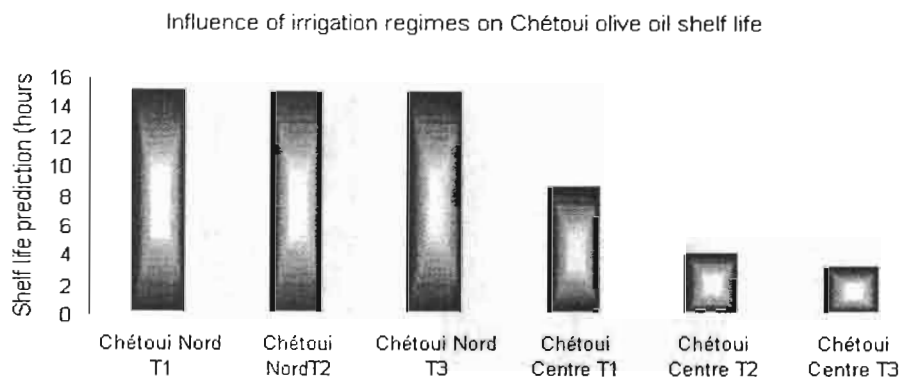


**Figure 3:** Influence of irrigation regimes on Chétoui olive oil shelf life

### 3.3. Aroma profiles of Chétoui olive oils

Taking into consideration the effect of irrigation regimes on aromatic composition of Chétoui olive oil, we found a very important modification of this matrix under the applied of the amount of water on the tree (Fig.4.).

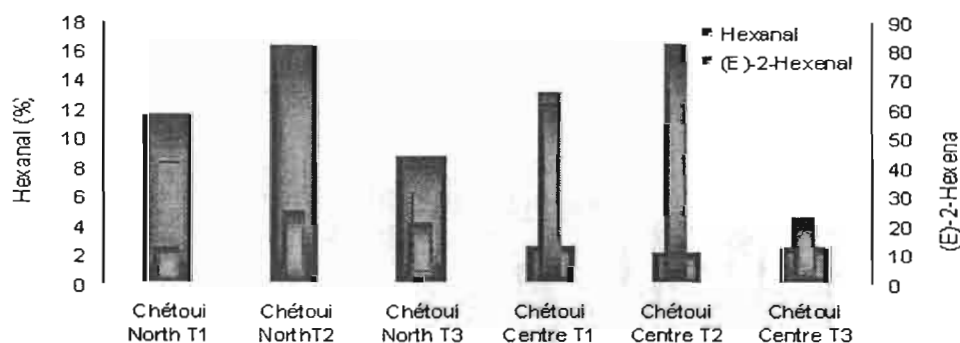


**Figure 4:** Influence of irrigation regimes on volatiles of Chétoui olive oils

The (E)-2-Hexenal, known by their green and almonds sensory attributes as described by Tena et al (2007), was detected at the highest level in both Chétoui olive oils produced by the extraction of fruits from the tree irrigated by 75% of water. Regarding the effect of water on the C6 esters such as (Z)-3-hexenyl acetate and 1-hexyl acetate (related with sweet, fruity and green leaves notes as described by Gómez-Rico et al. (2009)), these were present in small amounts in Chétoui olive oil from fruits irrigated by the lowest amount of water, indicating little activity of the alcohol acyl transferase (AAT) in the presence of the great amount of water.

### 4. Conclusion

In conclusion, it is important to note that the effect of irrigation regimes on olive oil quality depends more on the geographic zones.

### Acknowledgements

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## Osmoprotection et antioxydation dans les cellules foliaires de deux cultivars d'olivier soumis au déficit hydrique sévère

Mustapha Ennajeh, Ahmadou Mohamed Vadel, Habib Khemira

### Resumé

Dans cette étude, nous avons comparé l'efficacité des mécanismes de défense contre un déficit hydrique sévère dans les feuilles de deux cultivars d'olivier (*Olea europaea* L.), 'Chemlali' et 'Meski', réputé résistant et sensible à la sécheresse, respectivement. Des boutures âgées de deux ans, repiquées sur des pots de 10-dm<sup>3</sup> rempli de sol sableux, ne sont pas arrosées pendant deux mois. La variation de la teneur en malondialdéhyde dans la feuille en fonction de la diminution de la teneur relative en eau (TRE) a montré que 'Chemlali' maintient une bonne intégrité fonctionnelle et structurelle cellulaire que 'Meski'. A une hydratation foliaire faible, 'Chemlali' a accumulé du mannitol et de composés phénoliques et a augmenté l'activité de catalase (CAT) et de peroxydase (POD). Ces observations suggèrent que 'Chemlali' est plus capable de maintenir l'intégrité des cellules foliaires sous des conditions de déficit hydrique sévère grâce à l'efficacité des mécanismes d'osmoprotection et d'antioxydation.

**Mots-clés:** Enzymes antioxydantes, malondialdéhyde, mannitol, *Olea europaea*, tolérance.

## Osmoprotection and antioxidation in the leaf cells of two olive cultivars subjected to severe water deficit

### Abstract

In this study, we compared the efficacy of defense mechanisms against severe water deficit in the leaves of two olive (*Olea europaea* L.) cultivars, 'Chemlali' and 'Meski', reputed drought resistant and drought sensitive, respectively. Two-year old plants growing in sand filled 10-dm<sup>3</sup> pots were not watered for 2 months. Changes in malondialdehyde content as leaf relative water content (RWC) decreased showed that 'Chemlali' was able to maintain functional and structural cell integrity longer than 'Meski'. At low leaf hydration levels, 'Chemlali' leaves accumulated mannitol and phenolic compounds and had increased catalase (CAT) and peroxidase (POD) activities. These observations suggest that 'Chemlali' was more capable of maintaining its leaf cell integrity under severe water stress because of more efficient osmoprotection and antioxidation mechanisms.

**Keywords:** Antioxidant enzymes, drought tolerance, malondialdehyde, *Olea europaea*, mannitol

### 1. Introduction

Under Mediterranean climatic conditions, olive trees are often subjected to a variety of abiotic stresses affecting their productivity and even their survival. Water deficit is the most frequent of these constraints (Fernández and Moreno 1999; Sofó et al. 2007). Its impact is commonly aggravated by exposure to high light intensities typical of this region. Over-excitation of photoreceptor pigments can lead to the production of reactive oxygen species (ROS) which cause serious oxidative damage to vital cell components (Smirnoff 1993).

The accumulation of compatible solutes (osmotic adjustment) is one of the most common responses of plants to water deficit (Morgan 1984). In addition to improving water absorption capacity of the plant, these compounds are involved in the osmoprotection of membranes and macromolecules by replacing water molecules in their vicinity thus preventing the formation of intra-molecular hydrogen bonds that can cause irreversible structural disturbances (Chaves et al. 2003). Carbohydrates are among the most accumulated solutes in olive tree tissue under water deficit conditions (Bacelar et al. 2006; Ennajeh et al. 2006; Rejšková et al. 2007).

Plants have antioxidant mechanisms to scavenge ROS (Smirnoff 1993). These mechanisms consist of antioxidant enzymes and non-enzymatic molecules (Blokina et al. 2003). For instance, catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathion reductase (Gr) were demonstrated to play a major role in protecting olive leaf tissue against oxidative stress caused by water deficit (Sofo et al. 2005).

In this study, we compared the antioxidant defense mechanisms used by two olive cultivars differing in their resistance to drought, 'Chemlali' and 'Meski'. To evaluate structural and functional cell integrity, we assessed leaf lipid membrane peroxidation (MDA, malondialdehyde) changes depending on water deficit severity. Furthermore, we measured the abundance of soluble carbohydrates and phenolic compounds, and the activity of three antioxidant enzymes: CAT, POD and APX.

## 2. Materials and Methods

### 2.1. Plant material and experimental design

Two-year-old, self-rooted cuttings of olive cultivars 'Chemlali' and 'Meski' were transplanted in 10-dm<sup>3</sup> litre pots filled with a mixture of sandy soil and vermiculite (4:1, v/v). The plants were kept under a lath house covered with a shade net at the Faculty of Sciences of Gabès. The trees were watered weekly to field capacity alternately with tap water or a complete nutrient solution. After one month of growth under these conditions, water was withheld for 60 day for 45 trees of each cultivar. During this drought treatment, three trees of each cultivar were randomly selected every four days to perform physiological measurements and biochemical assays.

### 2.2. Plant water status

Plant water status was evaluated by measuring leaf relative water content (RWC;  $RWC = (FM - DM) \times 100 / (TM - DM)$ ), where FM is fresh mass, DM is dry mass and TM is the turgid mass of the leaf.

### 2.3. Carbohydrate content

Samples of 100 mg freeze-dried leaves were extracted for 15 min with 1 ml of 80% ethanol at 80°C in a shaking water bath. The extract was centrifuged at 10000×g for 10 min. The supernatant was saved and the pellet was re-extracted twice with boiling ethanol as described above. The three supernatants were pooled then filtered on a cartridge of activated charcoal and polyvinyl-polypyrrolidone (PVPP) to eliminate polyphenols. Aliquots of 20µl from each sample were analyzed with an HPLC (Shimadzu, LC-10AT; Detector: RID-10A; Integrator: C-R8A).

### 2.4. Lipid peroxidation

The degree of membrane lipid peroxidation was estimated by measuring MDA content using the thiobarbituric acid method described by Hodges et al. (1999). The MDA content was estimated as described by Heath and Packer (1968):

$$\text{MDA equivalent (nmol cm}^{-3}\text{)} = [(A_{532} - A_{600}) / 155000] 10^6$$

where  $A_{532}$  nm represents the maximum absorbance of MDA,  $A_{600}$  nm is a correction for non-specific turbidity and 155 000 is the molar extinction coefficient for MDA.

### 2.5. Enzyme activities

The activity of CAT was estimated as described by Aebi (1984). The H<sub>2</sub>O<sub>2</sub> decomposition was assessed spectrophotometrically by the decrease in absorbance at 240 nm. One unit of CAT activity corresponds to the amount of enzyme that decomposed 1 µmol of H<sub>2</sub>O<sub>2</sub> per minute. We used an extinction coefficient of 39.4 mM<sup>-1</sup> cm<sup>-1</sup>.

Total POD activity was determined spectrophotometrically by monitoring the formation of a colored complex from 3-dimethylamino benzoic acid (DMAB) and 3-methyl-2-benzothiazolinone hydrozone hydrochloride monohydrate (MBTH) at 590 nm in the presence of H<sub>2</sub>O<sub>2</sub>, according to the method described by Ngo and Lenhoff (1980). The specific enzyme activity expressed as the change in absorbance at 590 nm per minute and per milligram of protein was determined.

The activity of APX was determined following the method described by Ushimaru et al. (1997). The activity of APX was determined spectrophotometrically by assessing the decrease of ascorbate content at 290 nm. One unit of APX was defined as the amount of enzyme that oxidizes 1  $\mu\text{mol}$  of ascorbate per minute at ambient temperature. The absorption coefficient of  $2.47 \text{ mM}^{-1} \text{ cm}^{-1}$  was used for calculation.

### 3. Results

#### 3.1. Lipid peroxidation

The degree of membrane lipid peroxidation was estimated by measuring MDA content of leaf tissue (Fig. 1). MDA content increased when leaf RWC decreased for both cultivars. However, this increase was more acute in 'Meski' leaves. At high leaf RWC (90-85%), both cultivars showed low and identical MDA levels of  $18 \text{ nmol g}^{-1} \text{ DW}$ . When leaf RWC decreased, lipid peroxidation increased more significantly for 'Meski' than 'Chemlali'. Below a RWC of 85%, 'Chemlali' leaves had a MDA content lower than 'Meski' leaves for the same hydration level. At RWC of 40%, MDA content was 28 and  $38 \text{ nmol g}^{-1} \text{ DW}$  in 'Chemlali' and 'Meski' leaves, respectively.

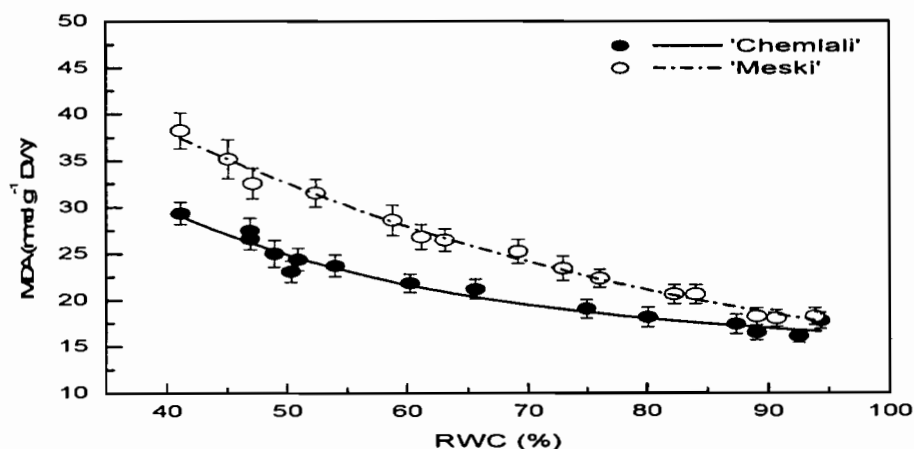


**Figure 1:** Changes in malondialdehyde (MDA) content as a function of leaf RWC in the leaves of two olive cultivars 'Chemlali' and 'Meski' subjected to water deficit by withholding watering. Means  $\pm$  SE,  $n = 3$ .

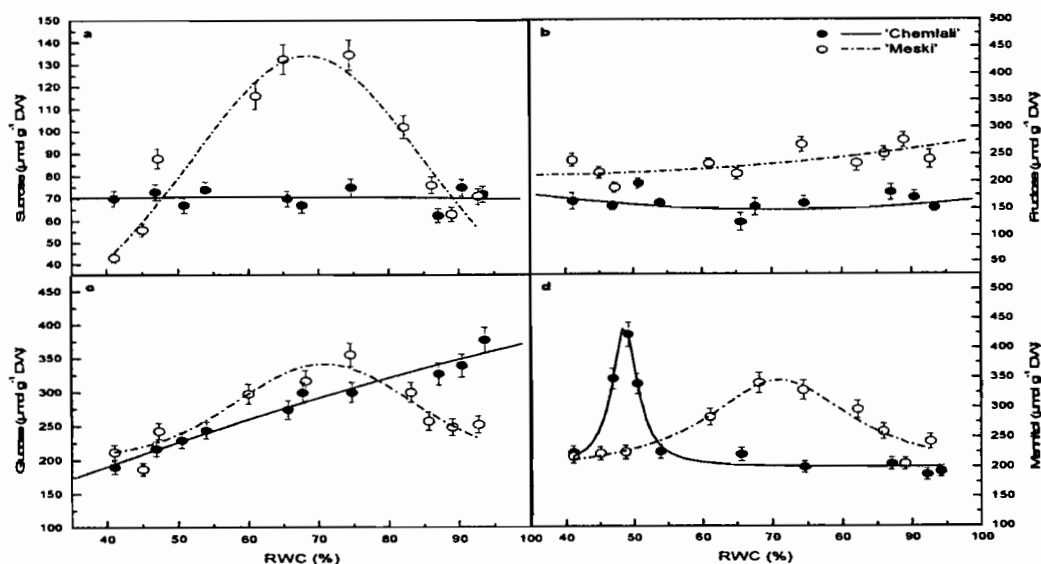


**Figure 2:** Changes in soluble sugar concentrations as a function of leaf RWC in the leaves of two olive cultivars 'Chemlali' and 'Meski' subjected to water deficit by withholding watering. a Sucrose, b Fructose, c Glucose and d Mannitol. Means  $\pm$  SE,  $n = 3$ .



### 3.2. Carbohydrates

Changes of leaf sucrose content as leaf RWC decreased differed by cultivar (Fig. 2a). In the case of 'Chemlali', sucrose content did not change. Whereas, in the case of 'Meski', sucrose content increased rapidly to reach its maximum at RWC of 70%. It, then, dropped to lower values than those found in 'Chemlali' leaves for RWC below 50%.

Fructose content was unchanged in both cultivars for leaf RWC ranging from 95% to 40% (Fig. 2b). However, 'Meski' showed fructose contents slightly higher than 'Chemlali'.

Glucose content did not differ between the two cultivars for RWCs between 80 and 40% (Fig. 2c). However, for leaf RWCs greater than 85%, glucose content in 'Chemlali' leaves was higher than that of 'Meski' leaves. This content increased, in 'Meski', until reaching a maximum value slightly higher than that of 'Chemlali' at RWC of 70%. Glucose content decreased progressively until reaching identical values for both cultivars at a RWC of 55%.

Both cultivars had similar mannitol content for leaf RWC above 85% (Fig. 2d). When leaf RWC decreased, mannitol content in 'Meski' leaves increased until reaching a peak ( $350 \mu\text{mol g}^{-1} \text{DW}$ ) at RWC of 70%. Below this hydration level, the concentration of this sugar decreased gradually to its initial level. In the leaves of 'Chemlali', mannitol started to accumulate at leaf RWC of 55% and reached a maximum ( $425 \mu\text{mol g}^{-1} \text{DW}$ ) at a RWC of 48%. At a RWC of 40%, both cultivars had similar mannitol contents.

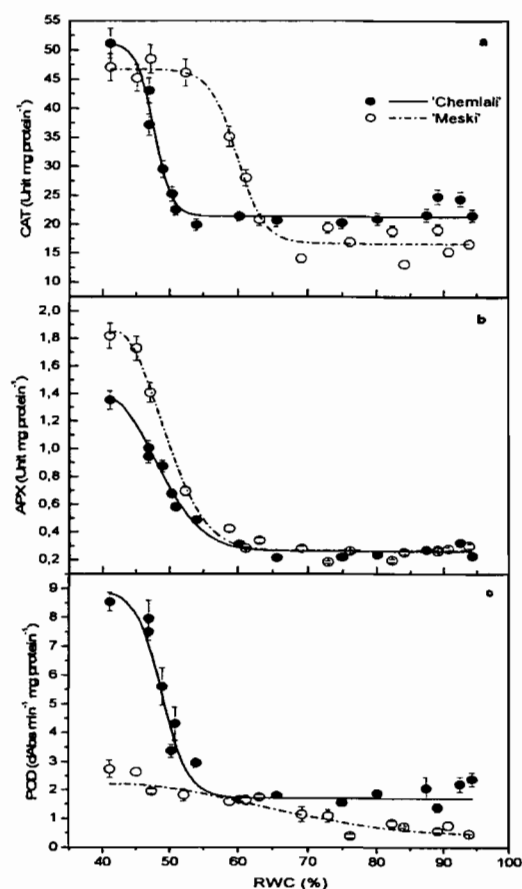
### 3.3. Antioxidant enzymes

Initial CAT activity was similarly low in the leaves of both cultivars (Fig. 3a). When RWC decreased, CAT activity increased, but this increase was more precocious in 'Meski'.

APX activity as function of leaf RWC showed a similar trend for both cultivars (Fig. 3b). This activity was low at RWC between 95-60%. Induction of APX started at a RWC below 60%. The increase in APX activity was more gradual in the leaves of 'Chemlali' compared to those of 'Meski'. At a RWC of 40%, APX activity was 6- and 4-times its initial values in 'Meski' and 'Chemlali', respectively.

Specific POD activity differed between the two cultivars (Fig. 3c). It was lower in 'Meski' leaves than in 'Chemlali' leaves. In the case of the latter cultivar, induction of POD activity was at a leaf RWC of 60%. At a RWC of 40%, this activity was 4-times and two times higher its initial value in 'Chemlali' and 'Meski', respectively.

**Figure 3:** Changes in the activities of three antioxidant enzymes as a function of leaf RWC in the leaves of two olive cultivars 'Chemlali' and 'Meski' subjected to water deficit by withholding watering. a catalase (CAT), b ascorbate peroxidase (APX) and c peroxidase (POD). Means  $\pm$  SE,  $n = 3$ .



#### 4. Discussion

Leaves of both cultivars showed increases in MDA content when RWC decreased (Fig. 1). This confirms that under severe water deficit conditions, olive leaf chloroplasts become more sensitive to oxidative damage, and this may be the main cause of photosynthetic apparatus functionality decline (Angelopoulos et al. 1996). However, it appears that 'Meski' is more sensitive to this type of damage than 'Chemlali'. This confirms that 'Chemlali' is more drought-tolerant than 'Meski'.

Many compatible solutes amongst carbohydrates can be accumulated in plants to ensure osmotic adjustment. In addition to their role in osmotic adjustment, these solutes can be also involved in osmoprotection (Toldi et al. 2009).

As water deficit intensified, soluble sugar content and composition changed differently depending on the olive cultivar considered (Fig. 2).

'Meski' showed a high diversity of soluble sugars involved in osmotic adjustment. Early accumulation of soluble sugars in this cultivar suggests that these solutes were involved in osmotic adjustment more than in osmoprotection. However, 'Chemlali' accumulated mainly mannitol. Large and delayed mannitol accumulation, especially under severe water stress conditions in 'Chemlali' leaves, suggests that this polyol was more important for osmoprotection than for improving water uptake (osmotic adjustment). Mannitol plays a role in the destruction of ROS (Vendruscolo et al. 2007).

Furthermore, under severe water stress, plants used enzymatic antioxidant system to destroy ROS (Foyer et al. 1994). The activity of three antioxidant enzymes, CAT, POD and APX, increased with the desiccation of leaf tissue for both olive cultivars. Our results confirmed those of Sofo et al. (2005). However, the activity of CAT and POD increased earlier in 'Meski', but was more acute in 'Chemlali'. These differences may explain, at least partially, the better ability of 'Chemlali' to maintain its cell membrane integrity.

In conclusion, the better tolerance of 'Chemlali' to severe water deficit can be attributed to a better osmoprotective and antioxidative responses. A larger accumulation of antioxidant molecules, carbohydrates (especially mannitol) and phenolic compounds, and increased antioxidant enzyme activities can, at least partly, justify the resilience of 'Chemlali'.

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## Short term responses of Chemlali olive tree to water salinity

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### Abstract

The water scarcity in Mediterranean region appears as one of the main factors limiting agricultural development. The use of saline water is a promising alternative. Olive is considered as a moderately salt tolerant plant, however salt tolerance in olives appears to be cultivar-dependent. Few data concerning responses of 'Chemlali' olive trees to water salinity are available in literature. An experiment was performed to ascertain the effects of short term irrigating of 'Chemlali' olive trees with saline water on the chemical properties of a sandy soil on salt accumulations in roots, leaves and fruits. The effect of water salinity on oil quality was also examined. These evaluation were made through three periods: nine months, two years and four years. The trees were subjected to one of three irrigation treatments: Trees cultivated in rain-fed conditions (T1), trees irrigated with moderate saline water (T2) and others receiving high saline water (T3). For both saline treatments an annual amount of 5,000 m<sup>3</sup> of water was supplied. Soil chemical characteristics were evaluated by measuring soil electrical conductivity (ECe) and levels of Na and Cl. Salt concentrations were determined in plant tissues (roots, leaves and fruits). Fresh fruit weight, oil content, fatty acid composition, induction time, total polyphenol and total chlorophyll contents were determined and reported. Data showed that irrigating with saline waters caused a significant increase of salt (Na and Cl) concentrations in soil and in plant tissues throughout the entire period of the experiment. Results revealed that root sodium and chloride concentration in olive trees increases with increasing NaCl in the soil. Leaf concentrations of Na<sup>+</sup> and Cl<sup>-</sup> were always under toxicity levels (0.2 and 0.5%, respectively) at short term, however continuous irrigation with high saline water could cause leaf Cl<sup>-</sup> toxicity. Results reported that different levels of water salinity did not affect the fresh fruit weight, the chlorophyll and the oleic acid contents. However, relative to 'Chemlali' cultivar, the high saline treatment increase the oil content and decrease the total polyphenol contents.

**Key words:** Olive tree, irrigation, high saline water, moderate saline water, rainfed conditions.

### 1. Introduction

Olive tree (*Olea europaea* L.) is the major tree crops in some Mediterranean countries. Under the arid and semi arid climate of Tunisia the severe droughts and the climate changes are between the most important factors limiting olive trees productivity (Ben Ahmed *et al.* 2007; Ben Rouina *et al.* 2007). Hence, these last years irrigated olive orchards are being extended and the yearly increase of irrigated areas exceeds 6000 hectares. In such regions, the competition for scarce water resources among users will inevitably reduce the supplies of freshwater available for crop irrigation. As a consequence, agriculture will increasingly be forced to utilize marginal waters such as brackish water or reclaimed wastewater to meet its increasing demands, which in turn increases the risks of soil salinization and yield reduction (Paranychiakis and Chartzoulakis, 2005).

Opportunities exist as saline treated wastewater (TWW) from municipal origin is rich in organic matter and also contain appreciable amounts of major and micronutrients (Bedbabis *et al.* 2008). Accordingly nutrient levels of soils are expected to improve considerably with continuous irrigation with TWW. Again these effluents may contain variable amounts of sodium and chloride, which may limit the long-term use for agricultural purposes as a likelihood of soil salinization and negative environmental effects (Grattan and Grieve, 1999; Parida and Das 2005).

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In fact, the long-term impact of irrigation with poor quality waters on physico-chemical properties of soils has been reported to depend upon the interaction of several factors like the unique water quality limiting parameters and site-specific crop, soil and climatic conditions (Loreto *et al.* 2003; Chartzoulakis *et al.* 2004). Although the concentrations of heavy metals in TWW are low, long-term use of this sewage on agricultural lands often results in the build-up of the elevated levels of these metals in soils (Rattan *et al.*, 2002).

In order to cope with salinity stress most plants generate a variety of mechanisms, which differentiate substantially among species, varieties, soil texture and climate conditions (Munns, 2002; Ben Ahmed *et al.* 2008 a & B. Ben Rouina *et al.* 2006). These mechanisms operate in a coordinated manner both at a cellular and a whole-plant level. In olive trees, salt tolerance is associated with their ability to restrict salt accumulation in leaves and shoots (Chartzoulakis *et al.* 2004; Ben Ahmed *et al.* 2006; 2008a & b; Ben Rouina *et al.* 2006) where damage is closely associated with  $\text{Na}^+$  and  $\text{Cl}^-$  accumulation.

Several studies focused on the effect of irrigation with fresh water on olive oil composition (Baccouri *et al.* 2007), yet only a few studies took into account the effect of TWW, as a water source of irrigation, in both olive tree growth, yield and oil quality parameters (Bedbabis *et al.* 2008). Therefore, the scope of the work discussed here was to study the effect of irrigation with TWW on "Chemlali" olive trees leaf nutrients, yield and oil quality.

## 2. Material and methods

The trial was carried out during two crop seasons (2006 and 2007), on the Chemlali olive trees at the site of El Hajeb (Sfax: 34°43N - 10°41E, Tunisia) in two experimental plots which are characterized by sandy soil (sand: 84.4%, clay: 9.8%, silt: 5.8%). Eighteen year old trees spaced 24 x 24 m, were used in a randomized complete block design with two different treatments. Each experimental plot consisted of 20 trees, with four replications of 5 trees each, and per treatment. Since January 2001, both plots were subjected to the following treatments: irrigation with well water and irrigation with treated wastewater (Tab. 1). All the plants were irrigated by submersion with the same amount of water (4000  $\text{m}^3 \text{ha}^{-1} \text{year}^{-1}$ ; 66% of  $\text{ETc}$ ) and subjected to the same common olive orchard practices of Sfax area.

**Table 1:** Characterization of irrigation with saline waters (Mean Values).

| Element           | Unit                | Well water | Treated wastewater |
|-------------------|---------------------|------------|--------------------|
| pH                | -                   | 7.95       | 7.60               |
| BOD               | $\text{mg L}^{-1}$  | -          | 22                 |
| COD               | $\text{mg L}^{-1}$  | -          | 73                 |
| S. S.             | $\text{mg L}^{-1}$  | -          | 59                 |
| E. C.             | $\text{mS cm}^{-1}$ | 3.44       | 6.30               |
| Dry matter(105°C) | $\text{mg L}^{-1}$  | 2700       | 4660               |
| P                 | $\text{mg L}^{-1}$  | 0.36       | 5.9                |
| $\text{K}^+$      | $\text{mg L}^{-1}$  | 35         | 220                |
| $\text{NH}_4^+$   | $\text{mg L}^{-1}$  | 12         | 85                 |
| $\text{NO}_3^-$   | $\text{mg L}^{-1}$  | 28         | 521                |
| $\text{Ca}^{2+}$  | $\text{mg L}^{-1}$  | 230        | 260                |
| $\text{Mg}^{2+}$  | $\text{mg L}^{-1}$  | 102        | 242                |
| $\text{HCO}_3$    | $\text{mg L}^{-1}$  | 512        | 749                |
| $\text{CO}_3$     | $\text{mg L}^{-1}$  | 66         | 99                 |
| $\text{Na}^+$     | $\text{mg L}^{-1}$  | 555        | 940                |
| $\text{Cl}^-$     | $\text{mg L}^{-1}$  | 780        | 1999               |
| $\text{Zn}^+$     | $\text{mg L}^{-1}$  | -          | 0.47               |

## 2.1. Soil sampling

Five topsoil samples (horizon 0 – 20 cm) were yearly collected randomly from each plot and evaluated for pH, electrical conductivity, organic matter and soil macro and micronutrients including N, P, K, Na, Cl, Zn, Mn, Cd and Pb. The principal chemical properties of soils examined were determined by conventional methods on samples that were previously air-dried, crushed and passed through a 2-mm sieve.

Briefly: (1) pH was determined at a ratio soil : water = 1:2.5; (2) EC was measured on water extracts obtained at a ratio soil : water = 1:5; (3) total organic C (TOC) by dichromate oxidation followed by titration with ferrous ammonium sulphate; (4) total N content by the Kjeldahl method; (5) available P and K contents were determined, respectively, by the Olsen method and by ICP on 1M BaCl<sub>2</sub> soil extracts using a ratio soil : extractant = 1:10; (6) Heavy metals were determined using a Perkin Elmer A Analyst 300 atomic absorption spectrophotometer.

## 2.2. Vegetal samples

Four current season shoots per experimental tree were randomly collected in paper bags and stored in a portable cooler twice by year (January and July) around the tree at heights of 1.5 – 2.0 m above the ground. In the laboratory, leaves and stems were separated from each shoot. Leaves were dried at 60 °C for 48 h in oven and ground with a grinder. Approximately 1 g of plant tissues was mineralised at 450 °C in a muffle furnace. After dissolution in 1N HNO<sub>3</sub> and dilution with distilled water, the ash samples were analyzed for N, P, K, Ca, Na, Cl and trace elements by using the same methods as for soil samples.

## 2.3. Statistical analysis

ANOVA analyses were conducted on the data considering both the treatments and the crop seasons as independent variables. All statistical analyses were carried out with the program SPSS 11.0 for Windows, statistical software (SPSS Inc., Chicago, IL, USA). The LSD test was used to determine significant differences in each parameter between treatments and crop seasons.

## 3. Results and discussions

### 3.1. Effects on soil fertility indicators and contaminants

All the waters were alkaline in reaction. The pH of the TWW (7.6) was lower than the ground water (7.95) while its salt content at 105 °C (4660 mg L<sup>-1</sup>) was considerably higher than this of well water (2700 mg L<sup>-1</sup>).

Results showed that the continuous use of TWW for irrigation decrease slightly soil pH (Tab. 2). The little alkali value was related to an elevated buffering capacity of south Tunisian soils loaded in limestone and the intense ammonification reactions of organic matter. Also, the analysis of soil samples reveals higher salt content on soil at the plot irrigated with TWW. Despite the leaching action, salts were accumulating in surface than the underlying soil layers. Hence, there was a gradual increase in EC at top soil depth (0-40 cm) from the beginning to the end of sub irrigation; with a marked differences in magnitude between the two different water qualities (3.6 - 5.4 mS cm<sup>-1</sup>, respectively in 2006 and 2007 for the TWW plot vs. 1.1 - 1.4 mS cm<sup>-1</sup> in well water irrigated soil). The increase in EC of the treated wastewater irrigated soil is one of a negative impact of treated wastewater reuse.

**Table 2:** Soil chemical characteristics after two sampling years (2006 – 2007) in relation with water qualities (TWW = treated wastewater; VW = well water).

| Water Qualities | year | pH  | EC mS | OM % | N ppm | P ppm | K ppm | Na ppm | Cl ppm | Zn ppm | Mn ppm | Cd + Pb ppm |
|-----------------|------|-----|-------|------|-------|-------|-------|--------|--------|--------|--------|-------------|
| TWW             | 2006 | 7.6 | 3.6   | 0.63 | 180   | 115   | 505   | 882    | 1709   | 25     | 114    | <0.004      |
|                 | 2007 | 7.6 | 5.4   | 1.17 | 229   | 105   | 670   | 998    | 1902   | 33     | 127    | <0.004      |
| VW              | 2006 | 7.9 | 1.1   | 0.51 | 107   | 47    | 250   | 401    | 512    | 8      | 18     | -           |
|                 | 2007 | 7.9 | 1.4   | 0.69 | 125   | 42    | 270   | 425    | 619    | 9      | 21     | -           |

After some years of continuous irrigation with TWW, subsurface soil EC increase until  $5.4 \text{ mS cm}^{-1}$  but remained within the tolerance range for olive trees of  $6\text{--}7 \text{ mS cm}^{-1}$  (Chartzoulakis *et al.* 2004; Ben Ahmed *et al.* 2008 a&b, Ben Rouina unpublished data; Tattini and Travesti, 2008). However, continued long-term irrigation with treated wastewater could cause further soil salinization and could lead to a reduction in crop yield (Muns and Tester, 2008).

Increasing organic matter (OM) inputs via TWW was observed. The OM content was higher in the arable layer and varied between 0.63% and 1.17% compared with 0.51% and 0.69% in the well water plots. Soil OM richness influences a wide range of physical, chemical and biological properties of soil and is considered the most important indicator of soil quality.

At TWW experimental plots, the concentrations of N, P and K was significantly better than in WW treatment, which reflected the higher NPK input levels ( $242 \text{ Kg N}$ ,  $50 \text{ Kg P}$  and  $185 \text{ Kg K ha}^{-1}$  per year) suggesting that treated wastewater increased levels of available N, P and K in the soil. These results agree with those of Emongor and Ramolemana (2004), who found that the use of sewage effluent for irrigation of horticultural crops will supply some of the plant requirements of macro nutrients.

Sodium and chloride soil concentrations increased significantly in TWW plots (Tab. 2). Sodium can cause soil aggregates to breakdown (disperse) and clay particles to swell, sealing the pores of the soil and making it impermeable to water flow. As well, suspended solids from the TWW ( $59 \text{ mg L}^{-1}$ ) can obstruct soil pores and reduce soil respiration and water holding. The water infiltration rate was decreased by half from  $19 \text{ cm h}^{-1}$  in the well water irrigated plots to  $10 \text{ cm h}^{-1}$  in TWW treatments (Fig. 1).

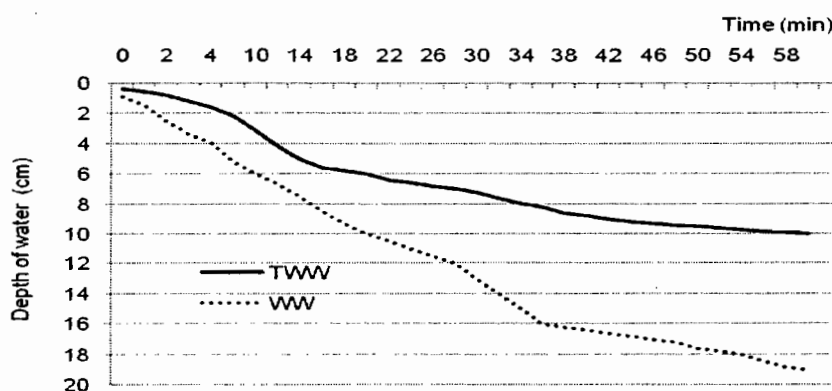


**Figure 1:** Water infiltration ( $\text{cm min}^{-1}$ ) in irrigated soil (TWW = treated wastewater irrigated plot, WW = well water irrigated plot).

These results suggest that irrigation with TWW has reduced water infiltration and agree with findings reported by Tedeschi and dell Aquila (2005), who found a reduction in soil permeability and a decrease of water infiltration.

Heavy metals can bind with organic matter, making them immobile in the organic rich topsoil layer. Similarly, soil richness of organics also influences the quantity of soil organic carbon development. Zn concentrations showed a high significant accumulation ( $p < 0.001$ ) in treated wastewater irrigated soil ( $33 \text{ vs. } 8 \text{ mg kg}^{-1}$ ) and may be attributed to the addition of Zn by irrigation water ( $0.42 \text{ mg L}^{-1}$ ). Several authors reported that treated wastewater, biosolids and sewage sludge application caused a significant build up of total Zn in the topsoil. Zinc accumulation in the treated wastewater irrigated topsoil can be caused by complexation phenomenon with organic matter. The build up of Zn concentration can be hazardous due to its antagonist role in controlling other metals availability, in conjunction with soil pH and organic matter, and its influence on nitrogen fixation, nitrification and decomposition microbial processes.

Soil Mn concentration varied significantly ( $p < 0.001$ ) between both experimental plots. Average Mn concentrations in the irrigated TWW soil was  $114 \text{ mg kg}^{-1}$  compared to  $18 \text{ mg kg}^{-1}$  in the WW irrigated soil (Table 2). Mn accumulation in the topsoil was likely a result of complexation with organic matter.

Soil Cd and Pb concentrations were lower than the detection limit of  $0.004 \text{ mg kg}^{-1}$ . Soil concentrations remained well below the toxic limits of  $3 \text{ mg kg}^{-1}$  for Cd and  $300 \text{ mg kg}^{-1}$  for lead. The treated



wastewater used in this experiment did not contain measurable concentrations of either Cd or Pb and therefore did not contribute to build-up of these heavy metals in soil.

### 3.2. Olive tree mineral contents

Leaf nitrogen and phosphorus status were enhanced by TWW (Tab. 3). Statistical analysis showed significant differences ( $p < 0.05$  for N and P) between them. N values were ranged between 0.95% and 1.79% in TWW irrigated plots and between 0.72% and 1.62% in WW plots. P concentrations involved between 0.03% and 0.16% in olive trees irrigated with treated wastewater and between 0.03% and 0.11% in these irrigated with water coming from well. These results suggested that treated wastewater can be considered as a complementary fertilization. The fertilized value of this type of water was reported by several authors.

Irrigation with saline treated wastewater induced the increase of both  $\text{Na}^+$  and  $\text{Cl}^-$  contents in all plant tissues (leaves, shoots and roots data no shown in this section). In leaves, both  $\text{Na}^+$  and  $\text{Cl}^-$  contents were statistically higher in TWW than in WW treated plants. At the end of the trial period (2007) leaf sodium concentrations ranged between 0.47 and 2.45  $\text{mg g}^{-1}$  of dry weight, respectively for WW and TWW irrigated trees.

Similarly to  $\text{Na}^+$  content pattern, the  $\text{Cl}^-$  was increased with the water salinity level. In contrast to the distribution patterns of  $\text{Na}^+$  and  $\text{Cl}^-$ , salt accumulation decreased  $\text{K}^+$  concentrations in leaves. Leaf  $\text{K}^+$  concentrations oscillated between 0.25% and 1.03% in olive trees irrigated with TWW and between 0.37% and 1.67% for olive trees irrigated with WW, respectively ( $p < 0.01$ ). Indeed,  $\text{Na}^+\text{Cl}^-$  accumulation in root zone causes the development of osmotic stress and disrupts cell ion homeostasis by inducing both the inhibition in uptake of essential nutrients such as  $\text{K}^+$  and the accumulation of toxic ions ( $\text{Na}^+$  and  $\text{Cl}^-$ ) at higher level (Paranychianakis and Chartzoulakis, 2005). This ionic disturbance led probably at long term to the decrease of K / Na ratio, and could alter photosynthetic performances and thus growth and yields (Ben Rouina *et al.* 2006; Ben Ahmed *et al.* 2006; 2008a et b).

**Table 3:** Olive tree Chemlali leaf nutrients concentrations during two sampling years (2006 – 2007) in relation with water qualities (TWW = treated wastewater; WW = well water). The mean values indicate the yearly average of each nutrient.

| Plot | year | Values | N (%) | P (%) | K (%) | Ca (%) | Na (mg/g) | Cl (mg/g) | Zn (mg/kg) | Mn (mg/kg) |
|------|------|--------|-------|-------|-------|--------|-----------|-----------|------------|------------|
| TWW  | 2006 | Max    | 1.79  | 0.147 | 1.03  | 2.96   | 2.45      | 2.35      | 48.4       | 73.7       |
|      |      | Min    | 1.23  | 0.072 | 0.42  | 1.09   | 2.11      | 1.36      | 30.5       | 19.2       |
|      |      | Mean   | 1.52  | 0.095 | 0.94  | 1.95   | 2.32      | 1.81      | 39.2       | 55.0       |
|      | 2007 | Max    | 1.44  | 0.107 | 1.12  | 2.69   | 2.51      | 0.73      | 42.3       | 55.1       |
|      |      | Min    | 0.95  | 0.056 | 0.25  | 1.52   | 2.33      | 0.18      | 33.2       | 13.0       |
|      |      | Mean   | 1.27  | 0.087 | 0.77  | 2.01   | 2.45      | 0.46      | 37.0       | 39.2       |
| WW   | 2006 | Max    | 1.62  | 0.113 | 1.67  | 2.92   | 0.63      | 2.59      | 26.1       | 44.3       |
|      |      | Min    | 1.12  | 0.028 | 0.70  | 1.87   | 0.36      | 1.82      | 11.0       | 14.3       |
|      |      | Mean   | 1.45  | 1.072 | 1.23  | 2.29   | 0.46      | 2.05      | 18.2       | 25.0       |
|      | 2007 | Max    | 1.53  | 0.091 | 1.32  | 2.89   | 0.71      | 0.78      | 16.5       | 35.1       |
|      |      | Min    | 0.72  | 0.035 | 0.37  | 1.12   | 0.25      | 0.37      | 15.5       | 15.5       |
|      |      | Mean   | 1.27  | 0.065 | 0.85  | 1.97   | 0.47      | 0.63      | 16.0       | 26.2       |

Both leaf Mn and Zn concentrations were higher in trees irrigated with treated wastewater than in those irrigated with water coming from well. Statistically significant differences were showed between them ( $p < 0.05$ ). Mn values oscillated between 39 and 55  $\text{mg kg}^{-1}$  in trees irrigated with treated wastewater and 25  $\text{mg kg}^{-1}$  in those irrigated with water of well. These concentrations were slightly higher in trees irrigated with treated wastewater (30.5 – 48.4  $\text{mg kg}^{-1}$ ) than in those irrigated with water taken from well (11 - 26  $\text{mg kg}^{-1}$ ). Leaf Mn and Zn concentrations in olive trees irrigated with TWW indicate that there was no pattern of metal accumulation in plants.

Leaf lead and cadmium concentration were very small as they are lower to 0.004  $\text{mg kg}^{-1}$ . These little concentrations were explained by the lower concentrations to 0.004 ppm as well as incorporations of



agent's chelators (detergents) in soil by treated wastewater that can decrease lead and cadmium accumulation in plant.

## Conclusion

Although treated wastewater was used since January 2001 in irrigation of Chemlali olive trees orchard, this study has given preliminary results on the medium term effect of TWW on soil chemical properties, leaf nutrients and oil chemical quality.

Five years after irrigation beginning data shown that irrigation affect positively the soil fertility but reveals higher toxic salt content on soil irrigated with TWW. Despite the leashing action,  $\text{Na}^+$  and  $\text{Cl}^-$  were accumulated in surface than the underlying soil layers with severe risks of soil salinization. Soil Cd and Pb concentrations were lower than the detection limit of  $0.004 \text{ mg kg}^{-1}$  and remained well below the toxic limits of  $3 \text{ mg kg}^{-1}$  for Cd and  $300 \text{ mg kg}^{-1}$  for lead. Irrigation with saline treated wastewater induced the increase of leaves  $\text{Na}^+$ ,  $\text{Cl}^-$ , N and P contents. However, leaf  $\text{K}^+$  concentrations were reduced by  $\text{Na}^+\text{Cl}^-$  accumulation in root zone.

It seems that irrigation with TWW influenced fatty acid composition, UV specific extinction and polyphenols. In particular, irrigation with TWW increased palmitic, palmitoleic, linoleic and linolenic acid contents and decreased oleic acid rates. In addition, a decrease of polyphenol content was recorded. Yet in order to better clarify the obtained results further investigation should be conducted on the relationship between plant nutritional status and oil quality parameters.

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## Water use strategies of olive tree through an integrated approaches between sap flow and high resolution stem growth analysis

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### Abstract

The effect of different irrigation levels on olive plants was studied with the objective to improve the water use efficiency and to assess the drought adaptive strategy. The field trial was conducted in the experimental station of the CNR of central-southern Italy (41°06'N, 14°43'E). The irrigation treatments were a rainfed control and a fully irrigated treatment (100% of Etc). Stem Maximum Daily Shrinkage (MDS) and sap flux methods were compared. Results were evaluated to investigate water use strategies, as well as functional and structural acclimation of olive plants to partial watering. These observations were discussed for highlighting valuable parameters to be implemented in automatic management irrigation practices in olive trees.

**Keywords:** Point dendrometers, sap flow, irrigation, plant water status, water use efficiency.

### Résumé

Les effets de différents régimes d'irrigation sur l'olivier a été étudié avec l'objectif d'améliorer l'efficacité de l'utilisation de l'eau et de comprendre la stratégie de l'adaptation du plant au contrainte hydrique. L'expérience a été conduite près de la station expérimentale du CNR située dans le centre-sud de l'Italie (41°06'N, 14°43'E). Les traitements d'irrigation ont été : la complète satisfaction de la demande en eau pendant toute la saison d'irrigation (100% de l'Etc), un traitement pas irriguée. Avec cette étude on a comparée le *Stem Maximum Daily Shrinkage* (MDS) et le *sap flux*. Les résultats ont permis d'avoir une première évaluation de l'acclimatation fonctionnelle et structurale du plant d'olivier au contrainte hydrique. Ces observations peuvent être utilisées pour améliorer l'implémentation des systèmes d'arrosage automatique.

**Mots clés:** Dendromètre, sap flow, irrigation, état hydrique de la plante, efficacité de l'utilisation de l'eau.

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## **Thème 5**

### **Bonnes pratiques de fabrication de l'huile d'olive et de l'élaboration des olives de table**

## Polyphenols et photooxydation de l'huile d'olive vierge

I. Janati Idrissi & M. Rahmani

### Resumé

Le pouvoir antioxydant des composés phénoliques a été évalué par le suivi de l'indice de peroxyde au cours de la photooxydation de l'huile d'olive décolorée en présence de la chlorophylle a, à une intensité lumineuse de 2,62 W/m<sup>2</sup>. Le pouvoir antioxydant des composés phénoliques testés décroît dans l'ordre :hydroxytyrosol > acide caféique> tyrosol> acide syringique.

Le mécanisme de désactivation de l'oxygène singulet (<sup>1</sup>O<sub>2</sub>) par les composés phénoliques a été déterminé en étudiant la cinétique de photooxydation de l'huile d'olive décolorée en présence de la chlorophylle a et de différentes concentrations de composés phénoliques. Les résultats ont montré que les polyphénols réduisent la photooxydation en désactivant l'oxygène singulet.

**Mots clés :** Composés phénoliques, huile d'olive vierge, photooxydation, oxygène singulet.

## Phenolic compounds and photoxidation of virgin olive oil

### Abstract

The antioxidant effect of phenolic compounds was measured by peroxide value, during the photoxidation of decoloured olive oil, in presence of chlorophyll a and a light intensity of 2,62 W/m<sup>2</sup>. The tested phenolic compounds showed the following order of antioxidant effect: hydroxytyrosol > caffeic acid> tyrosol> syringic acid.

Mechanism of singlet oxygen (<sup>1</sup>O<sub>2</sub>) deactivation by phenolic compounds was determined from the kinetics of decoloured olive oil, in presence of various concentrations of chlorophyll a and phenolic compounds. The results showed that antioxidant effect of phenolic compounds was due to their ability to deactivate singlet oxygen.

**Key words:** Phenolic compounds, virgin olive oil, photoxidation, singlet oxygen.

## **Evaluation de la fraction volatile d'huiles d'olives vierges commerciales tunisiennes et italiennes: relation avec les attributs olfactifs**

Faten Kotti, Lorenzo Cerretani, Mohamed Gargouri, Emma Chiavaro and Alessandra Bendini

### **Résumé**

La fraction volatile de quatorze huiles d'olives vierges commerciales extraites à partir de variétés tunisiennes et italiennes de différentes zones géographiques, est analysée par micro-extraction en phase solide (HS-SPME) suivie d'une identification des composés volatils par chromatographie en phase gazeuse couplée à la spectrométrie de masse (GC-MS). Ces échantillons ont été également soumis à des analyses olfactives par des dégustateurs qualifiés afin de définir leurs attributs olfactifs. Les concentrations des composés volatils et les fragrances ont montré une large variabilité entre les différents échantillons affirmant que la typicité de l'huile d'olive vierge, en termes de composition et de caractéristiques organoleptiques, est étroitement dépendante de la variété et de la qualité des olives. Particulièrement, les huiles d'olives vierges de la variété Chétoui ont révélé des profils de volatils et des notes sensorielles très similaires aux échantillons italiens. Les résultats relatifs aux composés volatils majeurs ont été reliés aux notes olfactives positives et négatives. Les défauts sensoriels détectés dans certains échantillons ont été confirmés par la présence de composés volatils marqueurs.

**Mots clés:** Huile d'olive vierge, composés volatils, SPME/GC-MS, attributs olfactifs, origine géographique.

## **Evaluation of the volatile fraction of commercial virgin olive oils from tunisia and italy: relation with olfactory attributes**

### **Abstract**

The volatile profile of fourteen commercial virgin olive oils (VOOs) produced by Tunisian and Italian olive cultivars from different geographical areas was evaluated by means of Headspace-Solid Phase Microextraction (HS-SPME) and gas chromatography-mass spectrometry (GC-MS) analysis. The samples were also analyzed by a group of fully trained assessors to define their olfactory attributes. The concentrations of volatile compounds as well as the nuances exhibited large variability among different kind of samples confirming that the typicality of VOOs in terms of both organoleptic and compositional characteristics greatly depends on both olive variety and quality. In particular, VOOs from Chetoui were found to exhibit both volatile profiles and sensory notes very similar to Italian samples. Data relative to the main volatile compounds were related to both positive and negative olfactory notes and sensory defects found in some oil samples were well confirmed by volatile marker presence.

**Keywords:** virgin olive oil, volatile compounds, SPME/GC-MS, olfactory attributes, geographical origin.

## Optimisation des conditions de saumurage de la variété picholine

Hayet Fourati Nouri, Fatma Bakkeri

### Résumé

Dans le présent travail on se propose de déterminer les conditions optimales de saumurage des olives de la variété Picholine élaborées à la sévillane à travers des analyses physico-chimiques à savoir le pH, l'acidité libre, l'acidité combinée et la teneur en sel.

Les résultats obtenus montrent qu'une concentration en soude de 3% et une concentration initiale en sel de 9°Be permettent de garantir un produit fini de bonne qualité.

**Mots clés:** olive de table, Picholine, concentration de soude, concentration de sel, acidité libre.

## Optimization of the brining conditions of two Tunisian olive table varieties

### Abstract

There are three basic commercial preparations of olive table in the international market, namely Spanish style green olives, naturally olives (Greek-style) and black ripe olives (Californian style). In general, any processing method aims to remove the natural bitterness of the fruit, caused by the oleuropein. In this work we propose to study the suitability for seveillane development of two Tunisian olive table varieties, Picholine and Chemchali. This work focuses on the determination of the soda and salt concentrations through physicochemical analysis such as free acidity, combined acidity, salt content and pH. Obtained results shows that a concentration of 3° Be of soda and 9° Be as salt concentration, are noteworthy for the Picholine variety, while concentrations advisor for Chemchali are 2° Be and 7° Be respectively.

**Key words:** Table olive, Picholine, Chemchali, NaCl concentration, soda concentration, free acidity.

### 1. Introduction

En Tunisie, les oliviers de table occupent environ 24000 ha concentrés en grande partie au nord du pays (70-80%). La production nationale en olives de table est de l'ordre de 18000T. Les exportations tunisiennes sont en moyenne de 500 tonnes par an GICA (2007). Cependant, le produit tunisien est concurrencé sur le marché par ceux en provenance du Maroc et de l'Espagne principalement. Il est donc nécessaire de créer de nouvelles unités modernes capables de maîtriser les techniques de production répondant aux normes et de réduire les coûts de production dans le but de battre de nouveaux marchés.

En Tunisie, environ 50% des olives de table vertes sont soumises au processus d'élaboration à la sévillane. Ce procédé se base essentiellement sur le traitement alcalin à l'aide d'une solution de soude caustique, les lavages et la mise en saumure dans une solution de chlorure de sodium en vue d'une fermentation lactique naturelle.

(Kachouri et Boukaili 1993), Chammem (1999), Chammem et al. (2005) ont mené des travaux sur les techniques de saumurage de la variété Meski à la Sévillane en faisant varier un certain nombre de facteurs pouvant avoir une incidence sur la qualité du produit fini. Ces auteurs ont montré des concentrations en soude de 2% et 9% en sel permettent de garantir la qualité du produit fini pour cette variété. Etant donné que ces deux paramètres varient selon la variété (De La Borbolla et Alcalá 1981), Amiot et al. (1990), De la torre et al. (1993), la recherche de la concentration de soude ainsi que la concentration initiale de la saumure en sel s'avère donc primordial afin de garantir la qualité du produit fini.

Le présent travail s'inscrit dans ce cadre, il vise l'optimisation des conditions de saumurage de la variété Picholine, particulièrement la concentration de soude ainsi que la concentration initiale en sel de la saumure.

## 2. Matériel et méthodes

Les olives utilisées proviennent de la variété Picholine de calibre 30/32. Ces olives ont été soumises au processus d'élaboration type "séville". Le traitement des olives a été réalisé tout en faisant varier la concentration de soude (2.5, 3 et 3.5%) et la teneur initiale en sel (7 et 9°Be) (Tableau 1). La modalité de lavage est de (2h-12h). Le suivi des principales caractéristiques chimiques de la saumure permet de connaître l'allure de la fermentation.

Tableau 1: Différents types de préparation des olives Picholine

|    | Concentration de soude (%) | Concentration initiale en sel (°Be) |
|----|----------------------------|-------------------------------------|
| A7 | 2.5                        | 7                                   |
| B7 | 3                          | 7                                   |
| C7 | 3.5                        | 7                                   |
| A9 | 2.5                        | 9                                   |
| B9 | 3                          | 9                                   |
| C9 | 3.5                        | 9                                   |

**pH :** Le contrôle de pH est effectué à l'aide d'un pH-mètre

### 2.1. Acidité libre :

Définit par la somme des divers acides organiques libres présents dans la saumure.

**Acidité combinée ou lessive résiduelle:** Se sont les sels des acides organiques présents dans la saumure et jouant un rôle important dans la formation du système d'amortissement du pH.

### 2.2. Teneur en sel:

La teneur en chlorure de sodium de la saumure revêt une très grande importance dans la confiserie d'olives. Une prise de 0.5 ml de saumure, en présence de près de 100ml d'eau distillée, est dosée avec la solution de nitrate d'argent (0.086 N), en utilisant le chromate de potassium.

## 3. Résultats et discussion

### 3.1. Effet de la concentration du traitement alcalin sur l'évolution du pH

Le suivi du pH pendant environ cinq mois montre une grande variation au cours des six premiers jours de la fermentation des olives Picholine. En effet, ce paramètre passe de 9.77 à 5.88 environ quelque soit le traitement réalisé. Après le sixième jour, une faible variation du pH est observée d'un échantillon à un autre. La fin de la fermentation est marquée par une constance du pH, les valeurs les plus faibles sont observées pour le traitement à 3% de soude alors que les plus élevées sont attribuées pour ceux traités à 3.5%. Ceci est du à la modalité du lavage qui a été la même pour tous les échantillons (Figure1). Les olives soumises à un traitement alcalin de forte concentration subissent normalement un lavage plus énergique afin d'éliminer le maximum de soude restante dans le but d'éviter une évolution incorrecte du pH (Rejano Navarro 1983). La concentration initiale en sel paraît sans effet sur l'évolution du pH.

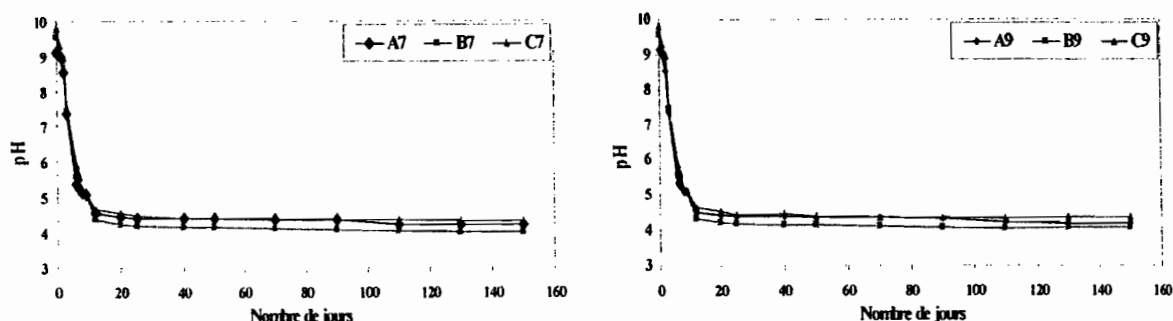


Figure1: Effet de la concentration du traitement alcalin sur l'évolution du pH lors de la fermentation des olives Picholine mises en saumure à 7°Be et 9°Be



### 3.2. Effet de concentration du traitement alcalin sur l'évolution de l'acidité libre

L'évolution de l'acidité libre au cours de la fermentation des olives Picholine montre que le traitement des olives avec une concentration élevée en soude (3.5%) masque nettement l'acide lactique produit. Au cours des premiers jours de fermentation l'évolution de l'acidité libre est pratiquement similaire pour tous les échantillons traités par une faible et moyenne concentrations de soude à savoir 2.5 et 3% de soude. Une concentration élevée en soude (3.5%) empêche l'acidification correcte de la saumure (acidité libre faible) (figure 2). La concentration initiale de la saumure affecte également l'évolution de l'acidité libre. En effet, les valeurs finales atteintes sont d'autant plus élevées que la teneur initiale en sel est faible. Ce phénomène est lié à l'inhibition du développement des lactobacilles qui est plus importante lorsque la teneur en sel de la saumure est élevée, Panagou Efsthios et al. (2006).

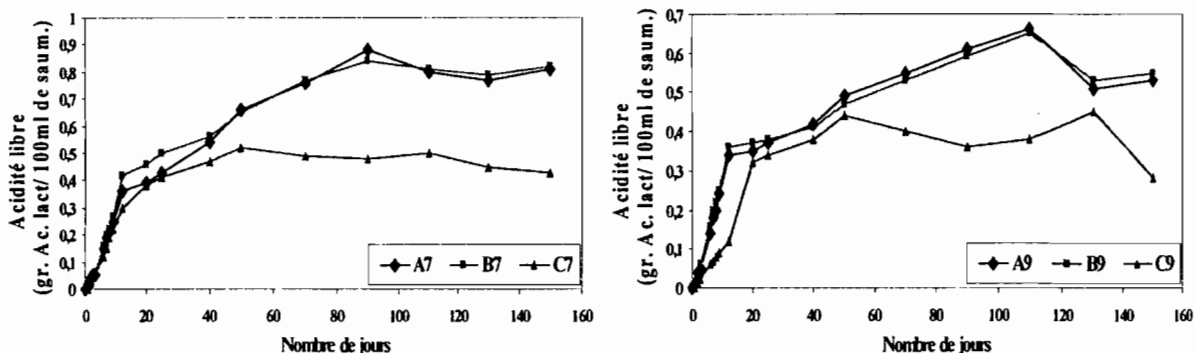


Figure 2: Effet de la concentration du traitement alcalin sur l'évolution de l'acidité libre lors de la fermentation des olives Picholine mises en saumure à 7°C et 9°C

### 3.3. Effet de concentration du traitement alcalin sur l'évolution de l'acidité combinée

Dès la mise en saumure et jusqu'à les vingt premiers jours de la fermentation, l'évolution de l'acidité combinée est d'autant plus importante que le traitement alcalin est de forte concentration. A partir du cinquantième jour, un certain équilibre s'établit pour tous les échantillons étudiés (figure 3).

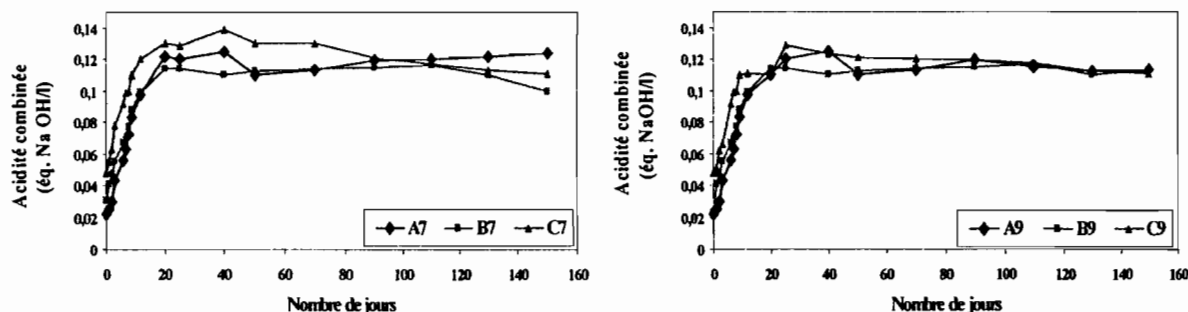


Figure 3: Effet de la concentration du traitement alcalin sur l'évolution de l'acidité combinée lors de la fermentation des olives Picholine mises en saumure à 7°C et 9°C

### 3.4. Effet de concentration du traitement alcalin sur l'évolution de la teneur en sel

La teneur en sel de la saumure des olives Picholine est influencée aussi bien par la concentration de soude utilisée que par la quantité de sel initialement employée. En effet, les saumures des olives traitées avec une moyenne concentration (3%) et une forte concentration de soude (3.5%) induisent une diminution plus rapide de la teneur en sel (au bout de 24 heures de la mise en saumure).

Pour un même traitement alcalin, on remarque que plus la concentration initiale en sel est faible, moins importante est la baisse de la teneur en sel dans la saumure (Figure 4).

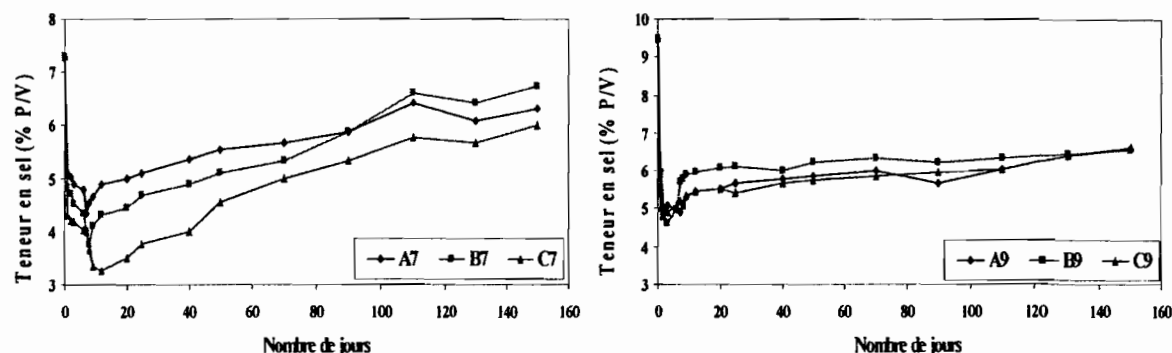


**Figure 4:** Effet de la concentration du traitement alcalin sur l'évolution de la teneur en sel lors de la fermentation des olives Picholine mises en saumure à 7°Be et 9°Be

#### 4. Conclusion

Etant donné que les olives Picholine présentent une amertume assez importante, l'utilisation d'une faible concentration de soude (2.5%) conduit à des olives encore amères à cause de la dégradation non complète de l'oleuropéine. Cependant le traitement avec une forte concentration en soude (3.5%) permet une bonne dégradation de ce composé mais affecte négativement la qualité organoleptique du produit fini en maintenant le pH à un niveau relativement élevé.

L'effet de la concentration initiale en sel de la saumure s'ajoute à celui du traitement alcalin qui à faibles doses engendre un développement des micro-organismes indésirables.

Les résultats obtenus montrent que le traitement des olives de la variété Picholine avec une concentration moyenne de soude (3%) combiné à une concentration initiale de sel (9°Be) permet un bon déroulement de la fermentation et conduit à un produit fini de bonne qualité organoleptique.

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## **Qualité des huiles d'olive produites par les huileries du gouvernorat de Sfax (Tunisie) lors de la campagne 2008-2009**

Mariam GHARSALLAOUI<sup>(1)</sup> et Mohamed AYADI<sup>(1)</sup>

### **Résumé**

Sfax est la deuxième ville et un centre économique important de la Tunisie. Cette région représente 11% de l'effectif oléicole national avec 304 huileries de différent type avec chaîne continue, classique et super presse. Avec 34% de la capacité de trituration elle contribue pour 37% à la production nationale et pour 68% aux exportations d'huile d'olive. Dans le but de promouvoir les industries oléicoles en Tunisie, dans chaque gouvernorat du pays, un comité régional des huileries constitué de plusieurs disciplines a été créé afin de sensibiliser les oléifacteurs pour l'application des bonnes pratiques de production de l'huile d'olive. Dans ce même cadre le comité régional des huileries de Sfax a réalisé 57 visites lors de la campagne 2008-2009 durant laquelle une collecte d'une trentaine d'échantillon a été effectuée afin de déterminer la qualité des huiles produites dans la région de Sfax. Les échantillons analysés au laboratoire de l'Unité Technologie et Qualité à l'Institut de l'Olivier ont présenté des qualités différentes avec des acidités variant de 0.4 % pour une chaîne continue à 4.3 % pour une huilerie classique (2ème pression). En ce qui concerne l'état d'oxydation les échantillons collectés ont des absorbances à 232 allant de 1.9451 à 3.1351 alors que l'absorbance à 270 varie de 0.1163 à 0.4474. Par ailleurs, les teneurs en polyphénols varient avec le système d'extraction utilisé et la date de collecte des échantillons. La qualité organoleptique des échantillons objet de cette étude présentent une variabilité très remarquable.

**Mots clés :** huile d'olive, huileries, qualité, gouvernera de Sfax.

## **Quality of olive oil produced by mills in the governance of Sfax (Tunisia) during 2008-2009**

### **Abstract**

Sfax, the second largest city and economic center of Tunisia. This region represents 11% of the national olive oil production with 304 mills between centrifugation classical and superpress, 34% of crushing capacity and contributes with 37% of national production and 68% of olive oil exports. In order to promote the olive oil industry in Tunisia, in each government, a regional committee consisting from several disciplines was created to educate oleifactors for the application of best practice of olive oil production. In this context the regional committee of Sfax has made 57 visits during 2008\_2009 during which it carried out the collection of thirty sample to determine the quality of oils produced in the region of Sfax. The samples analyzed in the laboratory of Technology and Quality Unit at the Olive Tree Institute present with different grades of acidity ranging from 0.4% for a continuous chain to 4.3% for classic mill (2nd press). Regarding the oxidation state the collected samples have absorbances at 232 from 1.9451 to 3.1351 while the absorbance at 270 ranges from 0.1163 to 0.4474. Moreover, the polyphenol content varies with the extraction system and date of sample collection. Organoleptic quality these samples a very remarkable variability.

**Keywords:** mills, olive oil, quality, Government of Sfax.

## Suivi de la dégradation thermique et photochimique d'huiles d'olive vierges dans des conditions d'oxydation contrôlées par IRTF et traitements chimiométriques

Yveline LE DRÉAU, Nathalie DUPUY, Jacky KISTER, Catherine RÉBUFA

### Résumé

Les huiles et les graisses tiennent une part importante dans l'alimentation humaine car elles procurent à notre alimentation un certain caractère gustatif et olfactif. Ceux sont aussi des aliments indispensables à notre santé car ils ne sont pas synthétisés par le corps humain. La cause la plus importante de détérioration des huiles et des graisses est due à des phénomènes d'oxydation qui réduisent la durée de conservation et la valeur nutritionnelle de certains composés qu'elles contiennent en générant en parallèle des produits toxiques. Ce rancissement est accéléré par une exposition à la chaleur, la lumière, l'humidité et la présence de métaux de transition. La lumière et le chauffage intense des huiles (friture) entraînent une oxydation importante conduisant à des modifications de leur composition chimique et de leurs propriétés physiques (densité, viscosité, indice de réfraction).

L'objectif de ce travail a été de démontrer l'apport de techniques spectroscopiques (IRTF, fluorescence) pour suivre l'évolution de la composition des huiles d'olive vierges françaises ayant subi une oxydation contrôlée (stress oxydant thermique et/ou photochimique (rayonnement visible ou ultra-violet)).

Des traitements chimiométriques (ACP, MCR-ALS) des spectres enregistrés en continu au cours du vieillissement ont permis de classer les huiles en fonction de leur résistance à l'oxydation, d'identifier des familles de composés présents avant et après altération, de suivre l'évolution de leur abondance relative au cours de la dégradation.

**Mots clefs:** Oxydation, huiles d'olives, Infrarouge à transformée de Fourier, indices spectrométriques, test de stabilité, ACP, MCR-ALS.

### 1. Introduction

La dégradation des huiles est due à des phénomènes d'oxydation (rancissement) qui réduisent leur durée de conservation et leur valeur nutritionnelle en générant en parallèle des produits toxiques. Ce rancissement est accéléré par la chaleur, la lumière, l'humidité ou la présence de métaux de transition (Guillén et Cabo, 2002; Russin et al., 2003; Van de Voort, 2008). La plupart des tests d'oxydation d'huiles (Isbell et al., 1999; European standard, 1996) conduisent rarement à une image globale du processus d'oxydation et prennent beaucoup de temps. Parmi les méthodes d'analyse utilisées, le moyen infrarouge à transformée de Fourier (IRTF) s'est avéré très performant (Guillén et Cabo, 2002; J.B. Russel, 1989) en apportant une information globale. Le but de ce travail a été de développer par IRTF une méthode simple et rapide pour suivre en continu l'évolution de la composition d'une huile d'olive vierge française ayant subi une oxydation contrôlée (thermique et/ou irradiation UV) et évaluer l'impact de ces deux traitements. Des indices spectroscopiques ont été utilisés pour comparer les cinétiques obtenues. Une Analyse en Composantes Principales (ACP) a été utilisée pour différencier les différents stades de l'oxydation. Une méthode multivariée (SIMPLISMA (SIMPLe-to-use Interactive Self Modeling Analysis)) a été employée pour extraire des informations spectrales sur les composants "purs" du système ainsi que leur profil de concentration associé (W. Windig et al., 2005).

### 2. Matériel et méthode

Huit tests de vieillissement ont été menés (résumés dans le tableau 1) sur une huile d'olive (AOC Aix en Provence) fournie par le Centre Technique de L'Olivier, Aix en Provence.

**Vieillissement thermique.** 3 g d'huile sont chauffés à 180°C (bain d'huile silicone) sous agitation et sous un flux d'oxygène (0,8 bar) dans une cuve en quartz.

**Vieillissement photochimique.** 3 g d'huile maintenus sous agitation et barbotage d'oxygène (0,8 bar) sont irradiés à l'aide d'une lampe à arc de mercure (ORIEL 6286, 250 watts).

**Tableau 1:** Récapitulatif des tests d'oxydation menés

| Essais | Traitement                             | Essais | Traitement                          |
|--------|----------------------------------------|--------|-------------------------------------|
| 1      | 60 min. à 180°C puis 750 min. sous UV  | 5      | 300 min. à 180°C / 180 min. sous UV |
| 2      | 180 min. à 180°C puis 180 min. sous UV | 6      | 345 min. à 180°C sous UV            |
| 3      | 390 min. à 180°C puis 390 min. sous UV | 7      | 810 min. sous UV                    |
| 4      | 180 min. sous UV puis 360 min. à 180°C | 8      | 810 min. à 180°C                    |

**Analyse IRTF.** Une goutte d'huile est déposée sur l'accessoire ATR monoréflexion (Golden Gate, Specac) d'un spectromètre AVATAR 370 FT-IR Thermo Nicolet. Les spectres sont enregistrés à l'aide du logiciel Omnic, de 4000 à 600  $\text{cm}^{-1}$  avec une résolution de 4  $\text{cm}^{-1}$  et 64 accumulations. Une référence sur l'air est enregistrée dans les mêmes conditions avant chaque mesure. La première mesure de la cinétique, correspond au temps  $t_0$ . Les suivantes sont réalisées, en prélevant un nouvel aliquot toutes les 15 ou 30 minutes. Les spectres correspondants sont alors indicés du temps de mesure.

### 3. Résultats et discussion

Les bandes IR de l'huile subissent des changements au cours du temps de chauffage (figure 1a) La diminution de la bande à 3006  $\text{cm}^{-1}$ , attribuée à la liaison C-H en position "cis" sur une double liaison C=C d'un acide gras ou d'un ester d'acide gras insaturé, correspond à la diminution des insaturations *cis*.

L'apparition de la bande vers 3500  $\text{cm}^{-1}$  correspond à la formation de produits primaires et secondaires d'oxydation renfermant des groupements OH tel que les hydroperoxydes, les alcools, les éthers, les acides. L'élargissement de la bande vers 1740  $\text{cm}^{-1}$  attribuée aux liaisons C=O correspond à la formation de composés oxygénés (aldéhydes, cétones, acides...). L'élargissement des bandes entre 1800 et 1000  $\text{cm}^{-1}$  caractérise un mélange complexe de produits secondaires d'oxydation (peroxydes, aldéhydes, cétones...).

Un accroissement de la bande à 960  $\text{cm}^{-1}$ , due à la liaison C-H en position "trans" d'une double liaison C=C (acide gras ou d'un ester d'acide gras insaturés, produits d'oxydation) est attribué à la formation d'insaturations *trans* caractéristiques d'un vieillissement. Ces mêmes variations sont observables lors d'une irradiation.

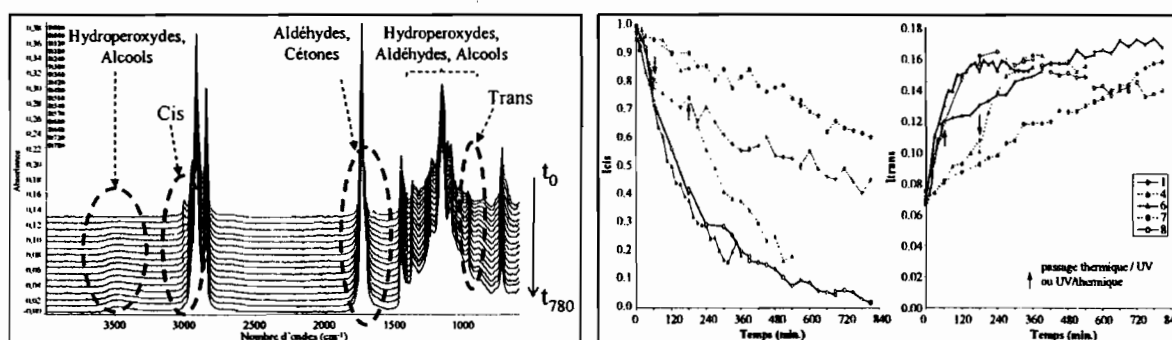


**Figure 1 (a) :** Changements des bandes lors d'une oxydation thermique, (b) évolution des indices "cis" et "trans" au cours du temps sous différentes conditions expérimentales.

La figure 1b montre l'évolution au cours du temps des indices  $I_{cis}$  (relatif à la bande à 3006  $\text{cm}^{-1}$ ) et  $I_{trans}$  (relatif à la bande à 960  $\text{cm}^{-1}$ ) calculés à partir de rapport d'aires de bandes (Le Dréau et al., 2009) pour les différents tests. Lorsque l'échantillon est chauffé ou irradié, une diminution de l'indice "cis" et une augmentation de l'indice "trans" sont observables. La chaleur dégrade d'avantage d'insaturations *cis* et induit d'avantage de liaisons *trans* et plus rapidement que l'irradiation. Cependant lorsque l'échantillon est irradié longtemps, l'indice "trans" atteint la même valeur que lorsque l'échantillon est chauffé. Par contre les valeurs de l'indice "cis" restent supérieures. Ceci suppose que, soit l'irradiation favorise la formation d'insaturations *trans*, soit le chauffage entraîne dans un deuxième temps la dégradation des insaturations *trans* après les avoir induites. Il ne semble pas y avoir d'effet de synergie entre les deux traitements.

L'ACP des données spectrales (centrage des données, validation croisée, retrait de la zone d'absorption du  $\text{CO}_2$ ) a permis de comparer la résistance de l'huile d'olive vis-à-vis des deux vieillissements sur une même durée (figure 2). La première composante principale (représentant 99% de la variance totale) discrimine dans la partie négative tous les spectres obtenus après une irradiation UV et ceux obtenus après chauffage entre 0 et 180 min. Elle regroupe dans sa partie positive les derniers temps du vieillissement thermique ( $T > 180$  min.). Le loading associé montre que les spectres de la partie négative se différencient par des bandes d'absorption localisées à 3006, 2920, 2850, 1750 et  $717 \text{ cm}^{-1}$ . Les derniers temps thermiques se différencient par un massif à  $3500 \text{ cm}^{-1}$ , la bande à  $1724$  et la zone entre  $800$  et  $1400 \text{ cm}^{-1}$  caractéristique des produits d'oxydation. Le deuxième axe discrimine dans la partie négative, les premiers temps des deux vieillissements ainsi que les derniers temps thermiques. Les bandes qui les différencient ( $3006, 1740, 1710, 1157, 707 \text{ cm}^{-1}$ ) caractérisent les structures "cis". Les temps intermédiaires du vieillissement thermique et les derniers spectres de l'huile irradiée se démarquent par des absorptions identifiant une isomérisation "trans" ( $2930, 2850$  et  $970 \text{ cm}^{-1}$ ).

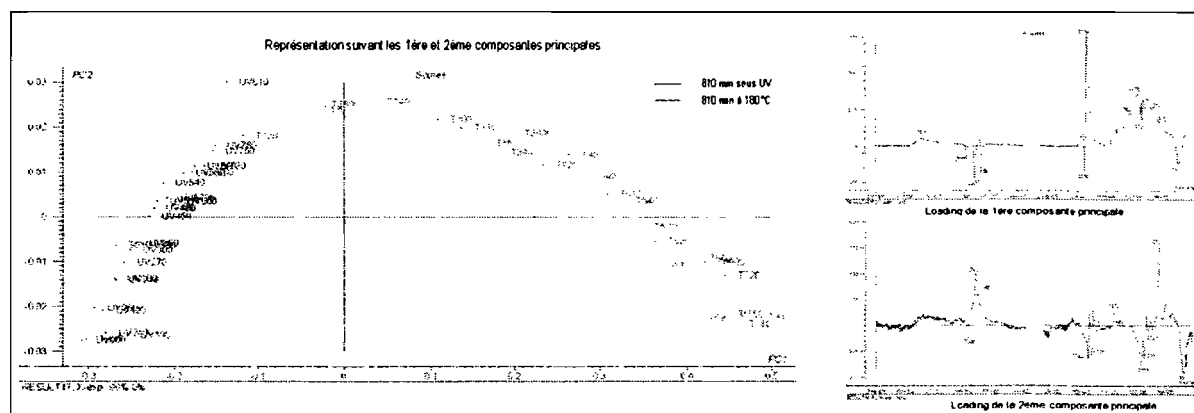


Figure 2: ACP des manipulations 7 et 8

Le traitement des données (manipulations 1 à 8) par la méthode SIMPLISMA a permis d'extraire mathématiquement 3 spectres (figure 3). Le premier décrit des triglycérides ayant des insaturations *cis* avec des bandes à  $3006, 2920, 2851, 1745, 1450, 1377, 1237, 1160, 1118, 1094$  et  $724 \text{ cm}^{-1}$ . Son profil de concentration montre une diminution de ces espèces au cours du temps qui est beaucoup plus importante après un stress thermique qu'après une irradiation UV. Le deuxième spectre peut être attribué aux hydroperoxydes des triglycérides présentant une isomérisation *trans* ( $3500, 2920, 2855, 1742, 1464, 1148, 1112, 970$  et  $891 \text{ cm}^{-1}$ ). Son profil de concentration traduit que ces produits se forment au cours des différents traitements et qu'ils sembleraient se dégrader après une longue période de chauffage (manipulation 8). Le troisième spectre est relatif aux composés d'oxydation avec de longues chaînes (bande à  $724 \text{ cm}^{-1}$ ) et des fonctions acides (large massif autour de  $3500 \text{ cm}^{-1}$  et bande carbonyle à  $1733 \text{ cm}^{-1}$ ). Ces derniers constituants se forment en plus grande quantité lors d'une oxydation thermique. Le rayonnement UV apparaît moins agressif en engendrant peu de ces structures.

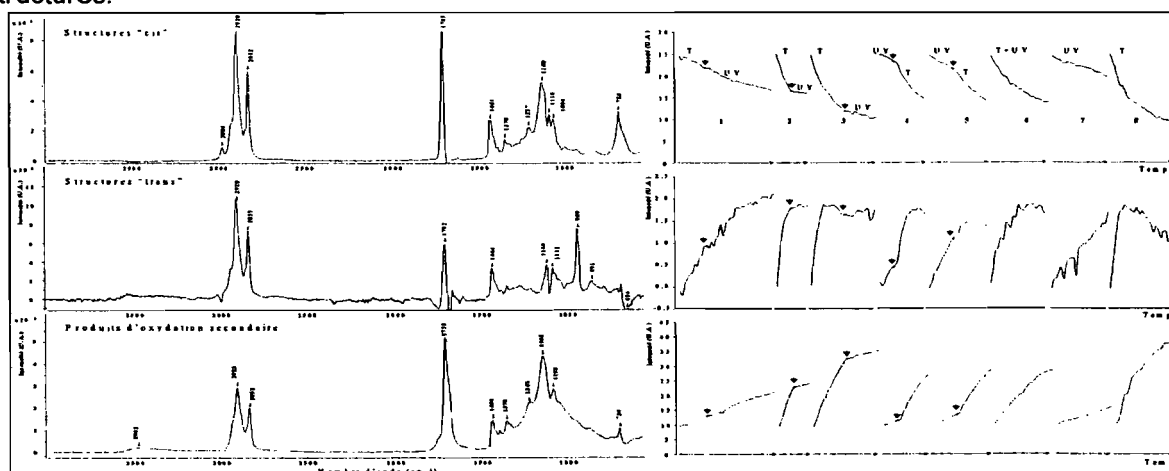


Figure 3: Spectres extraits mathématiquement et leur profil de concentration associé.

#### 4. Conclusion

Les deux modes d'interprétation des données spectrales sont complémentaires. La méthode des indices permet de comparer simplement différents échantillons ou différents traitements. L'ACP confirme le choix des bandes spectrales ( $3006$  et  $970\text{ cm}^{-1}$ ) utilisées dans le calcul des indices. L'utilisation de SIMPLISMA met en évidence la présence de familles de composés et de suivre leur répartition relative au sein de l'huile évoluant au cours du temps.

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## Vitamins evaluation of Tunisian olive oils by HPLC-RP

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### Abstract

The aim of this poster was to characterize fat-soluble vitamins and provitamins of Tunisian olive oils. Their extraction was carried out by saponification and analysed by HPLC-RP method. Results obtained showed the presence of four shapes of vitamins E: alpha( $\alpha$ ), beta ( $\beta$ ), gamma ( $\gamma$ ) and delta ( $\delta$ )-tocopherols. Tunisian olive oil contains also vitamin K<sub>1</sub> and  $\beta$ -carotene (provitamin A). Total fat-soluble vitamins (TFSV) content varied from 10.00 to 61.60 mg/100g of oil where  $\alpha$ -tocopherol represents 71.90% to 93.50% and vitamin K<sub>1</sub> constitutes 0.03% to 0.30% of TFSV.

**Key words:** HPLC-RP, olive oil, provitamins, vitamins, saponification.

### Résumé

Dans le présent travail, nous nous sommes proposé d'identifier les différentes vitamines et provitamines liposolubles présentes dans l'huile d'olive tunisienne en utilisant la technique de chromatographie liquide à haute performance en phase inverse (HPLC-RP). Les résultats obtenus ont permis de noter la présence des quatre formes de vitamine E:  $\alpha$ ,  $\beta$ ,  $\gamma$  et  $\delta$ -tocophérol ainsi que la présence de vitamine K<sub>1</sub>. Concernant les provitamines, l'huile d'olive tunisienne contient la provitamine A ( $\beta$ -carotène) et la provitamine D<sub>2</sub> (ergostérol).

La teneur en vitamines liposolubles totales varie de 11,89 à 70,8 mg/100g d'huile où l' $\alpha$ -tocophérol représente 55,81% à 87,90 %, tandis que la phylloquinone ou vitamine K<sub>1</sub>, ne représente que 0,029 à 0,26% des vitamines liposolubles totales et constitue de ce fait la vitamine mineure de l'huile d'olive.

### 1. Introduction

Virgin olive oil, obtained from *Olea europaea* fruits, is one of few vegetable oils consumed in its natural state out any technological procedures. It is rich in oleic acid, aromatic compounds and polyphenols (Dhifi *et al.*, 2005) and fat-soluble vitamins (FSV). The most abundant FSV are vitamin E with four forms:  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$ -tocopherols where  $\alpha$ -tocopherol is the most active in olive oil (Baldioli *et al.*, 1996) and  $\beta$ -carotene is the most important provitamin A source. The aim of the work is the analysis and quantification of Tunisian olive oils fat-soluble vitamins (E et K) and provitamin A ( $\beta$ -carotene).

### 2. Materials and methods

#### 2.1. Plant material

We used 41 samples obtained from homogeneous olive fruit of two Tunisian *Olea europaea* L. cultivars "Chetoui" and "Chemlali". "Chemlali" was grown in Sahel and in South of Tunisia and the variety "Chétoui" was grown in the north of Tunisia. Olive oil was extracted by using the continuous system.

#### 2.2. Sample preparation

Virgin olive oil was prepared with ethanol and ascorbic acid in a 30ml test tube. The sample was saponified with a 80% KOH for 10 min at 80 ° C. After saponification, the flask was placed in an ice bath; distilled water and hexan were added. After a strong mixture, the upper layer was transferred to a 125ml saparatory funnel. The pooled hexane layer was washed with distilled water three times, filtered through Na<sub>2</sub>SO<sub>4</sub>, and then evaporated under a stream of nitrogen.



### 2.3. Chromatographic conditions

The analysis and separation of fat-soluble vitamins was made by high-performance liquid chromatography reverse-phase (RP-HPLC) using a device type Agilent Technologies 1100, equipped with a UV detector and a C18 hypersil column (250 x 4.6 mm, 4  $\mu$  m). Analyses are carried out at room temperature. The mobile phase consists of the mixture: methanol and acetonitrile (95 / 5: v / v) at the flow-rate of 1ml/min. The volume injected was 20  $\mu$ l and peaks were monitored at 292 nm. Peaks identification was performed by co-chromatography of fat-soluble vitamins standards analyzed in the same experimental conditions.

### 3. Results & discussion

Different samples of oil presented a very important rate in vitamin E varying from 88.6 % to 99.1% where  $\alpha$ -tocophérol was the major form of tocophérols ranging from 71.9 % to 93.5 %. However,  $\beta$ -tocophérol was absent in the majority of the samples studied.  $\beta$ -carotene (provitamin A) had a proportion ranging from 0.8 % to 11.1%. In addition, vitamin K<sub>1</sub> presented the smallest percentage of TFSV (0.03 to 0.3%).

Total fat-soluble vitamins content of Tunisian olive oil ranged from 10.0 to 61.6 mg/100g.

$\alpha$ -tocopherol was the most abundant antioxidant of olive oil constituting 92.2% of total tocopherols; this vitamin had major content ranging from 7.87 to 57.74 mg / 100g. These values are comparable to those found in olive oils from different Mediterranean basin regions. Indeed, numerous works recorded contents varying from 12.1 to 36.9 mg/100g in Italian olive oil (Pellegrini *et al.*, 2001), from 18.4 to 28.4 mg/100g in Greek ones (Psomiadou and Tsimidou, 1998) and 15.6 to 25.3 mg/100g in Spanish samples (Gutierrez *et al.*, 2002).

Concerning the other forms of vitamin E,  $\delta$ -tocophérol was the second form varying in the range 0.37 to 4.07 mg / 100g. These contents were superior to those recorded by Lopez Ortiz *et al.* (2006) and which did not exceed 0.95 mg / 100g in Spanish olive oil samples. Compared to the two other forms of tocophérols ( $\beta$  and  $\gamma$ -tocophérols), they were less represented in our samples and so, Tunisian olive oil appeared less rich compared to the Spanish ones (Aguilera *et al.*, 2005). The second antioxidant present in olive oil is  $\beta$ -carotene whose content ranged from 0.28 to 1.43 mg/100g. This result was comparable to those of Giuffrida *et al.* (2007) concerning Italian olive oil and Gimeno *et al.* (2000) for the Spanish ones.

Regarding vitamin K<sub>1</sub>, our samples had very low contents (12 to 51  $\mu$ g/100g) compared with those of the tocopherols. (Table 1)

**Table 1:** Average rate and content of fat-soluble vitamins and provitamins of Tunisian olive oil samples.

| Vitamins and provitamins | % TFSV | % tocophérols | Average content of different of Tunisian olive oil samples from (mg/100g) |
|--------------------------|--------|---------------|---------------------------------------------------------------------------|
| Total tocopherols        | 97.50% | 100%          | 31.712 $\pm$ 2.55                                                         |
| $\alpha$ -tocopherol     | 89.90% | 92,26%        | 28.650 $\pm$ 2,2                                                          |
| $\beta$ -tocopherol      | 0.60%  | 0,64%         | 0.150 $\pm$ 0.03                                                          |
| $\gamma$ -tocopherol     | 2.00%  | 2,05%         | 0.650 $\pm$ 0.08                                                          |
| $\delta$ -tocopherol     | 4.90%  | 4,05%         | 1.560 $\pm$ 0.16                                                          |
| Vitamin K <sub>1</sub>   | 0.10%  |               | 0.032 $\pm$ 0.004                                                         |
| $\beta$ -carotene        | 2.40%  |               | 0.670 $\pm$ 0.08                                                          |

#### 4. Conclusion

As the Mediterranean olive oils, the Tunisian one is very rich in fat-soluble vitamins and provitamins. Indeed, it is almost the only one that contains four tocopherols forms ( $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$ ) and thus is the richest in antioxidants. Therefore, olive oil from Tunisia wins in stability compared to olive oils produced in other regions.

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## **Caractérisation de trois huiles d'olive vierge Algériennes de Kabylie et étude de l'effet du $\beta$ -carotène et de la vitamine E sur leur stabilité oxydative**

Ramdane MOUSSAOUI\*, Samia FODIL

### **Résumé**

Cette étude traite l'influence de la composition en  $\beta$ -carotène et vitamine E sur la stabilité oxydative de trois huiles d'olive vierge Algériennes de Kabylie issues de trois régions (montagne, côte et plaine). La composition en acides gras par chromatographie en phase gazeuse et celle en composés phénolique par chromatographie liquide haute pression (HPLC) de ces huiles ont été effectuées. Le  $\beta$ -carotène à une concentration de 10 ppm exerce un pouvoir antioxydant plus intéressant que la vitamine E. L'effet synergique des deux antioxydants n'est pas observé dans les conditions de stockage utilisées (obscurité et température de 40°C).

**Mots clés:** Huile d'olive, acides gras, composés phénoliques, indice de peroxyde, stabilité oxydative,  $\beta$ -carotène, vitamine E.

### **Influence of the composition in $\beta$ - carotene and vitamin E on the oxidative stability of three Algerian olive oils virgin of Kabylia**

### **Abstract**

This study treats the influence of the composition in  $\beta$ - carotene and vitamin E on the oxidative stability of three Algerian olive oils virgin of Kabylia exits of three areas (mountain, coast and plain). The composition in fatty acids by gas chromatography and that in compounds phenolic by liquid chromatography high pressure (HPLC) of these oils were carried out. The  $\beta$ - carotene with at the concentration of 10 ppm exerts an antioxydant power more interesting than the vitamin E. The synergistic effect of two antioxydants is not observed under the conditions of storage used (darkness and temperature of 40°C).

**Key words:** Olive oil, fatty acids, phenolic compounds, peroxide index, oxydative stability,  $\beta$ - carotene, vitamin E.

## Evaluation of the antioxidant capacity of four Tunisian virgin olive oils

Dabbou Samia<sup>1</sup>, Faten Brahmi<sup>1</sup>, Sifi samira<sup>2</sup> and Mohamed Hammami<sup>1</sup>

### Abstract

This work aimed to study the antioxidant capacity of phenolic extracts of four Tunisian olive oils from *Chaibi*, *Oueslati* and two mixture olive cvs in relation to their lipid composition and  $\alpha$ -tocopherol content. Oleic acid showed high levels (>70%) except for Mix1 (66.2%).  $\alpha$ -tocopherol contents were higher than 300 mg/Kg except for *Oueslati* oil (171.6 mg/Kg). Total phenol content ranged between 396 to 652 mg/Kg. Furthermore, the highest antioxidant capacity in virgin olive oil measured by the two methods TAA-ABTS test and RSA-DPPH assay, was observed in Mix2 (0.9 mmol TE/Kg and 72.3%, respectively) which showed the correlation between the antioxidant capacity of virgin olive oils studied with polar components and lipid profile, important components to their shelf life.

**Keywords:** olive oil, antioxidant capacity,  $\alpha$ -tocopherol, fatty acids, phenolic compounds.

## Evaluation de la capacité anti oxydante de quatre huiles d'olive vierge Tunisienne

### Résumé

Le présent travail étudie la capacité anti oxydante des extraits phénoliques de quatre huiles d'olive vierge Tunisiennes des variétés *Chaibi*, *Oueslati* et de deux mélanges d'olives (Mix1 et Mix2) en relation avec leurs teneurs en acides gras et en  $\alpha$ -tocopherol. La teneur en acide oléique montre des valeurs supérieures à 70% à l'exception de Mix1 (66,2%). Les teneurs en  $\alpha$ -tocopherol sont supérieures à 300 mg/Kg à l'exception de *Oueslati* (171.6 mg/Kg). Les quantités en phénols varient entre 396 to 652 mg/Kg. En outre, la capacité anti oxydante la plus élevée évaluée par les deux méthodes TAA-ABTS et RSA-DPPH, est observée chez Mix2 (0,9 mmol TE/Kg et 72,3%, respectivement) ce qui montre une corrélation nette entre la capacité anti oxydante des huiles et les composés polaires ainsi que le profil lipidique, qui sont des composantes indispensable pour sa conservation.

**Mots clés:** huile d'olive, capacité anti oxydante,  $\alpha$ -tocopherol, acides gras, phénols.

### 1. Introduction

Oil genuineness is a very important aspect of quality edible oils (Bendini *et al.*, 2007). Extra Virgin Olive Oil have been identified as one, if not the major healthful component of the Mediterranean diet, a diet that is synonymous with lowered rates of heart disease, cancers and extended life expectancy. All these properties were attributed to the very high content of mono-unsaturated fatty acids and phenolic compounds from simple to very complex structures (McDonald *et al.*, 2001; Robards *et al.*, 1999; Owen *et al.*, 2000<sup>a</sup>). Furthermore the health-promoting properties of the Mediterranean diet have been largely attributed to the antioxidant (Bendini *et al.*, 2007) and free radical-scavenging activity of polyphenols contained in olive fruit and oil (Kountouri *et al.*, 2007).

The concentration of these compounds in VOO are strongly affected by agronomical and technological factors, such as olive cultivar (Baccouri *et al.*, 2008; Tura *et al.*, 2007), place of cultivar (Cerretani *et al.*, 2006), climate, degree of maturation (Baccouri *et al.*, 2008), crop season (Gómez-Alonso *et al.*, 2002) and production process (Cerretani *et al.*, 2006; Ranalli *et al.*, 2001). However, interactions and recycling are very common mechanisms in the action of antioxidants. In addition, there are few reports about the antioxidant profile of field-grown olive cultivars of Tunisian origin. Therefore, this paper was designated to investigate the antioxidant and the free radical-scavenging potential of phenolic extracts of four virgin olive oils in relation to fatty acids,  $\alpha$ -tocopherol and pigments using two different tests RSA-DPPH and TAA-ABTS in order to find out the most valuable oil for disease preventing diets.

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## 2. Materials and methods

Plant material and growing areas selected. Olive fruits (*Olea europaea* L.) of the varieties Chaibi, Oueslati and two mixture olive cvs (Mix1 and Mix2) were collected in the 2007-2008 season from trees all located in the same orchard (a National Collection, 20 km away from Tunis, in North-East Tunisia) and which benefited from the same cultural practices. The experimental field in Béjaoua is characterised by a rainfall of 500 mm/year and a temperature varying from a minimum of 18°C to a maximum of 32°C. Olives are transformed into oil within 24h when only undamaged and uninfected fruits were processed at laboratory scale system (Abencor analyser). Finally, the oils were decanted and immediately stored in dark bottles at 4°C until analysis.

### Analytical methods

Quality parameters (Free Fatty acids, peroxide value and UV absorption characteristics) and Fatty Acid Methyl Esters were carried out following the analytical methods described in the EUC (1991). Total phenols and o-diphenols are determined colorimetrically as previously reported (Montedoro et al., 1992) and the results are expressed as 3,4-DHPEA equivalents. Pigments. Carotenes and chlorophylls were determined spectrophotometrically at 470 and 670nm respectively, as described by Minguez-Mosquera et al. (1991).  $\alpha$ -tocopherol was measured by the method reported by Gimeno et al. (2000); using the  $\alpha$ -tocopherol acetate as an internal standard. Radical Scavenging Activity (RSA) using DPPH assay (DPPH) was estimated according to the procedure described by Brand Williams et al. (1995). Total antioxidant status (TAA test with ABTS) was determined by ABTS radical cation decolorization assay (Rice-Evans et al., 1996; Re et al., 1999). The radical-scavenging activity of the samples was expressed as mmol Trolox equivalent (TE) per kg.

Accelerated Oxidation Tests evaluated as induction period (IP) was performed using the Rancimat operated at 120°C (Tura et al., 2007). Statistical analysis. Significant differences among varieties studied were determined by an analysis of variance which applied a Duncan test with a 95% significant level ( $P < 0.05$ ), using the SPSS programme, release 11.0 for windows (SPSS, Chicago, IL, USA). All analyses were carried out in triplicate and the results were presented as means of three repetitions.

## 3. Results and discussion

Analytical parameters of olive oils. Physico-chemical characteristics of all the oils produced and analysed showed values (Table 1) completely within the limits established by IOC (2009) as required for the 'extra virgin' category.

Fatty acid composition. The distribution of fatty acids, from all tested samples, covered the normal expected range for olive oil (Table 1). Results showed that fatty acid composition of the oils, one of the characteristics predominantly genetically determined (Hrnčirik & Fritsche, 2005) differ according to the variety. In fact, olive oils were low in palmitic acid and high in oleic acid (>70%) except for *Mix1*. Furthermore, studied olive oils seem to be rich in linoleic acid (>13.4%) except for *Oueslati*. The Oleic/Linoleic acid ratios of *Chaibi* and *Oueslati* (5.22 and 6.67, respectively) were relatively higher than those of *Mix1* and *Mix2* oils (<4.5).

**Table 1:** Quality indices and fatty acids composition (%) evaluated in the studied virgin olive oils.

| Fatty Acids                                           | Mix1                     | Chaibi                   | Mix2                     | Oueslati                 |
|-------------------------------------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Free fatty acids (% of Oleic acid)                    | 0.28±0.03 <sup>c</sup>   | 0.51±0.02 <sup>b</sup>   | 0.17±0.01 <sup>d</sup>   | 0.85±0.03 <sup>a</sup>   |
| Peroxide index (meq O <sub>2</sub> kg <sup>-1</sup> ) | 10.07±0.24 <sup>c</sup>  | 12.66±0.09 <sup>b</sup>  | 8.67±0.02 <sup>d</sup>   | 15.67±0.07 <sup>a</sup>  |
| K232                                                  | 2.56±0.13 <sup>a</sup>   | 2.14±0.00 <sup>c</sup>   | 2.29±0.07 <sup>b</sup>   | 2.19±0.04 <sup>b,c</sup> |
| K270                                                  | 0.30±0.02 <sup>a</sup>   | 0.28±0.02 <sup>a</sup>   | 0.30±0.01 <sup>a</sup>   | 0.27±0.02 <sup>a</sup>   |
| Palmitic                                              | 11.25±0.14 <sup>b</sup>  | 10.40±0.01 <sup>a</sup>  | 9.38±0.03 <sup>c</sup>   | 9.45±0.04 <sup>b</sup>   |
| Palmitoleic                                           | 0.48±0.01 <sup>a</sup>   | 0.57±0.01 <sup>b</sup>   | 0.39±0.00 <sup>c</sup>   | 0.49±0.00 <sup>c</sup>   |
| Margaric                                              | 0.09±0.00 <sup>a</sup>   | 0.08±0.00 <sup>b</sup>   | 0.09±0.00 <sup>a</sup>   | 0.05±0.00 <sup>c</sup>   |
| Margaroleic                                           | 0.12±0.00 <sup>b</sup>   | 0.11±0.00 <sup>c</sup>   | 0.12±0.00 <sup>a,b</sup> | 0.09±0.01 <sup>d</sup>   |
| Stearic                                               | 2.95±0.05 <sup>b</sup>   | 2.82±0.02 <sup>c</sup>   | 3.13±0.02 <sup>a</sup>   | 2.60±0.02 <sup>d</sup>   |
| Oleic                                                 | 66.21±1.12 <sup>c</sup>  | 69.94±0.40 <sup>b</sup>  | 69.03±0.01 <sup>b</sup>  | 72.81±0.66 <sup>a</sup>  |
| Linoleic                                              | 14.92±0.19 <sup>b</sup>  | 13.41±0.11 <sup>c</sup>  | 15.47±0.03 <sup>a</sup>  | 10.92±0.11 <sup>d</sup>  |
| Linolenic                                             | 0.89±0.01 <sup>a</sup>   | 0.82±0.02 <sup>b</sup>   | 0.90±0.00 <sup>a</sup>   | 0.73±0.01 <sup>c</sup>   |
| Arachidic                                             | 0.37±0.01 <sup>a</sup>   | 0.38±0.01 <sup>a</sup>   | 0.36±0.00 <sup>a</sup>   | 0.32±0.00 <sup>b</sup>   |
| Gadoleic                                              | 2.04±0.00 <sup>a,b</sup> | 1.47±0.55 <sup>a,b</sup> | 0.98±0.10 <sup>c</sup>   | 2.46±0.65 <sup>a</sup>   |
| Lignoceric                                            | 0.51±0.51 <sup>a</sup>   | 0.00±0.00 <sup>a</sup>   | 0.15±0.15 <sup>a</sup>   | 0.09±0.09 <sup>a</sup>   |
| Oleic/Linoleic ratio                                  | 4.44±0.02 <sup>d</sup>   | 5.22±0.01 <sup>b</sup>   | 4.46±0.00 <sup>c</sup>   | 6.67±0.01 <sup>a</sup>   |

Values are the means of the three different VOO samples ( $n=3$ )  $\pm$  standard deviations. Different letters indicate significant differences ( $P<0.05$ ) between cultivars.

**Pigments.** Results show significant differences between cultivars ( $p < 0.05$ ) where the levels of these pigments ranged from 8.28 to 12.51 mg kg<sup>-1</sup> and from 13.05 to 24.17 mg kg<sup>-1</sup>, for carotenes and chlorophylls respectively (Table 2). These levels of green–yellow color's virgin olive oil are influenced by olive cultivar (Minguez-Mosquera *et al.*, 1991), and could be considered as a product freshness indicator. Furthermore, pigments showed quite similar contents in *Mix1* and *Chaibi* oils whereas *Mix2* oil had the lowest levels.

**Total Phenols and O-diphenols.** The total phenols and o-diphenols evaluated by the colorimetric method (Table 2) showed significant differences ( $p < 0.05$ ) between different studied olive oils where the ranges were wide (from 395.67 to 651.55 mg kg<sup>-1</sup> for total phenol concentration and from 191.81 to 376.31 mg kg<sup>-1</sup> for o-diphenols). However, the highest amount of the total phenols and o-diphenols were present in *Mix2* olive oil followed by *Mix1* oil. *Oueslati* and *Chaibi* oils presented approximately the same content in total phenols whereas a wide difference was observed for o-diphenols.

**$\alpha$ -tocopherol** is the major constituent in olive oils with respect to tocopherols (Hrncirik & Fritsche, 2005). As shown in Table 2, the range of  $\alpha$ -tocopherol contents in studied olive oils is wide. There were significant differences in the  $\alpha$ -tocopherol contents which are highly variety-dependent ( $p < 0.05$ ) (Owen *et al.*, 2000<sup>b</sup>). In fact,  $\alpha$ -tocopherol amounts ranged from 171.64 mg kg<sup>-1</sup> (*Oueslati*) to 457.85 mg kg<sup>-1</sup> (*Mix1*).

**Table 2:** Stability parameters of virgin olive oils from studied varieties.

| Parameters                                  | Mix1                           | Chaibi                          | Mix2                            | Oueslati                        |
|---------------------------------------------|--------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Carotenes (mg kg <sup>-1</sup> )            | 12.51 $\pm$ 1.15 <sup>a</sup>  | 12.50 $\pm$ 1.48 <sup>a</sup>   | 8.28 $\pm$ 1.29 <sup>b</sup>    | 10.53 $\pm$ 0.55 <sup>a</sup>   |
| Chlorophylls (mg kg <sup>-1</sup> )         | 24.17 $\pm$ 2.80 <sup>a</sup>  | 23.55 $\pm$ 3.01 <sup>a</sup>   | 13.05 $\pm$ 2.24 <sup>b</sup>   | 22.25 $\pm$ 1.47 <sup>a</sup>   |
| $\alpha$ -Tocopherol (mg kg <sup>-1</sup> ) | 457.85 $\pm$ 2.08 <sup>a</sup> | 299.28 $\pm$ 34.25 <sup>c</sup> | 386.75 $\pm$ 15.95 <sup>b</sup> | 171.64 $\pm$ 5.14 <sup>d</sup>  |
| O-diphenols (mg kg <sup>-1</sup> )          | 247.63 $\pm$ 7.90 <sup>b</sup> | 240.01 $\pm$ 28.74 <sup>b</sup> | 376.31 $\pm$ 14.05 <sup>a</sup> | 191.81 $\pm$ 5.28 <sup>c</sup>  |
| Total phenols (mg kg <sup>-1</sup> )        | 492.35 $\pm$ 2.81 <sup>b</sup> | 403.07 $\pm$ 3.82 <sup>c</sup>  | 651.55 $\pm$ 29.94 <sup>a</sup> | 395.67 $\pm$ 18.01 <sup>c</sup> |
| IP (hours)                                  | 7.0 $\pm$ 0.09 <sup>c</sup>    | 6.97 $\pm$ 0.15 <sup>c</sup>    | 7.31 $\pm$ 0.08 <sup>b</sup>    | 9.11 $\pm$ 0.06 <sup>a</sup>    |
| DPPH (%RSA at 60min)                        | 48.50 $\pm$ 1.36 <sup>b</sup>  | 33.16 $\pm$ 0.08 <sup>d</sup>   | 72.33 $\pm$ 0.46 <sup>a</sup>   | 41.77 $\pm$ 0.53 <sup>c</sup>   |
| TAA (mM TE kg <sup>-1</sup> )               | 0.38 $\pm$ 0.01 <sup>c</sup>   | 0.47 $\pm$ 0.02 <sup>b</sup>    | 0.90 $\pm$ 0.04 <sup>a</sup>    | 0.33 $\pm$ 0.01 <sup>d</sup>    |

Values are the means of the three different VOO samples ( $n=3$ )  $\pm$  standard deviations. Different letters indicate significant differences ( $P<0.05$ ) between cultivars.

**Accelerated Oxidation Tests** (Table 2) is usually useful to evaluate the effects of natural or chemical antioxidants on olive oil resistance toward oxidation, and to compare the storage stability of different oils. Results showed that these values varied according to the cultivar. *Oueslati* olive oil had very high value of this parameter (>9h) followed by *Mix2* olive oil (7.31h) whereas the others olive oils (*Mix1* and *Chaibi*) showed similar values (7h). The different oxidative stabilities of the four samples are the combined result of the fatty acid composition and the effect of various pro/antioxidants present in the oils (Hrncirik & Fritsche, 2005). The explanation of the considerably higher stability of *Mix2* and *Mix1* oils lies probably in a relatively low content of polyunsaturated fatty acids, traditionally expressed by the O/L ratio and higher contents of antioxidants (phenols and tocopherols). Although the considerably higher levels of  $\alpha$ -tocopherol, total phenols and fatty acids in *Mix1* oil, no significant differences had been observed in its stability compared to *Chaibi* oil. These results can be attributed to the similarity in the contents of o-diphenols and pigments composition.

#### Antioxidant capacities

The results of the free radical scavenging properties of all olive oils studied evaluated by the two different methods are shown in Table 2. Differences had been observed between the two tests. In fact, *Oueslati* and *Mix1* olive oils showed the lowest antioxidant capacities (<0.4 mM) when evaluated by TAA, while oils from the Cvs. *Chaibi* and *Oueslati* had the lowest values by RSA. However, *Mix2* oil showed the same behavior for the two tests with the highest values (72.33% and 0.9mM). Contrary to the accelerated oxidation tests showing the highest stability for *Oueslati* oil, the higher stability evaluated by TAA and RSA was observed for *Mix1* and *Mix2* oils. This result can be explained by the higher contents of total phenols or o-diphenols when these tests are realized on the methanolic extract containing polar phenols.

#### 4. Conclusion

The different extra virgin olive oils from the four Tunisian olive oils analysed produced oils with different composition where *Chaibi* and *Oueslati* oils showed the best the fatty acid (lowest palmitic and linoleic acid and the highest oleic acid) and phenolic compositions. Secondly, VOOs studied varied greatly in oxidative stability, which depends on many intrinsic factors as fatty acid composition and natural level of pro/antioxidants. These natural antioxidants, despite their lower concentrations prevent them more from oxidation where *Mix1* and *Mix2* oils showed better RSA activity. Then, besides the single role of each antioxidants compound to monitor the oils stability, may be the effect of synergisms of all antioxidants matrix such as phenols, lipidic profile,  $\alpha$ -tocopherol, and pigments.

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## **Volatile compounds and sensory analysis of tunisian and italian monovarietal virgin olive oils with regard to two different extraction systems**

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### **Abstract**

Virgin olive oil has a fundamental role in the markets of alimentary oils because of its unique aroma, its stability and its healthy benefits. In this study the attention has been focused on two Tunisian olive oil cultivars (Chemlali, Chetoui) and on one Italian cultivar (Coratina). These different cultivars were extracted by centrifugation system (two phase and three phase). We have carried out this study to characterise the sensorial attributes of some monovarietal oils, to evaluate the preferences of a panel consumers, to explain these preferences by analytical data (sensorial and volatile compounds) and also to assess the influence of extraction method on consumer's preference. This study shows the significant impact on sensory analysis and volatile compounds on two different varieties and extraction systems. Furthermore, we demonstrate that consumer's preference is positively correlated to the various sensorial notes (fruity- bitter -pungent) and to the aromatic profile. Volatile compounds were extracted by dynamic headspace technique and characterised by Gas Chromatography and GC-Mass Spectrometry. Aromagrams showed a major content of most volatiles compounds in Coratina and Chetoui olive oil obtained by three phase extraction system with respect to two phase extraction system. On the other hand, aromagrams of Chemlali olive oil showed a minor content of some aroma molecules and no significant variation in the most volatiles, in olive oil obtained by three phase extraction system with respect to two phase extraction system. In particular, principal component analysis (PCA) of aroma compounds showed that 1-hexanol, trans-2-hexenal, trans-2-hexenol and cis-3-hexenyl-acetate which are produced by lipoxygenase pathway of olive oil could discriminate the varieties and the technological systems. Furthermore, PCA analysis disclosed that the two different systems were discriminated by Coratina and Chetoui samples, while for Chemlali olive oil the differences were not significant. The consumer's preference was satisfied by the oils of Chetoui and Coratina variety obtained by (tree and two centrifugation system) and in a less degree by the variety Chemlali. The latter seemed to attract young individuals less than 30 year old, who seemed to appreciate its particular sensorial profile.

**Key words:** Olive oil quality; Volatile compounds; Sensory evaluation; Centrifugation system.

## **Impact de la preference consommateur des composés volatiles, de la variété et du system d'extraction sur des huiles d'olives Tunisiennes et Italiennes**

### **Résumé**

L'huile d'olive vierge a un rôle fondamental dans les marchés des huiles alimentaires en raison de son arôme unique, sa stabilité et ses avantages sur la santé. Dans cette étude, l'attention s'est concentrée sur deux cultivars d'huile d'olive tunisienne (Chemlali, Chetoui) et sur un cultivar italien (Coratina). Ces différents cultivars ont été extraits par le système de centrifugation (deux phases et en trois phases). Nous avons effectué cette étude pour caractériser les attributs sensoriels de certaines huiles monovariétales, pour évaluer les préférences d'un panel de consommateur, pour expliquer ces préférences par des données analytiques (sensoriels et volatils) et également d'évaluer l'influence de la méthode d'extraction sur la préférence du consommateur. Cette étude montre l'impact significatif de l'analyse sensorielle, les composés volatils et des différents systèmes d'extraction de ces variétés. En outre, nous démontrons que la préférence du consommateur est positivement corrélée à diverses notes sensorielles (fruitée, amer, piquant) et au profil aromatique. Les composés volatils ont été extraits par la technique de l'espace de tête dynamique et marquée par chromatographie en phase gazeuse et GC-Mass Spectrometry.

L'Aromagrams montré une teneur importante de la plupart des composés volatiles pour les huiles d'olives de la variété Coratina et Chétoui obtenues par deux systèmes d'extractions (deux phase et trois phase).

D'autre part, l'aromagramme Chemlali de l'huile d'olive ont montré une faible teneur de certaines composés volatiles et une variation non significative de la plupart des composés volatils, de l'huile d'olive obtenue par le système d'extraction trois phase et le système d'extraction deux phase. En particulier, l'analyse en composantes principales (ACP) des composés d'arôme ont montré que les composés volatils 1-hexanol, le trans-2-hexénal, trans-2-hexénol et cis-3-hexényle-acétate qui sont produites par voie de la lipoxygénase d'huile d'olive permettrait de différencier les différences entre les variétés et les systèmes d'extractions. En outre, l'ACP des analyses a révélé que les deux systèmes ont été discriminés par les huiles des variétés Coratina et Chetoui, tandis que pour Chemlali huile d'olive, les écarts n'étaient pas significatifs.

La préférence du consommateur est satisfaite par les huiles de Chetoui et Coratina variété obtenue par les deux systèmes de centrifugations et à un degré moindre par la variété Chemlali. Ce dernier semblait attirer les jeunes individus de moins de 30 ans, qui semblait apprécier son profil sensoriel particulier.

**Mots clés:** qualité de l'huile d'olive, composés volatils, l'évaluation sensorielle; Centrifugation système.

## 1. Introduction

Olive oil is a natural product known for its popular taste and its desired effects on health. The quality of virgin olive oil is strictly correlated with the presence of a great number of volatile and non-volatile compounds such as phenolic compounds which are responsible for its flavour and contribute to the stability of the oil (Grati Kamoun, 2007, Aparicio et al, 1997).

In this study, we are interested on two Tunisian olive oil cultivars (Chemlali, Chetoui) and on one Italian cultivar (Coratina) which were extracted by centrifugation system (two phases and three phases).

The aim of this work is to evaluate the influence of extraction method on consumer's preference and to explain these preferences by analytical data (sensorial and volatile compounds).

## 2. Materials and methods

### • Determination of volatile compounds

Volatile compounds, stripped from the oil samples with N<sub>2</sub> (1.2 dm<sup>3</sup> min<sup>-1</sup>, 37°C for 2 h), trapped on 50 mg of activated charcoal and eluted with 1 ml of diethyl ether, were analyzed by GC using a Carlo Erba Mega Series 5160 gas chromatograph equipped with a Nordion silica capillary Carbowax 20 M column (50 m length; 0.32 mm i.d.; 0.5 mm @1m thickness), an on-column injection system, a CO<sub>2</sub> cryogenic accessory to hold the oven at 25°C and a Flame Ionization Detector (FID). The oven temperature program was in agreement with the method described in a previous paper (Angerosa et al. 1997).

### • GC±MS analysis

The identification of volatile compounds was carried out by GC±MS using the same operative conditions adopted for the gas chromatographic analysis. An HP model 5890A, equipped with an on-column injection system, and coupled with a mass selective detector model HP 5970B, was employed. Volatile compounds were identified by comparison of their mass spectra with those of authentic reference compounds, except for pentene dimers. These last were synthesized from trans-3-hexanoic acid and their mass spectra were obtained (Angerosa et al., 2000).

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## • Sensory analysis

The sensory evaluation was performed by two different tests:

1. The analytical tests, which determine the sensory characteristics of the oil and ranking it according to its sensory profile; this analysis is performed by an expert jury corresponding a group of subjects selected according to the guidelines of international standards. Panel members are trained to recognize the different flavor characteristics and measuring their intensities.
2. The hedonic tests are conducted by a panel of consumers through a questionnaire. The tasters choose, for each sample, the category that corresponds to their degree of appreciation. The different samples were coded and presented to consumers by randomization and repetition. All varieties were tasted by the same panel of consumers, not trained in order to know the olive oil most appreciated by consumers.

## 3. Results and discussion

The biplot in the Figure 1 reveals that the multivariate PCA technique, applied to the volatile compounds data set, was capable of grouping the volatile compounds with the specific preference consumer score.

In the bidimensional plot, the clear separation of the three clusters representing the preference consumer scores and volatile compounds are clearly displayed.

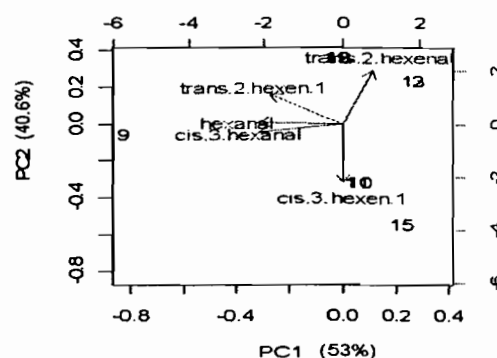


**Figure 1:** Multivariate approach toward volatile compound of some olive oil variety and preference consumer note.

The high scores of preference consumer (18) are positively correlated to the trans.2.hexenal volatile compound whereas the lowest scores (9) are correlated to the hexenal and cis.3.hexenal volatile compounds.

The biplot revealed that the multivariate PCA technique, applied to the volatile compounds factors and expert flavor scores, was capable grouping the volatile compounds and oil varieties as well as flavor expert opinion.

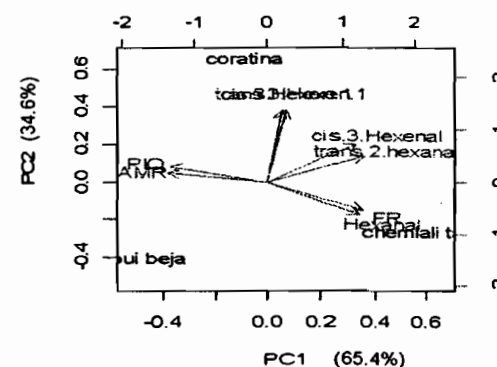


**Figure 2:** Multivariate approach toward volatile compound of some olive oil variety and sensorial attributes.

In the bidimensional plot (Figure 2), the clear separation of the three clusters representing the three varieties of Coratina, Chemlali and Chetoui are clearly displayed.

The Chemlali variety is positively correlated to the hexenal compound and fruity attribute. Whereas Coratina variety is highly correlated to the trans.2.hexen.1.ol and cis.3.hexen.1.ol compounds;

Regarding Chetoui variety, the major attributes which highly depend on are bitter and pungent

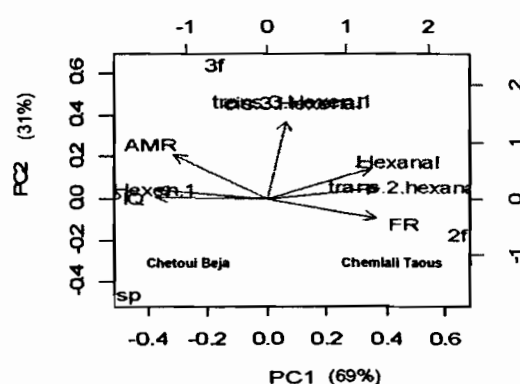


**Figure 3:** Multivariate approach toward volatile compound of some olive oil variety with sensorial attributes and extraction system.

The biplot (Figure 3) revealed that the multivariate PCA technique, applied to the volatile compounds factors as well as the expert flavor scores, was capable of grouping the volatile compounds, the expert opinions, and the extraction system by sensorial attributes.

The PCA shows that oils which are extracted by centrifugation system with two phases are rich in hexanal and trans.2.hexenal compounds and more fruity than others oils; whereas oils extracted by centrifugation system with three phases are rich in trans.3hexenal and trans.2.hexen1.ol.

Oils extracted by press system which contain the highest concentration in hexen.1.ol compounds are more pungent and bitter than oils extracted by centrifugation system.

#### 4. Conclusion

The present study has enabled us to demonstrate that the oils consumer's preferences regarding their extraction system and variety are positively correlated to the various sensorial notes (fruity- bitterness –pungency) and volatile compounds.

The above described profile showed appreciation for the oils rich in trans2.2hexenal extracted by centrifugation system with 2 phases. These compounds recall the attribute of almond and bitter (Angerosa et al, 2002). In a lower degree, a slight preference is indicated for oils rich in hexanal and cis.3.hexenal.

"Chemlali" variety is rich in hexanal and has a fruity attribute.

The pressure system guarantees oils which are rich in cis.3. Hexen.1.ol and having bitter and pungent attributes. These oils are less favored by consumers.

"Coratina" variety is rich in trans.2.hexen-1-ol and cis.3.hexen-1-ol. and "Chetoui» variety seems bitter and pungent.

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## Extra-virgin olive oil macerated with rosemary inhibits sterol oxidation

Laura D'Evoli, Massimo Lucarini and Ginevra Lombardi-Boccia

### Abstract

The aim of this study was to investigate the role of rosemary in preventing sterol oxidation in extra-virgin olive oil during heating. Total sterol and sterol oxide contents were determined in a extra-virgin olive oil macerated with rosemary for 72 h, 2 and 4 months. Heating experiments were performed at 180°C for 0, 1, 3, and 6 hours. The extent of sitosterol oxidation after heating was higher in the extra-virgin olive oil sample than in the rosemary extract. After 6 h heating extravirgin olive oil oxidized up to 11.5% of total sitosterol, only 6,1% oxides were detected in oil macerated with rosemary. Our findings suggest that olive oil macerated with rosemary may provide protection toward sterol oxidation in cooked foods. The study was supported be MiPAAF in the frame of the Project "BIOVITA"

**Key words:** Olive oil, rosemary, maceration, inhibition, sterol oxidation.

## Huile d'olive au romarin inhibe l'oxydation des stérols

### Résumé

L'objectif de cette étude était d'enquêter sur le rôle de romarin dans la prévention de l'oxydation des stérols en huile d'olive extra-vierge au cours du chauffage. Total des stérols et stérols, l'oxyde de contenu a été déterminé dans une huile d'olive au romarin macéré pendant 72 h, 2 et 4 mois. Chauffage expériences ont été réalisées à 180 ° C pendant 0, 1, 3 et 6 heures. L'étendue de sitostérol oxydation après chauffage était plus élevée dans l'huile d'olive extra vierge que dans l'échantillon extrait de romarin. Après 6 h de chauffage extra-vierge, huile d'olive oxydé jusqu'à 11,5% du total de sitostérol, seulement 6,1% d'oxydes ont été détectés en macération dans de l'huile de romarin. Nos résultats suggèrent que l'huile d'olive au romarin macéré peut fournir une protection envers l'oxydation des stérols dans les aliments cuits. L'étude a été soutenue par MiPAAF dans le cadre du projet "BIOVITA".

**Mots Clés :** Huile d'olive, romarin,, maceration, inhibition, oxidation des sterols.

### 1. Introduction

Olive oil is a good source of plant sterols, compounds regarded to have health-promoting properties. However, heating procedures commonly used in food cooking can lead to formation of oxides potentially harmful to human health.

Herbs and spices are known to keep the nutritional quality of foods and to increase their shelf life. Among aromatic herbs, rosemary, as well as other herbs, is used in Italy in the manufacture of typical food products and flavoured edible oils for its antioxidant properties.

The study was addressed to investigate the role of rosemary in preventing sterol oxidation in extra virgin olive oils during heating and to select the optimal methodology of flavoured oils preparation. Furthermore the stability of one flavoured oil was tested along time.

### 2. Materials and methods

Total sterol content and sitosterol oxides formation were evaluated in the following extra virgin olive oil samples enriched with rosemary and prepared at different maceration times:

- (sample A), native extra virgin olive oil (Italian brand, Monini)
- (sample B), extra virgin olive oil + rosemary, macerated for 72h
- (sample C), extra virgin olive oil + rosemary , macerated for 2 months
- (sample D), extra virgin olive oil + rosemary, macerated for 4 months
- (sample E), extra virgin olive oil + rosemary, macerated for 72h, filtered and stored for 4 months

Rosemary leaves were dried at 40°C in ventilated oven, ground and added to the olive oil samples at 10% concentration w/w at dark and room temperature. After the scheduled maceration time, samples were centrifuged and filtered before analyses. The same extra virgin olive oil as sample A was used for preparing the C-E rosemary olive oils. Maceration and storing were carried out in dark and room temperature.

Heating experiments of the samples were performed in an electric oven at 180°C for 0, 1, 3, 6 hours. The heating experiments for sitosterol oxides determination were performed only once for sample E and twice for samples A, B, C, D. All the analyses were carried out in duplicate.

Total sterols were determined by GC-FID after hot saponification, diethyl ether-heptane extraction and hot silylation (Piironen et al., 2002). Sterol oxides were determined by GC-FID and GC-MS after gentle cold saponification, diethyl ether-heptane extraction, oxide isolation through solid-phase extraction (SPE) and derivatization (Lampi et al., 2002; Soupas et al., 2004). As results derived from GC-FID were found comparable with those from GC-MS, only GC-MS data is presented. Analyses were carried out in dark and room temperature to prevent oxidation. Only sitosterol oxides were quantified.

### 3. Results and discussion

The total sterol content in samples before heating ranged from 275 mg/100 g in native olive oil (A) to 312 mg/100 g in rosemary olive oil macerated for 2 months.

A progressive decrease in sterol content occurred during heating up to 6 hours in all the samples analysed. Table 1 shows values both from total sterol content (mg/100 g) and from sterol loss (%) during heating.

**Table 1.** Total sterol content (T.S.) and sterol loss percentage (S.L.) during heating in A-E samples.

| time (h) | sample A        |          | sample B        |          | sample C        |          | sample D        |          | sample E        |          |
|----------|-----------------|----------|-----------------|----------|-----------------|----------|-----------------|----------|-----------------|----------|
|          | T. S. (mg/100g) | S. L.(%) | T. S. (mg/100g) | S. L.(%) | T. S. (mg/100g) | S. L.(%) | T. S. (mg/100g) | S. L.(%) | T. S. (mg/100g) | S. L.(%) |
| 0        | 275             | 0        | 292             | 0        | 312             | 0        | 301             | 0        | 310             | 0        |
| 1        | 235             | 15       | 280             | 4        | 313             | 0        | 262             | 13       | 255             | 18       |
| 3        | 189             | 31       | 270             | 7        | 310             | 1        | 251             | 17       | 252             | 19       |
| 6        | 170             | 38       | 237             | 19       | 289             | 7        | 209             | 31       | 240             | 23       |

The highest sterol losses were found after 6 hour of heating in all the samples, ranging from 7% in rosemary olive oil 2 month macerated (sample C) to 38 % in native olive oil (sample A).

The deterioration pattern can be clearly followed in Figure 1. Rosemary olive oil samples B and D showed a similar total sterol deterioration profile between 1 and 6 hours of heating. A different profile was exerted by rosemary olive oil 72 hour macerated and stored for 4 months (sample E): after 1 hour of heating a rather great sterol loss occurred (18%), but the degree of deterioration remained quite steady between 1 and 6 hours of heating.

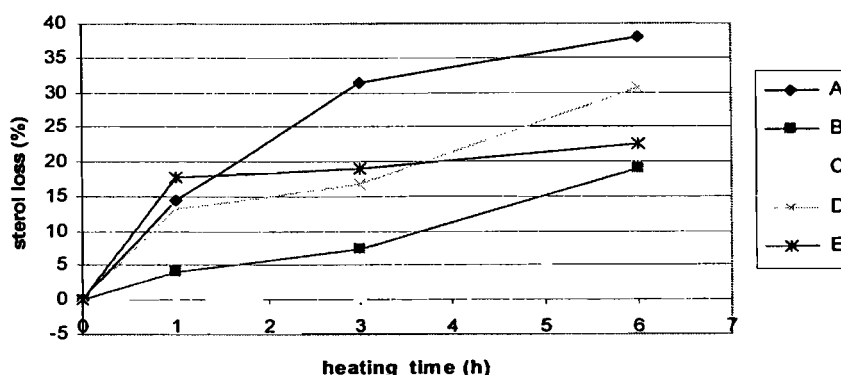


**Figure 1:** Total sterol deterioration profile during heating in A-E samples.

However, the highest sterol stability was found in the rosemary olive oil 2 month macerated (sample C), where the only slight sterol loss (7 %) was found in the 3-6 hour time. Indeed, hardly any sterol losses occurred up to 3 hours of heating in the same sample.

Oxidation of sterols was followed by measuring 8 oxides from sitosterol, the most abundant sterol found in each samples, accounting for over 40% of the total sterols. All the samples had a very small sitosterol oxide content before heating, around 0,7 %, expressed as a percentage of the initial sitosterol content. During heating the individual oxide contents increased both in the native olive oil (sample A) and in the rosemary olive oils (samples B, C, D, E) (Figure 2A).

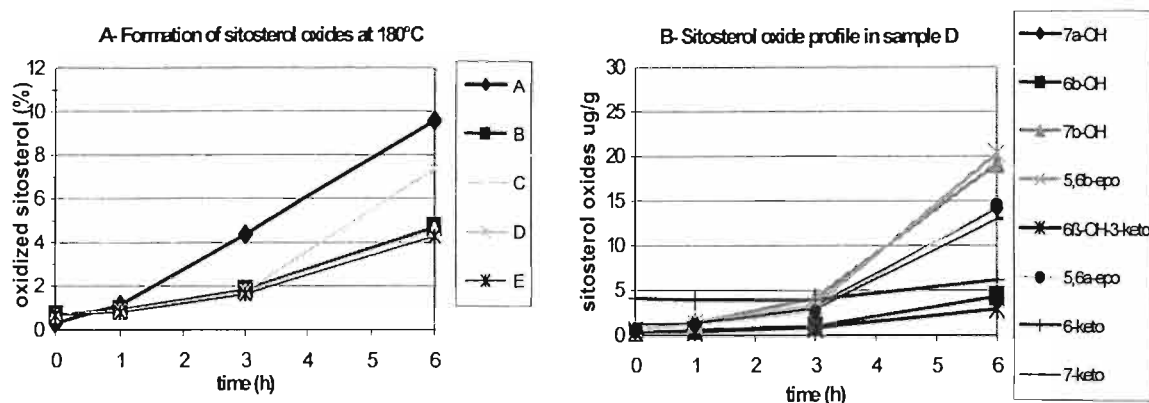


**Figure 2 A:** Formation of sitosterol oxides during heating in A-E samples; B-sitosterol oxide profile in sample D.

The degree of oxidation was much higher in the native olive oil compared to the rosemary olive oil samples. After 3 hours of heating more than 4% of initial sitosterol was oxidized in the native olive oil, and about 10% after 6 hours of heating. By contrast, the protective role of rosemary was mostly evident up to 3 hours of heating, when less than 2 % of sitosterol oxides were formed in samples B-E and less than 5% after 6 hours. However, over 7% sitosterol oxides were formed in the sample D after heating for 6 hours.

The sitosterol oxide profile during heating was fairly similar in all the samples analyzed. The sample D oxide profile is reported as an example in figure 2B. 7 $\alpha$ -OH sitosterol and 5,6 $\alpha$ -epoxy sitosterol were the main products formed during heating. The individual oxide contents increased greatly during heating except for 6-ketositosterol, already present in the non-heated samples.

Figure 3 shows as a percentage the sums of the total amounts of the quantified sitosterol oxides and the unaltered sitosterol, referred to the initial sitosterol contents, after 1, 3, 6 hours of heating.

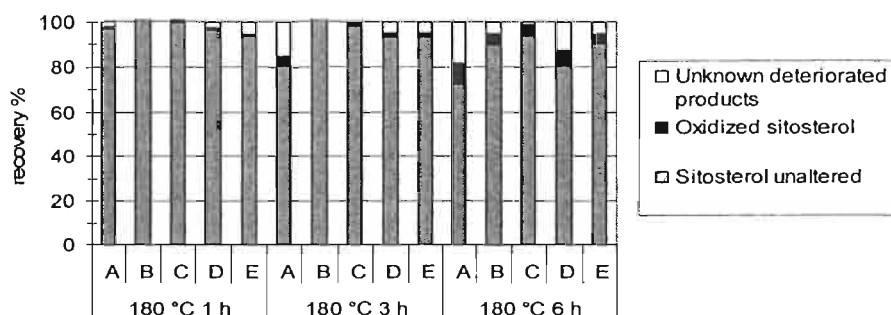


**Figure 3:** Amounts (%) of sitosterol, its oxidation products, and the unknown oxidation products in A-E samples during heating.

It should be noted that the sitosterol oxides do not count for all sitosterol losses during heating, but other unknown deterioration products were formed in heated samples. A progressive increase in oxidation products is evident in all the samples during heating, except for the rosemary olive oil 72h macerated (sample B): after 1 and 3 hours of heating no sitosterol losses were detected. The major

oxidation products occurred after 6 hours of heating in all the samples: rosemary olive oil 72 h macerated (sample B) and rosemary olive oil 72h macerated and stored for 4 months (sample E) had a similar profile after 6h, suggesting that the antioxidant power stability remained unchanged even after 4 month storing. When comparing the rosemary olive oil 2 month macerated (sample C) with the rosemary olive oil 4 month macerated (sample D), very small sitosterol oxide amounts were found in sample C after heating for 6 hours. This result suggests that most of the antioxidant compounds, responsible for the protective role against sitosterol oxidation, were extracted from rosemary after 2 month maceration period. By contrast, it might be that antioxidant rosemary compounds partially deteriorated after 4 month maceration time.

#### **4. Conclusion**

Rosemary is able to counteract the oxidation of plant sterols in extra-virgin olive oil during heating. The maceration time of rosemary in olive oils affects both sterol losses and sterol oxide formation during heating: 2 month maceration time resulted to be the optimum methodology for preparing flavoured oils, as the highest amount of total sterols was preserved during heating between 1 and 3 hours.

Stability test showed that most of the protective compounds from rosemary are probably deteriorated in prolonged storage time.

Phenolic compounds might be responsible for the antioxidant activity of rosemary in olive oil. However, further studies are needed to identify such compounds.

#### **Acknowledgement**

The study was supported by MiPAAF in the frame of the Project "BIOVITA"

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## **Profil des composés phénoliques d'une huile d'olive au romarin**

Laura D'Evoli, Massimo Lucarini and Ginevra Lombardi-Boccia

### **Résumé**

Les plantes aromatiques et les épices sont largement utilisées comme agents aromatisants et des aliments cuits dans la fabrication de conserves de denrées alimentaires traditionnelles. Le romarin en raison de ses propriétés antioxydantes et antimicrobiennes est une alternative prometteuse aux antioxydants synthétiques pour le maintien de la sécurité des aliments durant leur durée de vie de stockage. L'objectif de cette étude était d'identifier les composés responsables de l'activité antioxydante du romarin présents dans l'huile d'olive macéré avec des feuilles de romarin. L'huile d'olive natif et l'huile ou romarin ont été analysées pour connaître leur profil phénolique. Le rôle protecteur de romarin contre la dégradation oxydative de l'huile a été principalement attribué au carnosol et à l'acide carnosique, deux composés phénoliques à fort pouvoir antioxydant présent dans la plante et en quantité raisonnable détectés dans les huiles ou romarin.

**Mots clés:** Huile d'olive extra vierge, huile au romarin, phénols.

## **Phenolic profile of a rosemary extra-virgin olive oil**

### **Abstract**

Aromatic plants and spices are largely used as flavouring agents of cooked foods and in manufacturing of traditional preserved foods. Rosemary because of its antioxidant and antimicrobial properties is a promising alternative to synthetic antioxidants in keeping safety of foods during storage increasing their shelf life. The aim of the study was to identify rosemary compounds responsible for the observed antioxidant activity of the extra-virgin olive oil macerated with rosemary. Both native extra-virgin olive oil and rosemary oil were analyzed for their phenolic profile. The protective role of rosemary against oxidative deterioration of the oil was mainly ascribed to carnosol and carnosic acid, two phenolic compounds with strong antioxidant capacity present in the herb and detected in reasonable amount in the rosemary oils.

**Key words:** Extra virgin olive oil, rosemary oil, phenolic profile.

## Characterisation of phenolic compounds from Dhokar olive cultivar

G. Rigane <sup>a,b</sup>, R. Ben Salem <sup>b</sup>, S. Sayadi <sup>a</sup>, & M. Bouaziz <sup>a,\*</sup>

### Abstract

The phenolic composition of methanol-water (20:80, v/v) extracts of Dhokar fruit was studied by using reverse phase high performance liquid chromatography followed by LC-MS analysis. This technique allowed the identification of some common phenolic compounds, namely: hydroxytyrosol glycoside, oleoside, oleoside 11-methyl ester, rutin, nuzhenid, and oleuropein aglycon was also detected. Moreover, this technique enabled us to identify the Oleuropein diglucoside, which had previously been identified in olive leaves of other varieties.

**Keywords:** phenolic composition, Dhokar cultivar, LC-MS

## Caractérisation des composés phénolique présent dans les fruits d'olive de la variété Dhokar

### Résumé

La composition phénolique de l'extrait eau-méthanol (80:20, v/v) des fruits d'olive de la variété Dhokar a été étudiée en utilisant la chromatographie liquide en haute pression (HPLC) suivi par LC-MS. Cette technique nous a permis d'identifier les composés phénolique, à savoir : l'hydroxytyrosol glucoside, l'oleoside, l'acide élenolique, la rutine, le nuzhenid et l'oleuropein aglycon. Cette technique nous a permis aussi d'identifier l'oleuropein diglucoside pour la première fois dans cette variété.

**Mot clefs:** composition phénolique, Dhokar, LC-MS

### 1. Introduction

Olive tree culture has a great economic and social importance in the Mediterranean area. In fact, it is one of the most important agricultural activities in the Mediterranean countries, where there are about 8 million hectares of cultivated olive trees. In Tunisia, olive trees cover an area of 1.6 million hectares, where Chetoui and Chemlali are the two main olive cultivars (Bouaziz et al., 2004, 2005, 2006). While, Dhokar-Ben Guerden olive cultivar is one of rare olive trees in Medenine zone (South of Tunisia) which is used as a pollinator for olive cultivar and is grown as a very small number of trees because of the limited quantity of its oil (Trigui et Msallem, 2002).

### 2. Methods and Materials

#### 2.1. Extraction of phenolic compounds from olive fruits

The extraction method adopted for phenolic compounds must be able to achieve the extraction of interest compounds and must avoid chemical modification. The olive fruits were blended using household blender. The resulting paste (100 g) was extracted according to the method described by Bouaziz et al. (2006).

#### 2.2. Olive sample

Fruits from *Olea europaea* L. cultivar, namely Dhokar- Ben Guerdane were picked by hand at full maturation from Medenine.

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### 2.3. Reverse phase HPLC and LC-MS conditions

A reverse phase HPLC and LC-MS techniques were developed to identify and quantify phenolic compounds. Analysis conditions were as described before by Bouaziz et al. (2005, 2006).

### 3. Results and Discussion

Aqueous-methanol extracts of Dhokar-Ben Guerdane olive fruit were analyzed by a reverse phase HPLC technique at 280 nm in order to identify the phenolic compounds in the olive fruits. The structure of the main phenolic compounds was based on the search for pseudomolecular  $[M-H]^-$  ions, using extracted ion mass chromatograms and comparing those with data in the literature (Damak et al., 2008, Bouaziz et al., 2005). Data obtained from the LC-MS analysis of olive extracts are summarized in Table 1.

**Table 1:** Phenolic compounds detected in Dhokar olive fruit cultivars.

| No. | Approximative r.t. (min) | Pseudo-molecular ion $[M-H]^-$ | Main fragments m/z | compound                      |
|-----|--------------------------|--------------------------------|--------------------|-------------------------------|
| 1   | 1.9                      | 315                            | 153, 123           | Hydroxytyrosol glucoside      |
| 2   | 2.1                      | 389                            | 345, 209, 165      | Oleoside                      |
| 3   | 2.6                      | 685                            | 523, 453, 299      | Nuzhenid                      |
| 4   | 3.1                      | 403                            | 371, 223, 179      | Oleoside 11-methyl ester      |
| 5   | 3.4                      | 377                            | 197, 153           | Oleuropein aglycon derivative |
| 6   | 4.3                      | 609                            | 301                | Rutin                         |
| 7   | 5.4                      | 623                            | 461, 161           | Verbascoside                  |
| 8   | 10.1                     | 701                            | 539, 377           | Oleuropein diglucoside        |

#### 3.1. Structure determination of compound 1.

The ESI-MS<sup>2</sup> spectrum (Figure 1) of compound 1 showed an ion at m/z 315 and fragment ions at m/z 153 and 123. The fragmentation profile of the pseudomolecular ion at m/z 315 showed a great similarity to that reported by Romero et al. (2002) for hydroxytyrosol-4- $\beta$ -glucoside.

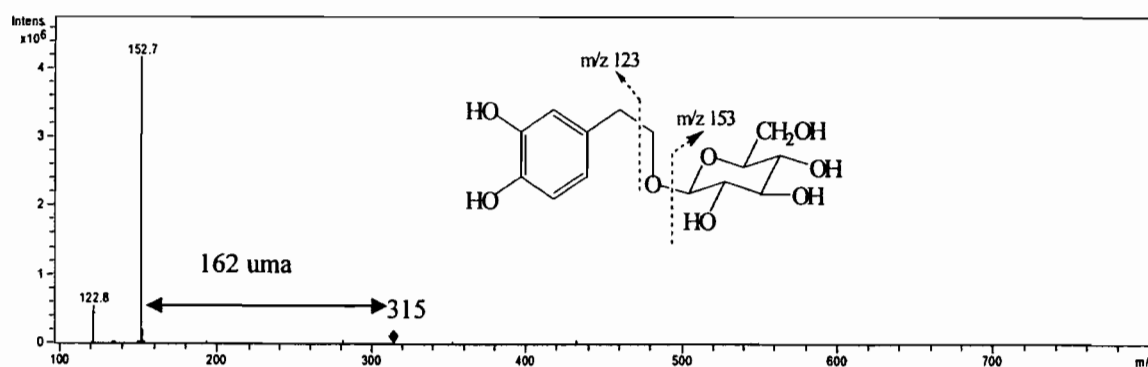


**Figure 1:** ESI-MS<sup>2</sup> spectrum of molecular ion at m/z 315.

#### 3.2. Structure determination of compound 3

The fragmentation of the pseudomolecular ion at m/z 685 ( $[M-H]^-$ ) yielded three fragments at m/z 523, 453 and 299. The ion fragment at m/z 523 was formed by the loss of a hexose unit, while the ion at m/z 453 has been obtained by the loss of C<sub>4</sub>H<sub>5</sub>O from the ion at m/z 523. The ion at m/z 299 was formed by the loss of the elenolic acid glucoside unit. As a consequence, the compound 3 was described in literature for nuzhenid (Jemai et al., 2009). This compound was detected in the Italy olive fruit (Servili et al., 2002).

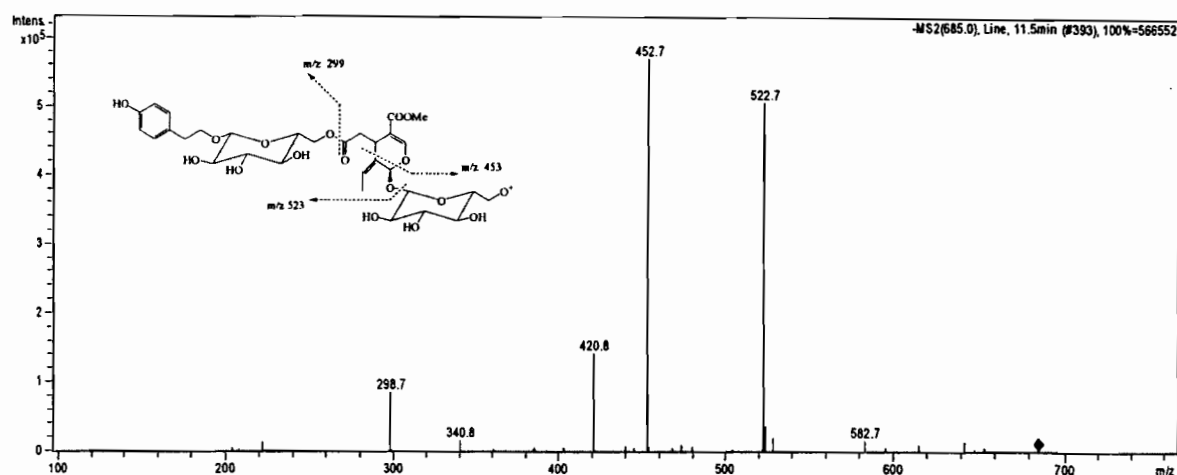


Figure 2: ESI-MS<sup>2</sup> spectrum of molecular ion at m/z 685.

### 3.3. Structure determination of compound 7.

The mass spectrum taken at 5.4 min displayed an abundant m/z 623 ion and two fragments at m/z 461, 161, which m/z 461 was obtained by the loss of caffeic acid moiety, while the peak at m/z 161 resulted from a proton transfer and the production of an anionic ketene (Ryan et al., 1999). These results are in agreement with the presence of Verbascoside.

### 4. Conclusion

The analysis of the methanol- water extracts by ESI-MS<sup>n</sup> allowed the detection of common phenolic compounds which have attracted interest in the last few years owing to their biological and pharmacological activity.

### Acknowledgements

70 uma                      162 uma                      685

The authors would like to thank Mr. Hédi Auissaoui and Mr. Adel Gargoubi for their help with LC-MS and HPLC analysis.

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## Oil quality evolution depending on the ripeness degree of olives from the empeltre variety

Garau, M. C., Negre, A., March, J., Canyellas, B., Luna, J.M., Sastre-Conde, I., Martorell, A.

### Abstract

The characteristic climate of Majorca determines the composition and quality of olive oil from Empeltre variety, establishing an optimum harvest period. Many studies show that the ripeness degree can modify the oil sensory characteristics. Usually, sweet type oil is elaborated with mature olives and fruity type oil with green olives. The main objective of the study was to determine the oil quality characteristics as a function of the ripeness degree. The organoleptical and physicochemical parameters of olive oil were determined following the official methods of analysis. Results exhibited a slight decrease of the peroxide index and k parameters with the increase of the maturation degree. Acidity values showed a constant value lower than 0.8. The organoleptical analysis reached adequate results for all samples, however, important differences on the fruity attribute were detected. In all cases, oil samples were cataloged as extra virgin olive oil.

**Keywords:** olive oil, oil quality, sensory quality, olive ripeness, Empeltre.

## **Thème 6**

**Nouveaux usages et alternatives de valorisation des produits de l'olivier (nutrition, santé, cosmétique, énergie, environnement)**

## Valorization of the solid by-product: olive pomace

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### Abstract

Olive pomace is a solid by-product obtained by virgin olive oil mechanical extraction. The most common utilization of olive pomace obtained by traditional methods is extraction of pomace oil by solvent. The olive pomace composition is variable and depends, principally, by different oil extraction technologies, variety and ripening index of fruits. In particular, the olive pomace obtained by centrifugal system has a low content of oil (3.0-4.5%) and an high content of moisture (55-75 %), respect to olive pomace derived from pressing method. In this study was investigated the alternative use of solid by-product derived to centrifugal system (2-3 phases). In particular, the separation of stone from fibre, realized with the stone-removal machine, represent the interesting solution for its valorization. The results confirmed the good propriety of the stones utilized as fuel because they produce a small quantity of ash (0.4-0.6 %), calorific power variable of 3200 to 4000 Kcal/Kg and smoke very poor of gas containing nitrogen and sulphur.

**Key-words:** Olive pomace, stone, fibre, stone-removal machine, by-product.

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## **Isolement de *Lactobacillus pentosus* capable de bioconvertir l'oleuropéine en hydroxytyrosol : application sur les feuilles d'olivier**

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### **Résumé**

Une bactérie lactique, identifiée comme *Lactobacillus pentosus*, a été isolée à partir de saumure d'olive. Elle est capable de produire l'hydroxytyrosol, composé d'intérêt biologique et commercial, sur MRS additionnée de 1g l<sup>-1</sup> d'oleuropéine. Cette souche est également capable de se développer sur un extrait aqueux de feuille d'olivier comme seule source de nutriments et comme source d'oleuropéine. La souche est apte à produire de l'hydroxytyrosol dans ce milieu.

**Mots Clés :** *Lactobacillus pentosus*; Identification microbienne; Valorisation; Feuilles d'olivier; Hydroxytyrosol.

### **Isolement of *Lactobacillus pentosus* able to bioconvert oleuropein to hydroxytyrosol: application on olive leaves.**

### **Abstract**

A lactic bacterium, identified as *Lactobacillus pentosus*, was isolated from olive brine. It has been proved able to produce hydroxytyrosol, compound with several interesting biological activity and commercial interest, on the medium MRS supplemented with 1g l<sup>-1</sup> oleuropein. This renewable method (comparing with chemical and enzymatic methods) was tested on an aqueous olive leaf extract, which was used as useful source for oleuropein for biotransformation. *Lactobacillus pentosus* was able to grow on this medium as sole substrate and produce hydroxytyrosol.

**Key Words:** *Lactobacillus pentosus*; Microbial identification; Valorisation; olive leaves; Hydroxytyrosol.

### **1. Introduction**

L'industrie oléicole engendre des quantités énormes de rejets, dont les feuilles d'olivier qui sont riches en composés phénoliques (notamment l'Oleuropéine).

L'Hydroxytyrosol, le produit dérivé de la dégradation de l'Oleuropéine (Fig 1), possède des intérêts commerciaux et scientifiques plus importants que ceux de l'Oleuropéine. Il est obtenu par hydrolyse chimique de l'Oleuropéine (Jemaia et al., 2008) ou par synthèse chimique (Capasso et al., 1999). Une technologie douce par utilisation de l'enzyme  $\beta$ -glucosidase a été aussi proposée (Briante et al., 2004; Jemaia et al., 2008). Cependant, ces procédés sont non renouvelables. Comme alternative à ces procédés, ce travail vise à tester la possibilité de produire l'Hydroxytyrosol par bioconversion microbienne de l'Oleuropéine présente dans les feuilles d'olivier, dans un but de valorisation de cette biomasse végétale pour un développement durable du secteur oléicole tout en respectant l'environnement et en utilisant un procédé renouvelable.

Le choix des bactéries lactiques pour cette bioconversion microbienne est due au fait que ces microorganismes sont impliqués dans la dégradation de l'oleuropéine en produisant l'hydroxytyrosol lors de la désamérisation biologique des olives (Marsilio et al., 1996; Servili et al., 2006; Agusti, J., comm. pers., 2007).

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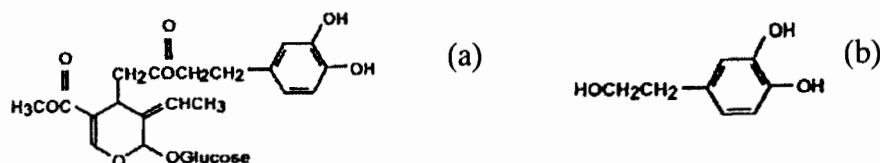


**Figure 1:** Structure chimique de l'Oleuropéine (a) et de l'Hydroxytyrosol (b)

## 2. Matériel et Méthodes

Un isolement des souches bactériennes a été réalisé sur MRS à partir de margine et de saumure d'olive. Après s'être assuré de la pureté des souches (par observation microscopique après au moins 3 repiquages successifs), différentes techniques d'identification (profil morphologique, profil biochimique sur galerie API 50CH, profil enzymatique sur galerie API zyme, profil fermentaire) ont été appliquées. Le profil fermentaire a été déterminé par HPLC selon le protocole décrit par Agusti (comm. pers., 2007).

Une culture de la souche sélectionnée SE4 a été effectuée sur MRS additionnée d'oleuropéine à 1 g l<sup>-1</sup> et sur un extrait aqueux de feuille d'olivier à 5% (m/v). L'analyse des phénols a été évaluée, avant et après une culture de 13 jours, par HPLC selon le protocole décrit par Agusti (2007). L'identification des pics a été confirmée par l'analyse des échantillons enrichis avec les standards.

## 3. Résultats et Discussion

Dix souches de bactéries ont été isolées à partir du biotope olive (margine et saumure d'olive) sur le milieu de culture MRS. Différents tests d'identification microbienne ont été réalisés : observation morphologie, test gram, test catalase, test oxydase, test indole, test mobilité, type respiration, profil fermentaire. Une seule souche parmi les dix souches isolées possède les caractéristiques des bactéries lactiques : gram (+), catalase (-), oxydase (-), indole (-), immobile, respiration anaérobie facultative, production d'acide lactique. La souche lactique est de forme cocobacille de petite taille. L'interprétation des résultats du profil biochimique a permis d'identifier la souche en tant que *Lactobacillus pentosus* avec ID 99,9%.

L'étape suivante a permis de tester la capacité de la souche *Lactobacillus pentosus* à bioconvertir l'oleuropéine en hydroxytyrosol. Pour ce faire, une culture de la souche a été réalisée sur un milieu MRS liquide additionné de 1 g l<sup>-1</sup> d'oleuropéine.

L'analyse par HPLC montre la dégradation de l'oleuropéine et la production de l'hydroxytyrosol suite à l'activité de *Lactobacillus pentosus* (Fig. 2). Ce résultat confirme les travaux de Servili et al., (2006) qui ont utilisé une souche de *Lactobacillus pentosus* pour la désamérisation biologique des olives à l'échelle pilote.

Les *Lactobacillus plantarum* sont plus connues dans la dégradation de l'oleuropéine lors de la fermentation des olives (Marsilio et al., 1996). Ces auteurs ont proposé un modèle de mécanisme de bioconversion biologique de l'oleuropéine en hydroxytyrosol. Ce modèle implique 2 enzymes : le beta-glucosidase et l'estérase.

Le profil enzymatique de la souche *Lactobacillus pentosus* montre la présence d'une activité beta-glucosidase uniquement à 30°C en anaérobiose et en aérobiose. L'activité estérase est absente quelque soit les conditions de culture testées (30°C, 37°C, aérobiose, anaérobiose). Ces résultats peuvent laisser supposer que le mécanisme de bioconversion de l'oleuropéine en hydroxytyrosol est différent selon les espèces de bactéries lactiques.

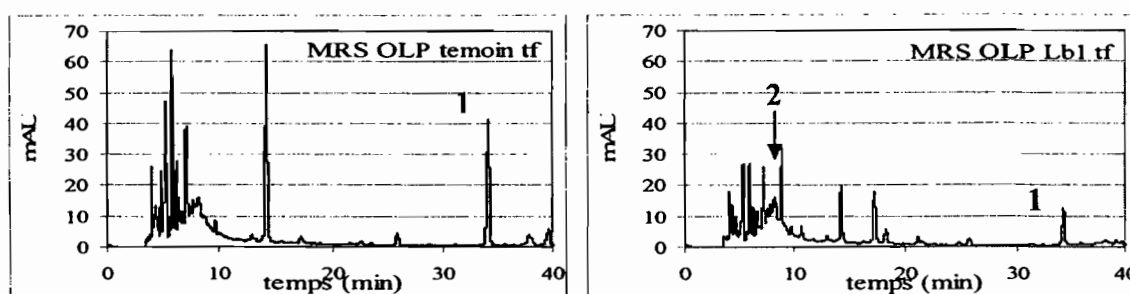


**Figure 2:** Profil HPLC sur milieu MRS contenant 1 g l<sup>-1</sup> d'oleuropéine avant et après bioconversion par la souche lactique *Lactobacillus pentosus*. Identification des pics: 1, Oleuropéine; 2, Hydroxytyrosol.

Les feuilles d'olivier sont connues par leur richesse en oleuropéine. Un extrait aqueux de feuille d'olivier a été utilisé comme seul substrat pour cultiver la souche *Lactobacillus pentosus* dans un objectif de produire l'hydroxytyrosol.

*Lactobacillus Pentosus* est capable de se développer sur ce milieu tout en produisant l'hydroxytyrosol et en dégradant l'oleuropéine (Fig. 3).

#### 4. Conclusions

Une bactérie lactique identifiée comme *Lactobacillus pentosus* est capable de dégrader l'oleuropéine et de produire l'hydroxytyrosol en impliquant l'enzyme beta-glucosidase. Cette souche est également capable de se développer sur un extrait aqueux de feuille d'olivier comme seule source de nutriments. La souche est apte à produire de l'hydroxytyrosol dans ce milieu.

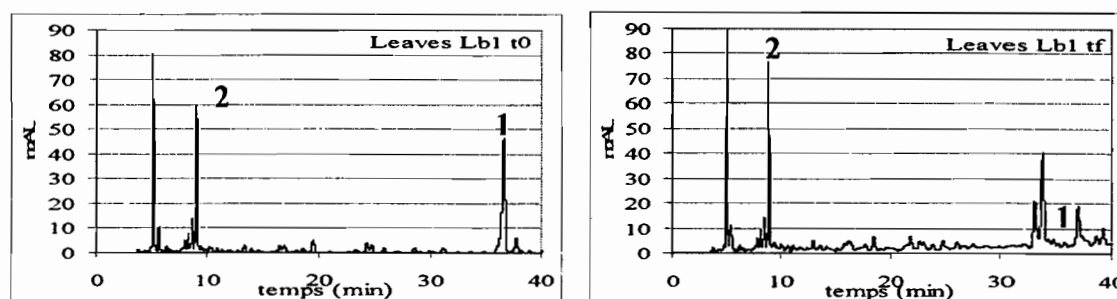


**Figure 3:** Profil HPLC d'un extrait aqueux de feuille d'olivier avant et après bioconversion par la souche lactique *Lactobacillus pentosus*. Identification des pics: 1, Oleuropéine; 2, Hydroxytyrosol.

#### Remerciements

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## Apport du SIG et de l'analyse multicritère dans l'optimisation du choix de complexes agro-industriels

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### Résumé

La région de Sfax souffre d'une situation environnementale critique causée par les rejets hydriques et atmosphériques des zones industrielles. Cette situation est accentuée par les rejets de margines générées par la trituration des olives. La gestion de ces effluents liquides ne répond pas aux conditions de préservation et de sauvegarde de l'environnement. En plus une divergence importante est marquée entre la capacité de trituration concentrée dans le milieu urbain et la production oléicole concentrée dans le milieu rural. Un transfert progressif des huileries du milieu urbain vers le milieu rural est nécessaire. Ainsi, ce travail vise à développer une approche optimale de gestion de l'environnement en vue de sélectionner des sites potentiels à la création de complexes agro-industriels dans cette région. Le SIG nous a permis la spatialisation des différents facteurs géologique, environnemental, social, infrastructurel et économique moyennant des outils d'analyse et de modélisation. Ceci a contribué à l'évaluation d'un grand nombre d'alternatives pour la localisation des sites selon des critères d'évaluation. Les méthodes d'analyse multicritères (AHP et ELECTRE) nous ont permis de faire ressortir les sites les plus adéquats à la création de complexes agro-industriels, et d'établir une liste de priorités pour les sites retenus.

**Mots clés:** Margine, Système d'Information Géographique (SIG), analyse multicritère, complexes agro-industriels.

### Abstract

Sfax suffer from a critical environmental situation caused by hydrous and atmospheric rejections of the industrial zones. This situation is accentuated by the OMWW generated by the olives triturating and the important divergence between the triturating capacity, concentrated in urban environment and the olive-growing production, concentrated in rural environment. A progressive transfer of the oil mills from the urban environment to the rural environment is necessary.

Thus, the objective of this work is to develop an optimal approach of environment management in order to select potential sites to create agro-industrial complexes in this region.

GIS has allowed the spatialization of the different factors: geological, environmental, social, infrastructural and economic using tools for analyzes and modelling. This contributes to the evaluation of a great number of alternatives for the localization of the sites according to the evaluation criteria. Multicriteria analysis (AHP and ELECTRE) allowed us the most adequate sites to the creation of agro-industrial complexes, and made a priority list of the selected sites

### 1. Introduction

L'activité oléicole a un poids important dans l'économie des pays producteurs, particulièrement dans le bassin méditerranéen. La Tunisie se classe comme quatrième producteur d'huile d'olive à l'échelle internationale, après l'Espagne, l'Italie et la Grèce [1]. L'extraction d'huile d'olive génère deux sous produits: solide (grignon), et liquide qui est nommé localement margine. La quantité de margine produite varie de 0,5 à 1,4 l kg (Sierra et al., 2001) selon le système de trituration adopté. Les margines présentent des caractéristiques variables liées essentiellement au procédé d'extraction adoptée, la variété des olives et leur maturité. Cet effluent de coloration noirâtre, très odorant et

turbide, contient des huiles émulsifiées, et des taux assez élevés de matière organique de l'ordre de 15%. La fraction inorganique présente des taux de l'ordre de 2%. Leur pH étant faible, la conductivité électrique est élevée, et présentent des fortes teneurs en polyphénols qui sont responsables de la coloration foncée et des propriétés phytotoxiques et antimicrobiennes (Martinez et al., 1986). La valorisation des margines consistent en des traitements aérobies pour la production des protéines ou des traitements anaérobies pour la production de biogaz (Fiestas, 1996).

En Tunisie, les oleifacteurs doivent construire des bassins temporaires d'évacuation des margines dans les huileries qui peuvent contenir les effluents d'une semaine. Cependant, ces petits bassins ne peuvent pas résoudre le problème. L'approche consiste en la construction de bassins régionaux plus grands qui sont aménagés sur des sols imperméables et dans des quels les margines seront naturellement évaporées pendant l'été.

Dans la région de Sfax (fig.1), principale zone de production d'olive en Tunisie, le non respect de la réglementation et la concentration des huileries dans le milieu urbain amplifient les problèmes relatifs à la gestion des margines. En plus, la divergence entre la production oléicole concentré dans le milieu rural et la capacité de trituration concentré dans le milieu urbain (Sahnoun et al., 2005) nous amène à proposer une nouvelle répartition spatiale des huileries à travers leur transfert progressif du milieu urbain vers le milieu rural où on peut appliquer l'épandage des margines aux champs dont les avantages ont été prouvée par la recherche (Ben Rouina et al., 2004).

Pour ce faire, on a eu recours à une approche multidisciplinaire qui a mis un équilibre entre les différents facteurs environnementaux, sociaux, économiques, infrastructurelles et géologiques. Les Systèmes d'Information Géographique (SIG) et l'Analyse Multicritère (AM) sont des outils et des méthodes bien adaptées à cette problématique et illustrant le mieux l'approche multidisciplinaire à la sélection de sites potentiels à la création de complexes agro-industriels. Les SIG sont spécialisés dans la saisie, le stockage et l'analyse de la base de données, et permettent d'intégrer plusieurs couches d'information. Les méthodes d'AM sont conçues pour fournir une information synthétique (Joerin, 1998). En effet, elles permettent une sélection des sites suite à une combinaison des critères de choix par des combinaisons linéaires pondérées, et leur classification. Cet article présente une étude détaillée d'utilisation de cette approche SIG-AM pour optimiser le choix de sites potentiels à la création de complexes agro-industriels dans la région de Sfax (figure 1).

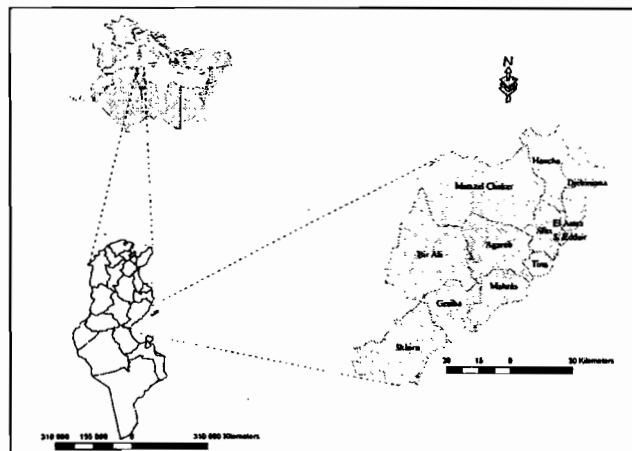


Figure 1: localisation de la zone d'étude.

## 2. Méthodologie de travail

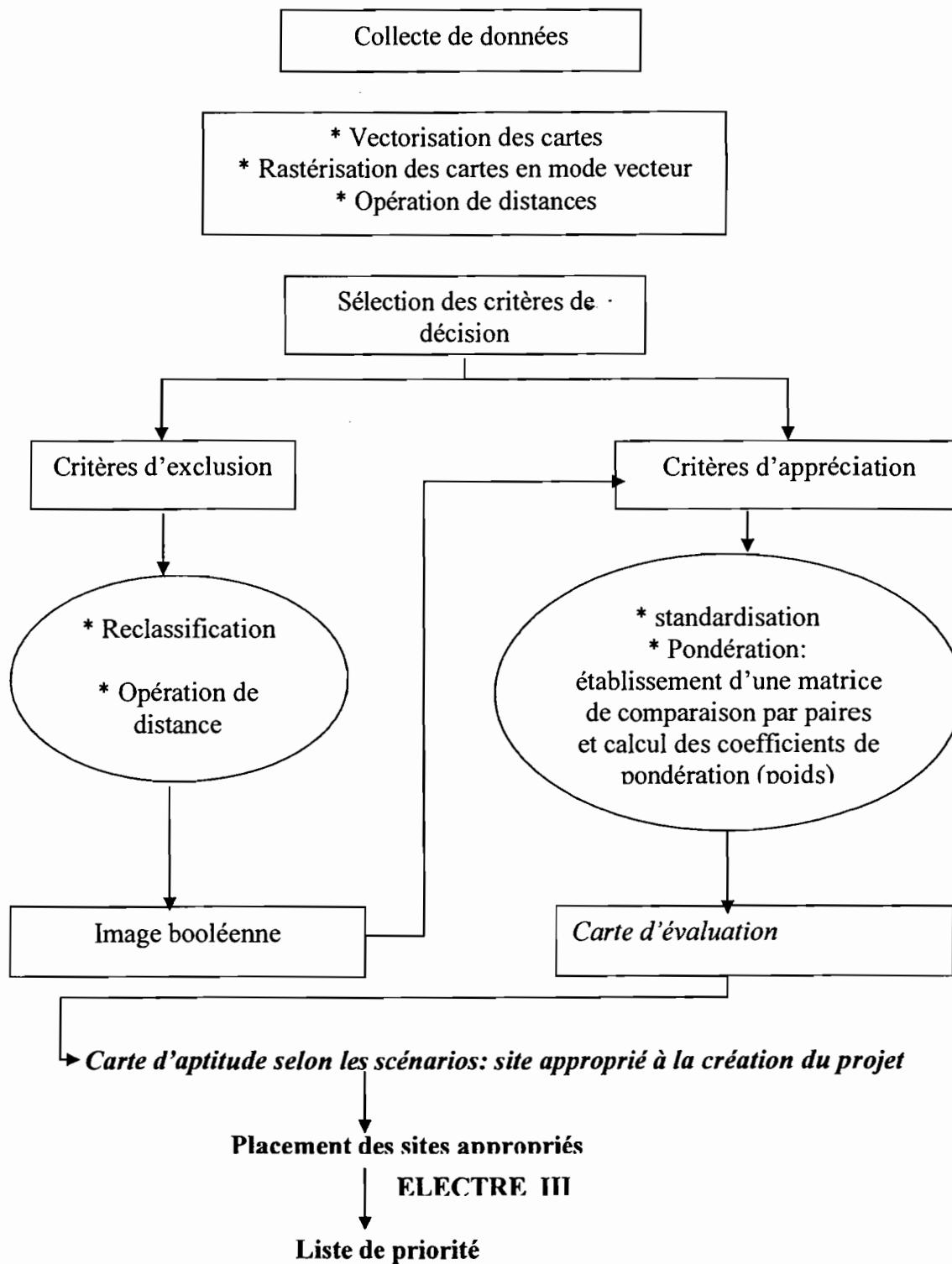


**Figure 2:** Illustration schématique de la méthodologie de travail

## 2.1. Les critères de décision et classification

## 2.2. Critères exclusifs

Ces contraintes ont pour objectif de limiter la recherche de sites appropriés dans des domaines à caractère exclusif qui ne tolèrent aucune concurrence. Suite à une étude bibliographique et en fonction des résultats d'une enquête entreprise auprès d'experts dans les différentes disciplines de l'étude, il a été convenu de fixer les contraintes suivantes:

## 2.3. Réseau hydrographique

Le site ne doit pas être aménagé à proximité des rivières, ruisseaux ou dans une région exposée à des risques de crue. Le choix de la distance minimale à respecter aux alentours des rivières varie d'un pays à l'autre et en fonction de leurs débits. Par exemple à Gölbaşı en Turquie, cette distance peut varier de 100 à 300 m en fonction du débit moyen annuel de la rivière (Basagaoglu et al., 1997).

Ainsi, dans le cadre de cette étude, la distance minimale n'a pas été déterminée sur la base des débits annuels des oueds, car la plupart ils sont intermittents et ne sont alimentés que lors des crues. Nous avons choisi une zone tampon de 100 m, depuis le plan du réseau hydrographique (fig. 3).

Cette distance nous paraît suffisante puisque les huileries sont équipées de bassins temporaires pour l'évacuation des margines. Le risque d'écoulement latéral est alors négligeable.



Figure 3: Image booléenne du critère "réseau hydrographique"

## 2.4. Zones protégées

Dans le but de préserver et conserver la diversité biologique des salins de Sfax, zone humide protégée à l'échelle internationale d'après la convention de Ramsar, en plus de sa localisation sur la côte, nous avons exclu, pour l'emplacement du complexe, toutes les zones qui se trouvent à une distance de 20 Km de la côte pour la partie sud où existe les salins de Sfax. Pour la partie nord de la région, nous n'avons exclu que les zones qui se trouvent à une distance de 5 Km uniquement (fig. 4).



Figure 4: Image booléenne du critère "zones protégées"

## 2.5. Faciès dominant

Un complexe doit être créé sur des roches imperméables et semi-perméables. Nous avons associé donc la valeur 1 pour ces régions et la valeur 0 pour les pixels qui ne vérifient pas cette condition (fig. 5).

L'image résultante issue de la combinaison des trois images relatives aux critères d'exclusion (perméabilité, zones protégées et réseau hydrographique) montre que les zones potentielles à la création de complexes agro-industrielles dans la région de Sfax correspondent à un nuage qui s'étend de la délégation de Hencha à la délégation de Skhira en passant par les délégations de Manzel Chaker, Agareb et Bir Ali (fig. 6).



**Figure 5:** image booléenne du critère Perméabilité.

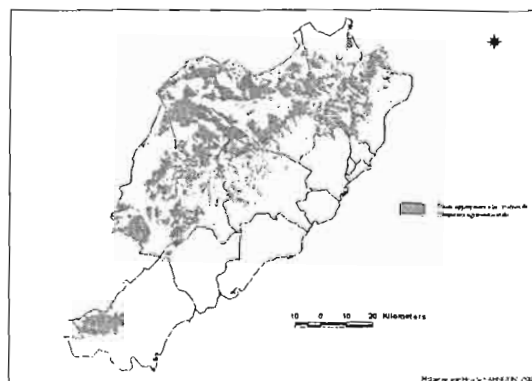


**Figure 6:** Zones appropriées à la création de complexes agro-industriels.

## 2.6. Critères d'appréciation

Les critères d'appréciation ont pour effet de renforcer ou de réduire la pertinence des sites retenus lors de l'étape précédente. Ils permettent donc de sélectionner les meilleures zones pour la création de complexes agro-industriels. Notre étude s'est concertée de cinq types de facteurs: Environnement, Social, Economie, Infrastructure et Géologie qui ont été standardisés dans un intervalle commun de 0 à 10 (Eastman, 1993).

## 2.7. Facteur géologique

Il est à noter que dans ce genre d'étude, il faut prendre en considération le facteur morphologique comme facteur déterminant dans le choix des zones appropriées à la création de complexes agro-industriels. Cependant, dans la région de Sfax, la pente ne varie pas beaucoup (on note un maximum de 5%), c'est pourquoi, ce facteur n'est pas pris en considération dans notre cas.

## 2.8. Facteur environnemental

Ce facteur est présenté par les critères: vocation du sol, la proximité des bassins de stockage des margines, la proximité des unités d'extraction d'huile de grignon et les unités de conditionnement des huiles.

Le facteur de la vocation (fig.7) du sol a été produit à partir de la carte de l'occupation du sol en assignant à chaque classe une notation sur une échelle numérique continue de 10 points.

Quant aux critères: proximité des bassins de stockage des margines (fig. 8), proximité aux unités d'extraction d'huile de grignon (fig. 9), ils ont été évalués par l'opérateur de distance pondérée en standardisant les valeurs de distance par la fonction décroissante monotone sigmoïdale. Les points d'inflexion choisis dans cette étude sont (c) 10 et (d) 35 Km; sur ce domaine de définition, le degré d'appartenance est une valeur possible sur une échelle de 0 à 10: ainsi, les pixels de distance inférieure à 10 km (seuil choisi lors de l'analyse des critères d'exclusion) auront une note de 10 optimale au complexe à ceux de distance supérieure à 35 Km, auront un degré d'appartenance de 0 et sont moins favorables au complexe.

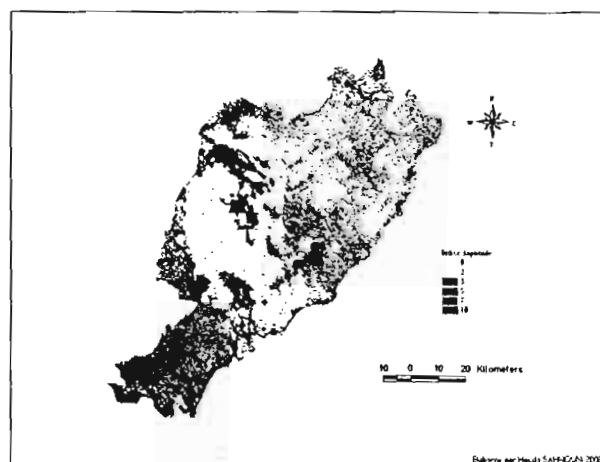


**Figure 7:** Notation de l'occupation du sol.

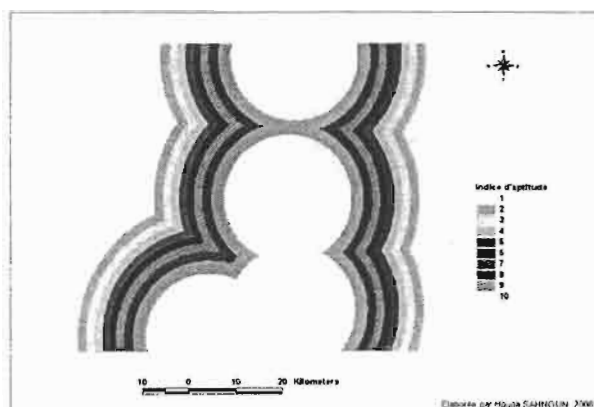


**Figure 8:** Evaluation spatiale du critère d'appréciation "proximité des bassins d'évacuation des margines existants et projetés".

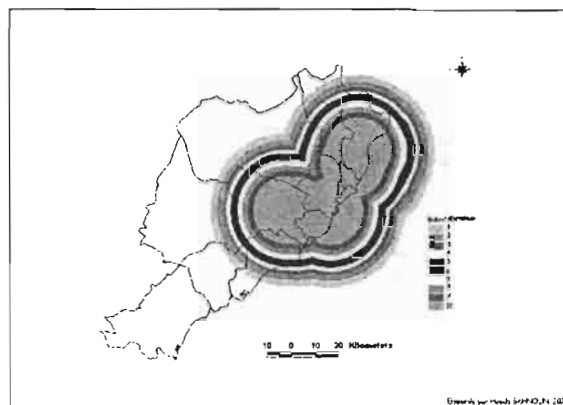


**Figure 9:** Carte d'évaluation spatiale du critère d'appréciation "proximité des unités de valorisation des grignons existants".

## 2.9. Facteur infrastructurel

Le facteur de proximité du réseau routier a été évalué à partir d'une standardisation par la fonction décroissante monotone sigmoïdale. Les points d'inflexion choisis dans cette étude sont (c) 2 et (d) 5 Km; sur ce domaine de définition, le degré d'appartenance est une valeur possible sur une échelle de 0 à 10 : ainsi, les pixels de distance inférieure à 2 km (seuil choisi lors de l'analyse des critères d'exclusion) auront une note de 10 optimale au complexe à ceux de distance supérieure à 5 Km, auront un degré d'appartenance de 0 et sont moins favorables au complexe.

Le facteur de proximité du réseau de l'eau potable a été évalué à partir d'une standardisation par la fonction décroissante monotone sigmoïdale. La note maximale 10 a été attribuée aux pixels qui ont une distance inférieure à 1 km du réseau. Aux pixels se trouvant à une distance supérieure à 10 Km, a été attribuée la valeur 0.

Le facteur de proximité du réseau électrique a été évalué à partir d'une standardisation par la fonction décroissante monotone sigmoïdale; La note maximale 10 a été attribuée aux pixels qui ont une distance inférieure à 1 km du réseau. Aux pixels se trouvant à une distance supérieure à 10 Km, a été attribuée la valeur 0.

Il est à noter que le réseau téléphonique fixe est très utile dans l'installation d'un complexe agro-industriel. En effet, la commercialisation de l'huile d'olive à l'étranger nécessitent des communications sur internet. Cependant, sa présence n'est pas obligatoire, puisque la société de télécommunications offre aux clients appartenant au milieu rural le service "Mobirif". Il s'agit d'une ligne téléphonique qui est implanté dans les zones rurales et qui offre les mêmes services qu'une ligne téléphonique fixe. C'est pourquoi dans ce travail, on n'a pas tenu compte de ce paramètre.

## 2.10. Facteur social

La proximité aux aires résidentielles reflète le facteur social primordial dans l'aide à la décision pour la sélection des meilleurs sites d'installation d'un complexe agro-industriel. En effet, ce facteur a la possibilité de freiner ou d'empêcher la réalisation du complexe engendrant des mécontentements de la population si le site est trop proche des habitations. C'est dans ce sens que nous avons introduit dans cette analyse multicritère cet aspect pour réduire les risques de nuisance des odeurs et des bruits à la population.

La carte du degré d'urbanisation a été construite à partir de l'extraction des zones urbanisées depuis la carte de couverture du sol; nous avons standardisé les valeurs de cette image par la fonction croissante sigmoïdale. La note maximale 10 a été attribuée aux pixels qui ont une distance supérieure à 5 km de l'habitation. Aux pixels se trouvant à une distance inférieure à 1 Km, a été attribuée la valeur 0.



## 2.11. Facteur économique

L'aspect économique est défini par le taux de chômage des personnes entre 15 et 60 ans, les primes d'investissement (avantages spécifiques accordés par l'Etat au titre de développement régional) et le prix du terrain en zones industrielles.

Pour combiner ces différents critères, nous avons établi leur importance relative. Les poids des facteurs sont donc déterminés à partir d'une série de comparaison par paire selon la méthode AHP développée par Saaty (1977).

Leur importance est déterminée sur une échelle numérique de 9 niveaux, et arrangée dans une matrice de décision ou une matrice de comparaison par paire (tableaux 1, 2, 3).

**Tableau 1:** Matrice de comparaison par paire déterminant le coefficient de pondération du facteur environnemental

|                                                                                 | Vocation du sol | Proximité des bassins de stockage des margines | Proximité des unités de valorisation des grignons frais (extraction + tamisage) | Vecteur propre | Coefficient de pondération |
|---------------------------------------------------------------------------------|-----------------|------------------------------------------------|---------------------------------------------------------------------------------|----------------|----------------------------|
| Vocation du sol                                                                 | 1               | 1/7                                            | 1/3                                                                             | 0,36           | 0,08                       |
| Proximité des bassins de stockage des margines                                  | 7               | 1                                              | 5                                                                               | 3,27           | 0,73                       |
| Proximité des unités de valorisation des grignons frais (extraction + tamisage) | 3               | 1/5                                            | 1                                                                               | 0,84           | 0,18                       |

**Tableau 2:** Matrice de comparaison par paire déterminant le coefficient de pondération du facteur infrastructurel

|                                      | Proximité du réseau routier | Proximité du réseau électrique | Proximité du réseau de l'eau potable | Vecteur propre | Coefficient de pondération |
|--------------------------------------|-----------------------------|--------------------------------|--------------------------------------|----------------|----------------------------|
| Proximité du réseau routier          | 1                           | 5                              | 7                                    | 3,27           | 0,73                       |
| Proximité du réseau électrique       | 1/5                         | 1                              | 3                                    | 0,84           | 0,18                       |
| Proximité du réseau de l'eau potable | 1/7                         | 1/3                            | 1                                    | 0,36           | 0,08                       |

**Tableau 3:** Matrice de comparaison par paire déterminant le coefficient de pondération du facteur économique

|                        | Prix du terrain | Taux de chômage | Prime d'investissement | Vecteur propre | Coefficient de pondération |
|------------------------|-----------------|-----------------|------------------------|----------------|----------------------------|
| Prix du terrain        | 1               | 5               | 7                      | 3,27           | 0,73                       |
| Taux de chômage        | 1/5             | 1               | 3                      | 0,84           | 0,18                       |
| Prime d'investissement | 1/7             | 1/3             | 1                      | 0,36           | 0,08                       |

## 3. Evaluation multicritère: agrégation des critères

Une fois que les couches des critères d'appréciation ont été établies et auxquelles ont été assignées des coefficients de pondération, il est aisé de les combiner pour synthétiser ainsi une décision composite sur l'implantation optimale des complexes agro-industriels. Cette opération s'appelle évaluation multicritères ou agrégation des critères. La technique la plus courante et la plus connue de cette approche est la moyenne pondérée, qui intègre totalement tous les critères considérés en un seul selon la formule suivante:

$S = \sum w_i x_i \cdot \Pi c_j$  avec  $S$  : indice d'aptitude,  $w_i$  : poids du facteur  $i$ ,  $x_i$  : valeur du facteur  $i$ ,  $c_j$  = valeur de la contrainte  $j$  et  $\Pi$  : opérateur produit (Eastman, 1993).

Elle consiste à multiplier chaque couche facteur par son coefficient de pondération respectif, et ensuite d'additionner ces résultats pour produire un indice d'aptitude, pour chaque site, situé sur une échelle de 0 à 10 identique à celle des facteurs et dont la somme des poids est égale à 1. Cette couche d'aptitude résultante est combinée avec les couches contraintes par multiplication pour

délimiter les pixels les plus propices aux sites de stockage de la margine. Cette démarche a été adoptée individuellement pour les cinq facteurs de décisions chacun à part (Figure 11 a, b, c, d).

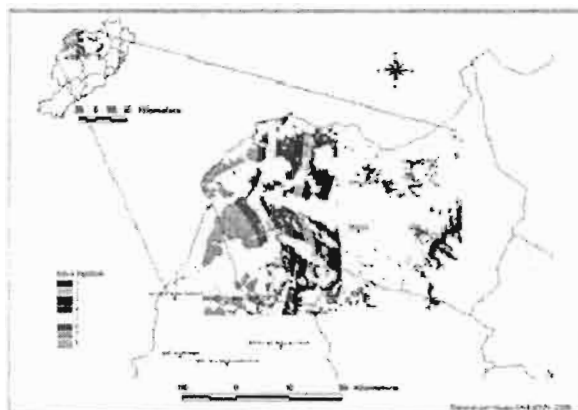


**Figure 11a:** Agrégation multicritère par moyenne pondérée pour le facteur environnemental

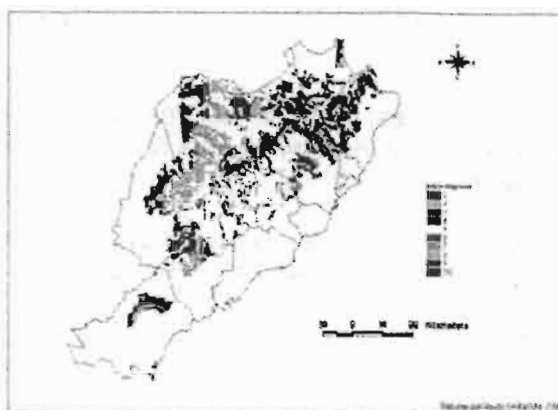


**Figure 11b:** Agrégation multicritère par moyenne pondérée pour le facteur infrastructurel

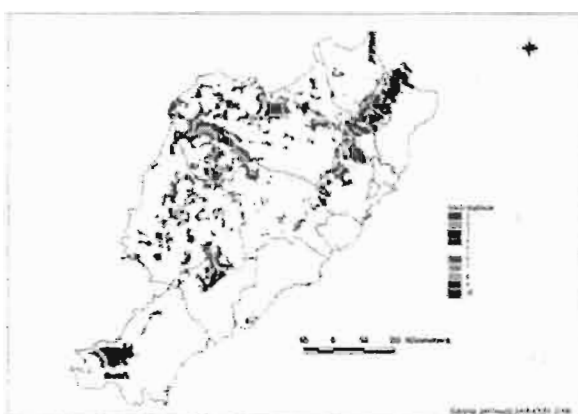


**Figure 11c:** Agrégation multicritère par moyenne pondérée pour le facteur social.

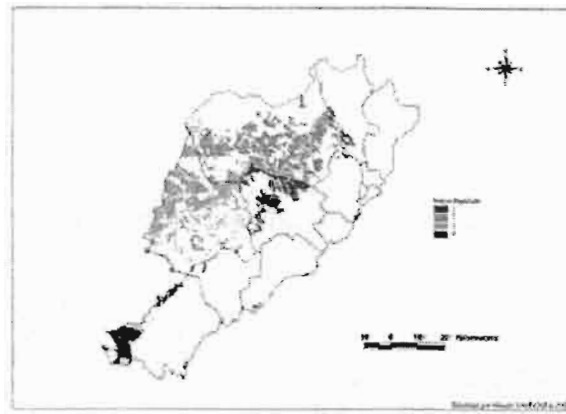


**Figure 11d:** Agrégation multicritère par moyenne pondérée pour le facteur économique.

#### 4. Etablissement de scénario répondant à la notion de développement durable

L'environnement et le développement durable sont au centre des préoccupations de ce début de siècle, c'est pourquoi, nous avons décidé de compléter le travail avec le scénario répondant à cette notion (fig. 12). Les coefficients de pondération de chaque facteur sont calculés dans le tableau 4.

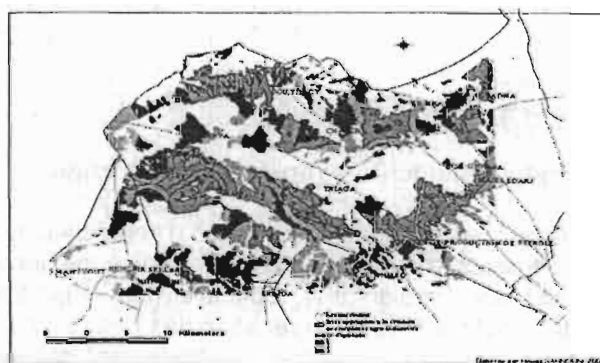


**Figure 12:** Localisation des sites appropriés à la création de complexes agro-industriels.

**Tableau 4:** Calcul des coefficients de pondération pour les facteurs de décision pour le scénario répondant à la notion de développement durable

| Critères de décision                                    | Environnement | Social | Infrastructurel | Economique | Vecteur propre | Coefficients de pondération |
|---------------------------------------------------------|---------------|--------|-----------------|------------|----------------|-----------------------------|
| Scénario répondant à la notion de développement durable |               |        |                 |            |                |                             |
| Environnement                                           | 1             | 1      | 3               | 1          | 1.31           | 0.3                         |
| Social                                                  | 1             | 1      | 3               | 1          | 1.31           | 0.3                         |
| Infrastructurel                                         | 1/3           | 1/3    | 1               | 1/3        | 0.43           | 0.1                         |
| Economie                                                | 1             | 1      | 3               | 1          | 1.31           | 0.3                         |

## 5. ELECTRE et mesure de préférence

La liste des sites potentiels étant fixée, celle-ci sera le sujet d'une analyse en vue d'établir un classement selon un ordre de priorité faisant appel à la méthode analytique ELECTREIII. Pour ce faire, les sites retenus sont évalués par rapport à des critères jugés les plus renseignant en matière de classement de priorité. L'illustration de cette évaluation prend la forme d'une matrice dite de performance pour chacune des sept actions potentielles, et qui prend une note d'évaluation pour chaque critère défini. La note étant la valeur numérique de la grandeur évaluée par le critère, qu'on retient par des requêtes spatiales. Cette partie est réalisée par les critères: surface, insertion dans le paysage, proximité du sol nu, proximité des champs d'olivier, proximité des sebkhas. Ces critères sont standardisés sur une échelle numérique continue de 10 points (tableaux 5, 6, 7, 8, 9).

**Tableau 5:** Standardisation du critère: "surface"

| Surface | Note |
|---------|------|
| Site 1  | 10   |
| Site 2  | 3    |
| Site 3  | 8    |
| Site 4  | 5    |
| Site 5  | 10   |
| Site 6  | 10   |
| Site 7  | 6    |
| Site 8  | 7    |
| Site 9  | 8    |

**Tableau 6:** Standardisation du critère: "insertion dans le paysage"

| Localisation     | Note |
|------------------|------|
| Bord de la route | 10   |
| Distance = 2 Km  | 7    |
| Distance = 3 Km  | 5    |
| Distance = 7 Km  | 1    |

**Tableau 7:** Standardisation du critère: "proximité du sol nu"

| Distance séparant le complexe du sol nu | Note |
|-----------------------------------------|------|
| Distance = 0 Km                         | 10   |
| Distance = 10 Km                        | 7    |
| Distance = 12 Km                        | 6    |
| Distance = 20 Km                        | 4    |
| Distance = 23 Km                        | 3    |
| Distance = 25 Km                        | 2    |
| Distance = 30 Km                        | 1    |

**Tableau 8:** Standardisation du critère: "proximité des champs d'olivier"

| Distance séparant le complexe des champs d'olivier | Note |
|----------------------------------------------------|------|
| Distance = 0 Km                                    | 1    |
| Distance = 4 Km                                    | 5    |
| Distance = 5 Km                                    | 6    |
| Distance = 7 Km                                    | 10   |

**Tableau 9:** Standardisation du critère: "proximité des sebkhas"

| Distance séparant le complexe des sebkhas | Note |
|-------------------------------------------|------|
| Distance = 0 Km                           | 1    |
| Distance = 7 Km                           | 3    |
| Distance = 11 Km                          | 5    |
| Distance = 14 Km                          | 6    |
| Distance = 17 Km                          | 7    |
| Distance = 22 Km                          | 10   |

Une fois les critères évalués, nous avons procédé à la détermination des poids pour exprimer l'importance relative accordée à chaque critère. Dans ce contexte nous avons sollicité les appréciations émanant d'experts connaisseurs dans la matière (tableau 10).

**Tableau 10:** Pondération des critères

| Critère | Surface | insertion dans le paysage | proximité du sol nu | proximité des champs d'olivier | proximité des sebkhas |
|---------|---------|---------------------------|---------------------|--------------------------------|-----------------------|
| Poids   | 15      | 30                        | 10                  | 25                             | 20                    |

Il faut noter que l'indice d'importance (tableau 11) n'a pas la même signification physique que les poids des méthodes d'agrégation totale car il ne s'agit pas d'un coefficient de majoration pour l'évaluation des secteurs proposés mais d'une appréciation relative (Maystre et al, 1994).

**Tableau 11:** Tableau des performances

| Secteurs | Surface | Proximité du sol nu | Proximité des champs d'olivier | Proximité des sebkhas | Proximité du réseau routier |
|----------|---------|---------------------|--------------------------------|-----------------------|-----------------------------|
| 1        | 10      | 10                  | 5                              | 3                     | 1                           |
| 2        | 3       | 10                  | 10                             | 10                    | 10                          |
| 3        | 8       | 6                   | 6                              | 5                     | 10                          |
| 4        | 5       | 10                  | 10                             | 7                     | 5                           |
| 5        | 10      | 3                   | 6                              | 3                     | 10                          |
| 6        | 10      | 4                   | 1                              | 3                     | 10                          |
| 7        | 6       | 2                   | 1                              | 1                     | 10                          |
| 8        | 7       | 1                   | 1                              | 1                     | 7                           |
| 9        | 8       | 7                   | 1                              | 6                     | 10                          |

**Tableau 12:** Représentation des résultats selon le pré-ordre final

| Rang | Action         |
|------|----------------|
| 1    | site 2         |
| 2    | site 3, site 9 |
| 3    | site 4, site 5 |
| 4    | site 1, site 6 |
| 5    | site 7         |
| 6    | site 8         |

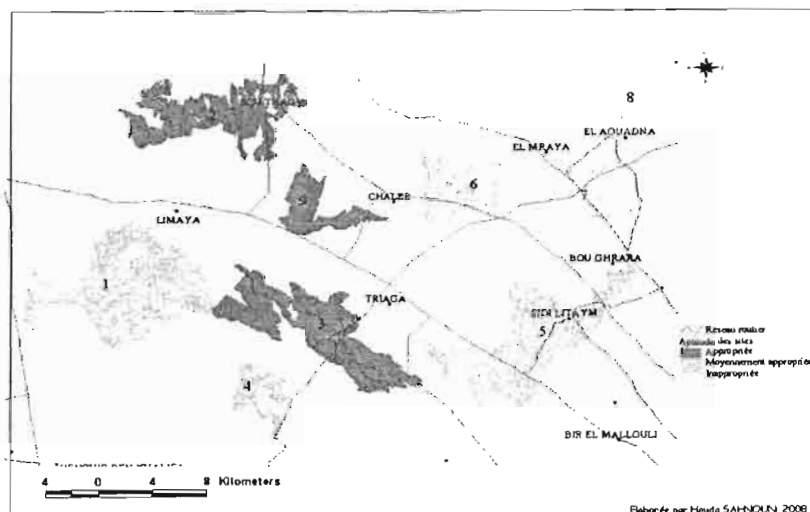


**Figure 13:** Classement des 9 sites par l'algorithme d'ELECTRE III pour la création de complexes agro-industriels.

## 6. Conclusion

Cette étude à vocation environnementale, s'inscrit dans le cadre d'élaboration d'outils de prospections futures en matière de gestion et de préservation de l'environnement, entre autres le choix de sites potentiels à la création de complexes agro-industriels qui résout le problème de gestion des margines. Elle s'est basée sur le traitement de données numériques spatiales collectées et relatives à la zone d'étude, en l'occurrence le gouvernorat de Sfax. Le vif de l'étude est concrétisé par la définition des critères qui s'imposent, et leur évaluation par des calculs et par sollicitation des avis des experts via enquêtes ciblées, et dont les résultats sont maintenus pour la standardisation de ces facteurs. Ces derniers sont sujets d'une démarche de pondération pour établissement de l'importance relative de chaque critère par rapport aux autres, par la méthode AHP. Ceci a abouti à plusieurs sites basés sur une configuration illustrée par un scénario répondant à la notion de développement durable à la base d'une pondération spécifique des familles de critères ou facteurs (environnemental, social, économique, géologique et infrastructurel). La combinaison de ces facteurs a décelé neuf sites répondant au mieux à cette exigence. En vue de raffiner l'étude, on s'est proposé de générer un ordre de priorité des sites retenus entre eux, par une approche de surclassement faisant appel à la méthode ELECTREIII. Les sites 2, 3 et 9 sont classés les plus aptes pour le stockage et le séchage de margines. Toutefois, ce travail cette méthode mériterait d'être testée sur d'autres régions productrices d'huiles d'olives et confrontées aux mêmes problèmes d'évacuation des margines pour localiser les secteurs adéquats à la création de complexes agro-industriels.

Certes, ce travail n'est pas exhaustif et, pour l'enrichir, nous proposons de réaliser : (i) une étude de la vulnérabilité à la pollution des aquifères par l'outil SIG; (ii) une analyse du risque sismique par la réalisation des cartes de zonage sismique pour aider à prendre une décision adéquate quant à la sélection appropriée des sites des complexes agro-industriels.

Nous proposons également d'approfondir l'analyse multicritère en intégrant l'aspect politique et en consultant plusieurs partenaires concernés par ce projet environnemental (autorités communales et municipales, associations de protection de la nature et de l'environnement, agriculteurs, forestiers, industriels, experts..) pour une meilleure sélection des sites.

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## Etude physico-chimique du sol après épandage des margines

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### Résumé

Les margines effluents d'huilerie d'olive sont caractérisées par une forte charge organique avec une demande chimique en oxygène (DCO) généralement supérieur à 80 g/L, une demande biologique en oxygène (DBO) élevée et une forte concentration en composés phénoliques. Ces derniers sont à l'origine de la toxicité des margines et des difficultés de traitement et d'élimination. Par ailleurs, l'épandage des margines constitue une alternative à cette problématique et peut être considéré comme fertilisant liquide. Ainsi plusieurs essais d'épandage à des doses variant de 50 à 200 m<sup>3</sup>/ha sur le sol sableux de la région de Châal ont été effectués. L'étude physico-chimique du sol après dix mois d'épandage a montré un apport bénéfique en matière organique, en éléments fertilisants (phosphore, azote) et en éléments minéraux. Après cette période, on constate que le sol retrouve son état d'équilibre et surtout pour le cas des faibles doses. Pour le cas des composés phénoliques, on constate que ces derniers sont susceptibles de migrer et atteindre les horizons subjacents.

**Mots clés:** Fertigation, sol, marge, composés phénoliques, épandage.

### Abstract

Olive mill waste water (OMW) is produced in large amounts in Mediterranean countries. OMW is characterized by a high chemic oxygen demand (COD), biological oxygen demand (BDO) as well a high level of phenolic compounds. The use of OMW as liquid manure is a valuable options for its disposal, provided that its impact on sandy ground (Châal region). The soil was amended with 0, 50, 100 and 200 m<sup>3</sup>/ha. Ten month after spreading, the soil shows a change of various chemical physical properties essentially organic matter, nitrogen and available phosphorus increases. Therefore, ground keeps its equilibrium state even with the highest dose applied. In addition, phenolic compound increases with dose amended and joins depth.

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## Impact de l'épandage successif des margines sur l'évolution des propriétés chimiques d'un sol sablonneux

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### Résumé

L'effluent liquide des huileries, dénommé «margines», posent plusieurs problèmes environnementaux vu leur forte charge en matières organiques et leur acidité très élevée. D'où divers essais, de valorisation et de traitement des margines ont été entrepris durant ces dernières années. Parmi eux, l'utilisation des margines comme fertilisant dans les terres agricoles tunisiennes à des doses différentes, peut être une solution envisageable qui contribue à l'évacuation de ce produit et à l'amélioration de la fertilité du sol. C'est dans ce cadre que s'insère ce travail qui a pour objectif l'étude de l'incidence de l'épandage annuel des margines brutes, pendant trois années successives sur l'évolution des caractéristiques chimiques du sol. Pour ce faire, nous avons appliqué 4 traitements (0, 50, 100 et 200 m<sup>3</sup> de marge /hectare / an) dans les oliveraies situées dans le site expérimental de Taous qui se trouve à 26 km de la région de Sfax. Les résultats obtenus montrent qu'après 3 années d'épandage successif des margines, le PH du sol reste inchangé malgré que cet effluent est acide. Les teneurs du sol en matière organique, potassium et azote augmentent d'une manière significative par rapport avec le témoin sans margines et l'amélioration du sol en ces éléments est très nette, surtout en potassium qui passe d'une teneur de 170 ppm chez le témoin à 985 ppm pour la dose 200 m<sup>3</sup>. Les teneurs du sol en phosphore sont presque identiques et ne présentent pas de différence significative avec le témoin. La conductivité électrique du sol présente un accroissement considérable par rapport au témoin. La valeur de la conductivité électrique initialement de 0,49 mS/cm atteint une valeur de 3,21 mS/cm après 3 épandages successifs. Les teneurs en chlorures et en sodium augmentent proportionnellement avec la dose appliquée et passent de 30,28 ppm et 43 ppm chez le témoin à 280,04 ppm et 105 ppm pour la dose la plus élevée respectivement pour les chlorures et le sodium.

**Mots clés :** Margines propriétés chimiques sol sablonneux.

## Impact of olive mill waste water spreading on some soil chemical properties over a 3-year period

### Abstract

The objective of this work was to study the effect of untreated olive mill waste water (OMW) spreading applied at different doses on soil chemical properties under natural field conditions in an olive grove during three successive years. In this study, four treatments were applied (0, 50, 100 and 200 m<sup>3</sup> of OMW / hectare / year) in the experimental site «Taous» of the Olive Tree Institute of Sfax (Tunisia). Results obtained showed that after 3-year spreading period, soil contents in organic matter, potassium and nitrogen increased significantly in comparison to the control treatment. Indeed, soil potassium content reached 985 ppm in soil treated with 200 m<sup>3</sup> of OMW. For the control this value was of only 170 ppm. Similarly, the OMW led to a significant increase of soil electrical conductivity (EC) which showed a value of 3.21 mS/cm after the three spreading, against a value of 0.4 mS/cm for the treatment unamended of OMW. This increase of EC was accompanied with those of sodium and chloride contents. However, the effect of OMW spreading was not significant on both soil pH and phosphorus contents.

**Keywords:** olive mill waste water; soil; spreading; chemical properties.

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## Quantification de la teneur en eau et en huile d'olives fraîches : Reconnaissance de l'origine variétale par spectroscopies proche et moyen infrarouge

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### Résumé

Le traitement chimiométrique des spectres proche (PIR) et moyen (MIR) infrarouge a été utilisé dans un premier temps pour prédire les teneurs en eau et en huile d'olives fraîches ( $n=240 \times 35$ ). Dans un deuxième temps, il est appliqué pour classer des olives provenant de cinq cultivars principaux français (*Aglandau*, *Cailletier*, *Olivière*, *Salonenque*, et *Tanche*). Les échantillons proviennent de quatre récoltes successives (2005/2006 à 2008/2009) pour prendre en compte les variations saisonnières. La comparaison des résultats obtenus dans le MIR (REP = 5.1% pour la teneur en eau et 9.1% la teneur en huile) et dans le PIR (REP = 3.9% pour la teneur en eau et 5.8% la teneur en huile) montre que les résultats du PIR sont meilleurs que ceux du NIR. Les olives sont classées de manière satisfaisante selon leur cultivar en utilisant la méthode PLS-DA à partir des spectres PIR et MIR concaténés. L'utilisation de la méthode KNN sur les résultats de PLS-DA conduit à 100% d'échantillons bien classés. L'infrarouge est une méthode alternative aux méthodes existantes pour déterminer les teneurs en eau et en huile ainsi que l'origine variétale des olives.

**Keywords:** Olive, Cultivar, Teneur en eau, Teneur en huile, Chimie-métrie, Moyen Infrarouge, Proche Infrarouge

### Abstract

Chemometric treatment of near (NIR) and mid (MIR) infrared spectra was used first to predict oil and water contents in fresh olive fruit samples ( $n=240 \times 35$ ) and second ( $n=195$ ) to classify these samples into five principal French cultivar origins (*Aglandau*, *Cailletier*, *Olivière*, *Salonenque*, et *Tanche*). The study was carried out during four crop years (2005/2006 to 2008/2009) to take into account the seasonal variations. The comparison of the results obtained in the MIR range (REP = 5.1% for the water content and 9.1% for the oil content) and in the NIR range (REP = 3.9% for the water content and 5.8% for the oil content) provides an obvious advantage to NIR compared to MIR. Fresh olive fruit cultivars were classified satisfactory with PLS-DA method in the combined range. After the used of the K-means clustering on the PLS-DA scores, all the samples are well classified in their five groups of origin. The used of infrared combined spectra allows a considerable improvement in estimating olive fruit quality (oil and water contents, varietal origins).

**Keywords:** Partial Least Square-Discriminant Analysis, K-means, water content, olive fruit, cultivar, Chemometrics, Fourier transform infrared spectroscopy, Near infrared spectroscopy, Oil content

### 1. Introduction

La détermination de la traçabilité des produits de l'industrie agroalimentaire est devenue essentielle en raison des problèmes de santé publique qu'ils peuvent présenter ainsi que pour assurer la loyauté des échanges commerciaux. La filière oléicole n'échappe pas à cette nécessité de traçabilité. Bien que comportant environ 200 variétés différentes, la majeure partie de l'huile d'olive française est produite à partir d'une quinzaine de variétés principales d'olives. Pour optimiser l'extraction de l'huile dans les moulins, il est important de connaître la teneur en eau des olives afin de pouvoir affiner le réglage de l'installation. Cependant, la méthode de référence pour assurer cette détermination est longue à mettre en oeuvre.

Le premier but de ce travail a été d'étudier le potentiel des spectroscopies proche (PIR) et moyen (MIR) infrarouge associées à un traitement chimiométrique en tant que méthode alternative plus rapide pour les déterminations de la teneur en eau et de la teneur en huile. Le deuxième objectif est de prédire l'origine variétale d'olives provenant de cinq cultivars français parmi les plus importants (Dupuy et al. 2010).

## 2. Matériels et méthodes

### 2.1. Echantillons

Les échantillons d'olives ont été fournis par le centre technique de l'olivier (CTO). Un total de 240 lots de 35 olives a été rassemblé pendant trois récoltes successives (2005/2006, 2006/2007 et 2007/2008) pour tenir compte des variations saisonnières. Ils englobent les variétés : "Aglandau", "Cailletier", "Olivière", "Salonenque", "Tanche", "Grossane", "Picholine", "Négrette", "Bouteillan" et "Cayanne". Pour la prédiction de l'origine variétale, seuls 195 lots provenant des cultivars "Aglandau", "Cailletier", "Olivière", "Salonenque" et "Tanche" sont utilisés. Le nombre de lots provenant des autres cultivars n'est pas suffisant pour permettre de les intégrer dans cette étude.

### 2.2. Spectres proche infrarouge

Les spectres sont enregistrés à l'aide d'un spectromètre Antaris™ FT-Near Infrared. 30 olives sont dénoyautées puis disposées dans le passeur d'échantillons. Les spectres sont acquis en réflexion diffuse par la sphère d'intégration, de  $10\,000\text{ cm}^{-1}$  à  $4\,000\text{ cm}^{-1}$ , résolution de  $4\text{ cm}^{-1}$  avec 16 accumulations de scans.

### 2.3. Spectres moyen infrarouge

Une fraction de fruit est déposée entre le diamant et l'enclume en saphir de l'accessoire ATR monoréflexion (Golden Gate, Specac) positionné dans un spectromètre AVATAR 370 FT-IR THERMO NICOLET muni d'une source IR Ever-Glo, d'un détecteur DTGS KBr et d'une séparatrice type KBr. Les spectres sont enregistrés à l'aide du logiciel Omnic, de  $4\,000$  à  $650\text{ cm}^{-1}$  avec une résolution de  $4\text{ cm}^{-1}$  et 64 accumulations.

### 2.4. Teneur en eau et matières volatiles : mesure de référence

Un échantillon d'olives d'environ 1,5 kg est broyé. Après homogénéisation de la pâte, une petite quantité (35 g) est pesée et mise à sécher sur un film résistant à la chaleur, pendant une nuit, dans une étuve à  $104^{\circ}\text{C}$ .

### 2.5. Teneur en huile : mesure de référence

Pour déterminer la teneur en huile, l'échantillon sec est analysé par spectroscopie RMN (spectromètre Minispec de BRUKER). L'échantillon sec, laissé à température ambiante pendant environ une heure avant la mesure, est placé avec son film dans un tube de mesure (en verre, 40 mm de diamètre).

### 2.6. Chimiométrie

Dans cette étude, nous avons eu recours à une seule méthode de prédiction, la régression PLS. Cette méthode est une alternative à la régression linéaire multiple classique. Les applications chimiométriques sont réalisées à l'aide du logiciel Unscrambler 9.6, (CAMO, Norway).

## 3. Résultats et discussion

### 3.1. Détermination de la teneur en eau et de la teneur en huile

Les 240 spectres d'olives sont divisés en deux séries de façon aléatoire : deux tiers des échantillons, soit 150 échantillons, permettent le calcul des modèles à partir des spectres proche infrarouge (PIR); le tiers restant, soit 90 échantillons, permettent de valider les modèles précédemment calculés. La Figure 1 donne les résultats de la prédiction des teneurs en eau et en huile obtenues sur ces échantillons de validation en PIR par PLS.

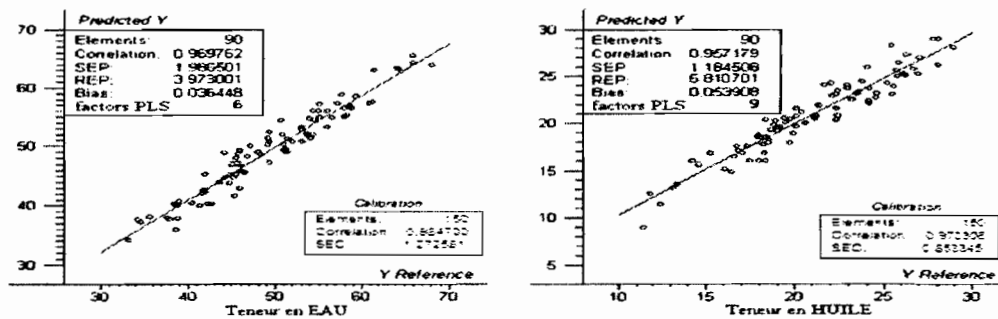


Figure 1: Résultats de la prédiction des teneurs en eau et en huile à partir des spectres PIR

La Figure 2 présente les résultats obtenus à partir des spectres MIR. Les résultats obtenus dans le MIR sont beaucoup moins bons que ceux obtenus dans le PIR puisque les coefficients de corrélations sont plus faibles et les erreurs de prédiction (REP) sont plus importantes. Les prédictions des teneurs en eau et en huile à partir des spectres PIR donnent des résultats satisfaisants avec des REP n'excédant pas 5%. Cette méthode analytique serait donc une bonne alternative aux méthodes de références.

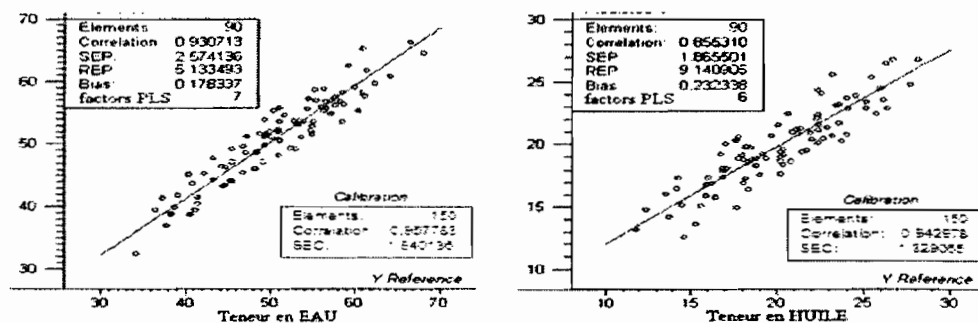


Figure 2: Résultats de la prédiction des teneurs en eau et en huile à partir des spectres MIR

### 3.2. Détermination de l'origine géographique

Les 195 lots sont divisés de façon aléatoire en deux séries: 118 permettent le calcul des modèles d'étalonnage ("Aglandau" (n=41), "Caillietier" (n=21), "Olivière" (n=12), "Salonenque" (n=20), "Tanche" (n=24)). Les 77 restants permettent de valider les modèles ("Aglandau" (n=25), "Caillietier" (n=16), "Olivière" (n=11), "Salonenque" (n=11), "Tanche" (n=14)).

La méthode PLS-DA permet de construire des modèles indépendants pour chaque origine. Chaque modèle considère une origine contre toutes les autres. Les résultats (**tableau 1**) de la prédiction obtenus à partir des spectres PIR ou MIR pour les cinq modèles PLS-DA sont résumés sous la forme d'une matrice de confusion.

Tableau 1: Prédictions de l'origine des olives par traitement PLS-DA des spectres PIR et MIR

|                    | Modèles PIR |             |          |            |        | Modèles MIR |             |          |            |        |
|--------------------|-------------|-------------|----------|------------|--------|-------------|-------------|----------|------------|--------|
|                    | Aglandau    | Caillietier | Olivière | Salonenque | Tanche | Aglandau    | Caillietier | Olivière | Salonenque | Tanche |
| Nombre de facteurs | 9           | 9           | 6        | 8          | 8      | 8           | 7           | 7        | 8          | 9      |
| Aglandau (n=25)    | 24          |             |          |            |        | 25          |             |          |            |        |
| Caillietier (n=16) |             | 15          |          |            |        |             | 16          |          |            |        |
| Olivière (n=11)    |             |             | 11       |            |        |             |             | 11       |            |        |
| Salonenque (n=11)  |             |             |          | 9          |        |             |             |          | 10         |        |
| Tanche (n=14)      |             |             |          |            | 14     |             |             |          |            | 13     |

La Figure 3 est un exemple des prédictions des origines variétales des échantillons de validation par le modèle PLS-DA des spectres MIR concernant l'origine "Aglandau".

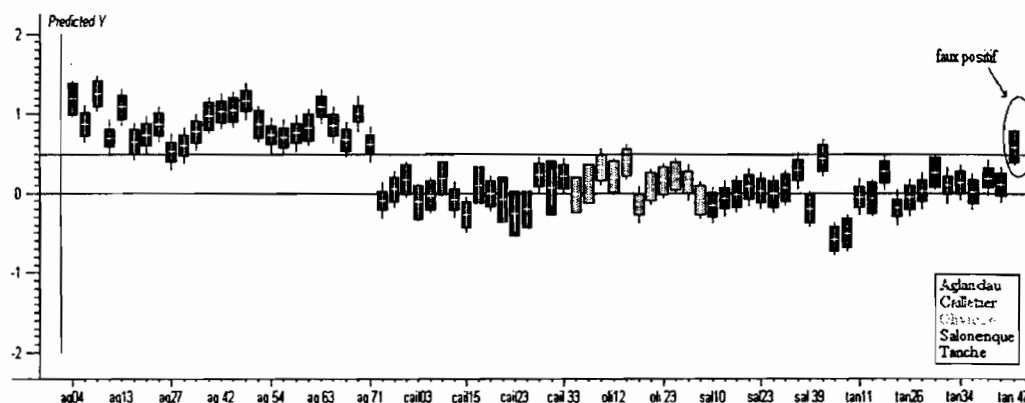


**Figure 3:** Prédiction de l'origine variétale des échantillons de validation par le modèle PLS-DA "Aglandau" à partir des spectres MIR

Tous les échantillons dont la valeur est supérieure à 0,5 sont prédits comme appartenant au cultivar "Aglandau". Tous les échantillons *Aglandau* sont prédits comme *Aglandau*. Un seul des autres échantillons (appartenant au cultivar "*Tanche*") a une valeur prédite supérieure à 0,5. C'est un faux positif car il est prédit à tort comme appartenant au cultivar "*Aglandau*".

Les résultats obtenus sont satisfaisants quelle que soit la technique spectrale utilisée. Il apparaît que les quelques échantillons mal prédits à partir des spectres MIR sont différents de ceux mal prédits à partir des spectres PIR. L'information apportée par chaque technique est différente, et peut être complémentaire. Une alternative consiste à effectuer une PLS-DA sur les spectres proche et moyen infrarouge concaténés et d'appliquer sur ces résultats intermédiaires une autre méthode de classification. Nous avons décidé d'utiliser la méthode des K plus proches voisins (K-nearest neighbors, KNN) pour s'affranchir de la valeur seuil à 0,5. La classification KNN est réalisée en spécifiant au départ un nombre total de cinq classes.

Les valeurs prédites obtenus pour les cinq modèles PLS-DA sont regroupées dans un tableau Excel®. En effectuant une classification KNN sur les résultats obtenus par PLS-DA, tous les échantillons sont bien classés, sans faux-positifs ni faux-négatifs. L'avantage de classer les échantillons avec cette étape supplémentaire, est que non seulement un échantillon est classé dans une origine si la valeur prédite pour ce modèle est grande, mais en plus, on a une information supplémentaire sur les autres modèles pour lesquels les valeurs prédites pour cet échantillon seront petites.

Une analyse en composantes principales (ACP) sur les valeurs du tableau (Figure 4), donne une représentation où figurent cinq groupes distincts correspondant aux cinq origines.

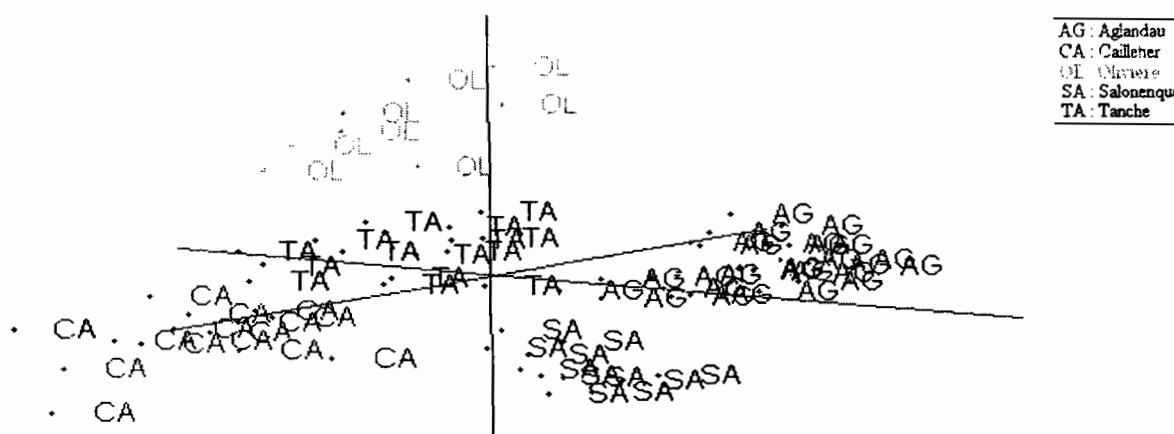


**Figure 4:** ACP sur les résultats (scores) des modèles PLS-DA des échantillons de validation.

#### 4. Conclusion

La détermination des teneurs en eau et en huile par spectrométrie infrarouge et traitement chimiométrique des spectres par la méthode PLS donne des résultats de qualité différente suivant les techniques MIR ou PIR utilisées. La spectroscopie PIR donne de meilleurs résultats que ceux obtenus par spectroscopie MIR et peut être proposée comme méthode alternative aux dosages de l'eau et de l'huile dans les olives.

La détermination de l'origine variétale des olives appliquée à cinq cultivars majeurs français par spectroscopie MIR et PIR combinés et traitement des spectres par les méthodes PLS-DA puis KNN a conduit à 100% d'échantillons bien classés. L'infrarouge peut être un moyen rapide et objectif de reconnaissance variétale des olives.

#### Référence

Dupuy N., Galtier O., Le Dréau Y., Pinatel C., Kister J., Artaud J. 2010. Chemometric analysis of combined NIR and MIR spectra to characterize French olives. *European Journal of Lipid Science and Technology*, 112: 463-475.

## **Etude des huiles extraites des différentes variétés d'olivier: Valeur nutritionnelle et formulation d'une crème de soins**

Ellouze Ghorbel Raoudha Slim Smaoui- Nozha Kamoun et Mohamed Damak

### **Résumé**

Au cours de ce travail, nous nous sommes intéressés à la caractérisation physico-chimiques des différentes variétés d'huiles d'olives tunisienne et particulièrement de la partie insaponifiable (polyphénols, stérols, tocophérols et composés aromatique). Cette étude a révélé une valeur nutritionnelle importante de certaines variétés d'huile d'olive vierge. Des essais de formulations des crèmes de soins à base d'huile d'olives vierges ont montré une stabilité physicochimique et une salubrité microbiologiques à différentes températures de stockages des crèmes élaborées.

**Mots clés:** Huiles d'olive, Tunisie, fraction insaponifiable, valeur nutritionnelle, applications en cosmétologie.

## **Oils extracted from various varieties: Nutritional value and formulation of a cream of care**

### **Abstract**

In this work, we interested in the physicochemical characterization of the various varieties of Tunisian olive oils and particularly of the insaponifiable part (polyphenols, sterols, tocopherols and compounds aromatic).

This study showed an important nutritional value of some varieties of virgin olive oil. Tests in the formulations of the creams of care containing virgin olive oil showed a physico-chemical stability and a healthiness microbiological at various temperatures of storages of the elaborate creams.

## Effet antimicrobien de quelques produits cupriques et des polyphénols extraits de la margine contre *Pseudomonas savastanoi* pv *savastanoi* agent de la tuberculose de l'olivier

Samira Krid Hadjtaieb<sup>1</sup>, Ali Rhouma<sup>2</sup> et Ali Gargouri<sup>1</sup>

### Résumé

La maladie de la tuberculose de l'olivier, causée par *Pseudomonas savastanoi* pv (Pss), est rencontrée actuellement dans le nord et le centre de la Tunisie où elle a commencé à inquiéter les agriculteurs en raison notamment de la difficulté de trouver une solution adéquate de lutte. Ce travail a pour objectif de tester l'effet du sulfate de cuivre, de l'oxychlorure de cuivre et des polyphénols extraits de la margine sur la croissance de Pss.

Une gamme de concentration de sulfate de cuivre allant de 0,3 à 20 % et d'oxychlorure de cuivre allant de 0,3 à 6 % a été préparée. Les polyphénols ont été extraits à partir de la margine par l'acétate d'éthyle. Les résultats des tests *in vitro* ont révélé que les produits à base de cuivre aux différentes concentrations ainsi que les polyphénols montrent une activité antimicrobienne contre Pss.

**Mots clés:** *Pseudomonas savastanoi* pv *savastanoi*, olivier, margine, cuivre, polyphénols.

### Abstract

OMW and Polyphenols extracts, used at different concentrations *in vitro* displayed a high level of antibacterial activity against *Pseudomonas savastanoi* pv *savastanoi* (Pss), the causal agent of olive knot disease. In planta, treatment with polyphenols at concentration of 1000, 500 and 100 mg/l inhibited completely the formation of excrescence on twigs of olive plants inoculated with strains IVIA 1628 and Aw9. However, treatment with copper sulphate resulted in a percentage of inhibition of 6 % but no weight reduction was observed when plants were inoculated with IVIA 1628. In the case of strain Aw9, percentage of inhibition and the reduction in weight were 12 and 51 % respectively. Both strains were susceptible for polyphenols but strain Aw9 appear more sensible than IVIA 1628 for CuSO<sub>4</sub>.

These results suggest that polyphenols extracted from OMW can be used to control and to prevent the causal agent of olive knot disease.

### 1. Introduction

Les margines sont obtenues lors de l'extraction de l'huile d'olive, à partir de l'eau de fruit et de l'eau ajoutée au cours de broyage et de séparation. Elles sont caractérisées par une concentration élevée en sucres, lipides, protéines, acides, polyphénols et en matière organique. Il est connu que les margines présentent un effet antimicrobiens contre plusieurs champignons et bactéries (Rodriguez et al., 1988; Özdemir, 2009) et sont aussi phytotoxiques en raison de la forte teneur en polyphénols (Aziz et al, 1998 ; Capasso et al, 1995). Il a été reporté dans d'autres études que les extraits phénoliques obtenus à partir de la margine présentent un effet antioxydant, hypoglycémique (Hamden et al., 2009), hypocholestérolémique (Feki et al., 2007) et antimicrobien contre plusieurs phytopathogènes tels que *Alternaria solani*, *Fusarium sambicinum*, *Verticillium dahliae* et *Agrobacterium tumefaciens* (Yangui et al., 2008, 2009).

La tuberculose de l'olivier causée par Pss est une maladie répandue dans toutes les aires de culture de l'olivier dont surtout les pays méditerranéens. En Tunisie, elle est rencontrée surtout dans le nord et le centre de la Tunisie. Les symptômes apparaissent sous formes d'excroissances sur les branches, les rameaux, les feuilles et même sur les fruits. La maladie cause de sérieuses pertes économiques et affecte même le rendement et la qualité de l'huile d'olive (Schroth et al., 1973).

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Actuellement, seules les composés chimiques à base de cuivre sont disponibles pour réduire les symptômes. Cependant, la toxicité et la possibilité de l'acquisition d'une résistance par le pathogène envers le cuivre incitent le développement d'autres alternatives pour le contrôle de cette maladie.

Dans cette étude, nous examinons *in vitro* et *in vivo* l'effet antibactérien de la margine et des extraits phénoliques contre *Pss*.

## **2. Matériel et méthodes**

### **2.1. Microorganismes**

Deux souches de *Pseudomonas savastanoi* pv *savastanoi* ont été utilisées dans cette étude. La souche Aw9 isolée de la région de Ouedna (Sfax) et la souche IVIA1628 gracieusement fournie par (Centro de Proteccion Vegetal y Biotecnologia. Instituto Valenciano de Investigaciones Agrarias (IVIA), Apartado Oficial, Moncada, Valencia, Spain).

### **2.2. Extraction et identification des polyphénols**

Les polyphénols contenus dans la margine ont été extraits par l'acétate d'éthyle. La fraction organique obtenue est évaporée par Rootavap. Après dilution du résidu obtenu, les polyphénols ont été dosés par la méthode de Folin-Ciocalteu. Le résultat est exprimé par mg équivalent acide tannique. L'identification des polyphénols a été réalisée par GC/MS.

### **2.3. Test d'activité antibactérienne des polyphénols**

La méthode utilisée est celle de la diffusion du surnageant de culture dans les puits, décrite par Tagg et Mc Given (1971).

### **2.4. Détermination de la concentration minimale inhibitrice (CMI) et la concentration minimale bactéricide (CMB) des polyphénols et du sulfate de cuivre**

La méthode NCCLS (2000) de dilution dans la gélose a été choisie pour la détermination de la CMI et la CMB. La CMI est considérée comme la plus faible concentration en extraits phénolique qui ne montre pas une croissance bactérienne visible.

### **2.5. Essais in vivo**

Ces essais ont été réalisés sur des plants d'olivier âgés de 2 ans. Trois blessures ont été pratiquées sur la tige principale à l'aide d'un scalpel, 10 µl d'une suspension bactérienne de la souche IVIA1628 ou Aw9 contenant 10<sup>8</sup> UFC a été introduite dans la blessure. Trois jours après, 10 µl d'extraits phénoliques aux concentrations 1000, 500, 100 et 40 mg/l a été introduite sur les blessures. Ces dernières ont été couvertes par du Parafilm M. Pour la comparaison une solution de sulfate de cuivre à 0,5% a été utilisée. Pour chaque essai 6 plants ont été considérés. Les plants sont placés dans la chambre de culture à une température variant entre 25 et 30 °C. L'observation des symptômes et l'analyse des résultats se fait après 60 jours par la détermination du pourcentage des blessures qui ont développées des tumeurs par rapport au témoin non traité et par la détermination du poids de chaque tumeur.

## **3. Résultats et discussions**

Les polyphénols extraits de la margine ainsi que la margine filtrée montrent *in vitro* un effet antibactérien contre *Pss* se traduisant par la présence de zones d'inhibition autour des puits (Figure 1 A et B) atteignant 35mm de diamètre. La CMI et la CMB sont de 5-10 mg/l pour les deux souches testées. Ce qui témoigne de l'effet bactéricide des extraits phénoliques. Ce résultat est en concordance avec ceux trouvés par Capasso et al., 1995. Les résultats des tests *in vivo* ont confirmé l'effet inhibiteur des polyphénols. En effet, les extraits phénoliques ont permis une inhibition totale de l'apparition des tumeurs pour tous les sites inoculés aussi bien par la souche IVIA1628 que par la souche Aw9 (Figure 2). Ce qui correspond en un pourcentage d'inhibition de 100% obtenu en utilisant les concentrations 1000, 500 et 100 mg/l. Par ailleurs, l'utilisation du sulfate de cuivre à 0,5% n'a pas montré une réduction significative du poids des tumeurs pour le cas des plants inoculés par la souche



IVIA1628 alors que dans le cas de la souche Aw9 le pourcentage d'inhibition et la réduction du poids des tumeurs étaient de 12 et 51 % respectivement. La CMI et la CMB du CuSO<sub>4</sub> sont comprises entre 0,92 et 1,84 g/l pour le cas de la souche IVIA1628 alors qu'elles sont comprises entre 0,46 et 0,92g/l pour la souche locale Aw9. Le cuivre présente un effet bactéricide sur les deux souches mais la souche locale Aw9 est plus sensible que la souche espagnole. L'inefficacité du traitement par le CuSO<sub>4</sub> est due à la faible concentration utilisée et qui est inférieure à la CMI dans le cas de la souche IVIA 1628. Si nous pensons à augmenter la concentration en cuivre pour avoir une meilleure inhibition, nous risquons d'avoir des problèmes de phytotoxicité. Il est préconisé donc de ne pas dépasser le seuil fixé par l'état qui est de 5g/l.

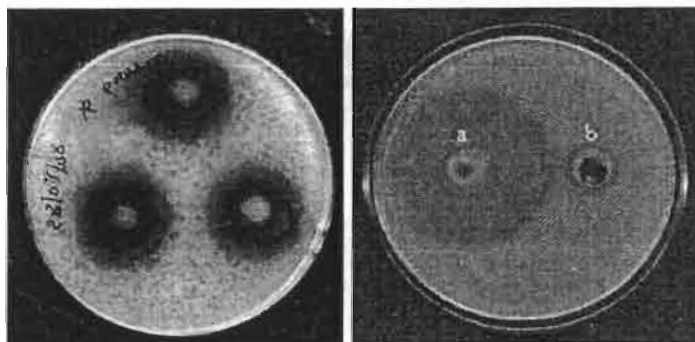


**Figure 1 (A):** Effet antibactérien de la margine sur la croissance de Pss IVIA1628.  
**(B)** Effet antibactérien des extraits phénoliques sur la croissance de Pss IVIA1628  
 (a) : Zone d'inhibition obtenue avec 1g/l d'extraits phénoliques  
 (b) : Zone d'inhibition obtenue avec 1% de sulfate de cuivre

L'observation des plants traités par les polyphénols et ceux du témoin négatif inoculés uniquement par les extraits phénoliques permet de constater qu'ils ne montrent aucun symptôme de phytotoxicité. De ce fait, nous pouvons conclure que les polyphénols extraits de la margine peuvent être utilisés pour le contrôle de Pss. La concentration 100 mg/l est une concentration adéquate pour obtenir une inhibition totale de la formation des tumeurs utilisant les deux souches. La suppression de l'apparition des symptômes est expliquée par l'effet inhibiteur des composés phénoliques sur la croissance du pathogène. Il a été démontré que les polyphénols affectent les fonctions cellulaires et l'intégrité membranaire (Cifardini et Zullo, 2003). Ils adhèrent très bien à la membrane cellulaire en la détruisant. Certains composés phénoliques tel que les flavonoides agissent sur l'ADN en causant des perturbations au moment de la réplication de l'ADN (Wong et Kitts, 2006).

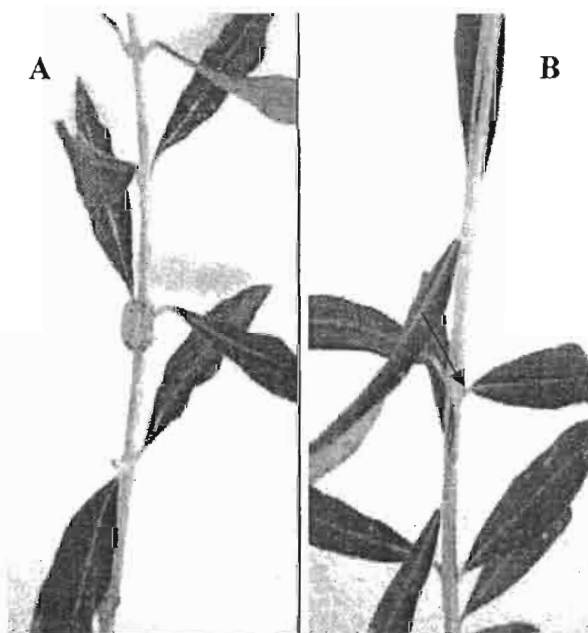


**Figure 2:** Formations des tumeurs après 60 jours de l'inoculation par la souche IVIA 1628.  
**A:** Tumeur sur une blessure non traitée ; **B:** Blessure traitée par 1g/l d'extraits phénoliques

L'analyse des polyphénols par GC/MS montre la présence de 3-4 dihydroxyphényléthanol (hydroxytyrosol), 4-hydroxyphényléthanol (tyrosol), catéchol, acide caféique et acide paracoumarique avec l'hydroxytyrosol et le tyrosol sont les plus majoritaires. L'effet inhibiteur des polyphénols semble être du à l'hydroxytyrosol et au cathécol. Ces deux composés sont toxiques pour Pss mais uniquement l'hydroxytyrosol peut être utilisé en agriculture car il est non phytotoxique (Capasso et al., 1995).

Ces résultats montrent que les extraits phénoliques peuvent être utilisés pour le contrôle de la tuberculose de l'olivier. D'autres essais peuvent être menés pour s'assurer que les polyphénols ne présentent pas d'effets secondaires.

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## **Les anthocyanes des olives et les polyphénols et tanins du bois de l'olivier**

AYED Naceur, CHALOUATI Wafa

### **Résumé**

Les olives noires sont appréciées par leurs anthocyanes et polyphénols, celles en saumure sont obtenues en majorité par traitement des olives vertes par des procédés chimiques, une analyse du fer permet de différencier les olives traitées chimiquement et celles qui sont naturelles. Par ailleurs le Bois d'olivier est apprécié en termes de sa teneur en polyphénols et en tanins afin de mieux orienter son emploi. Une caractérisation du Bois chimiquement, physico-chimiquement et mécaniquement amène à une meilleure connaissance de ce bois assurant une confection d'articles destinées comme contenir compatibles avec les olives.

Mots clés : Olives noires, anthocyanes, Bois d'olivier, conservation.

### **Abstract**

Black olives are appreciated because of their polyphenols and anthocyanins content. Those in brine are obtained thanks to a chemical treatment of green olives with alkali first and after exposition to air with iron gluconate, which differentiate them from naturally black olives.

Olive tree wood is also very appreciated because of its high content in polyphenols and tannins. A good characterization of this wood both chemically and mechanically is necessary to the production of different handicrafts objects as containers to be used for olive storage.

## Use of olive leaves for the recovery of lipophilized polyphenolic derivatives

Zouhaier Bouallagui, Mohamed Bouaziz and Sami Sayadi\*

### Abstract

Hydroxytyrosol, a polyphenolic derivative recovered from olive leaves was submitted to the enzymatic lipophilization aiming the synthesis of lipophilic derivatives largely researched for foods, cosmetics and pharmaceuticals. Lipases were shown to be a regioselective biocatalyst during esterification and transesterification reactions. Hydroxytyrosol esters were synthesized and their structures were determined using mass spectrometry and  $^1\text{H}$  and  $^{13}\text{C}$  RMN analysis. DPPH assay revealed interesting antioxidant activities of the lipophilic derivatives. Similarly, cytotoxic effects in human cancer cell model showed a synergistic effect between hydroxytyrosol and the acyl group subsequent to the ester hydrolysis. Furthermore, the enzymatically prepared derivatives used at non cytotoxic concentrations resulted in a remarkable cell protection from the oxidative stress induced by  $\text{Fe}^{2+}$  ions.

**Key words:** Olive leaves; Polyphenols; Lipase; Antioxidants.

### 1. Introduction

Chemical modification of Hydroxytyrosol to lipophilized derivatives is performed under drastic conditions and mixtures of products are obtained (Capasso et al. 1999). Nowadays there is much evidence in about the application of lipases in food and pharmaceutical industries. These enzymes are endowed with both hydrolytic and synthetic activities. The shifting between hydrolytic and synthetic lipases activities is monitored by the water content in the reaction medium. Indeed, water excess stimulates hydrolytic activity whereas decreased water amounts are in favor of esterification reactions. The common hypothesis is that both reactions are controlled by a thermodynamic equilibrium where water is revealed to be vital participant (Camacho Pérez et al.2003). Synthetic lipases activities are well explored in the production of lipophilized interesting molecules (Lai and O'Connor, 1999; Chamouleau et al. 2001).

Here we are describing a convenient and rapid method for the synthesis of oleate and acetate esters of Hydroxytyrosol using Lipase B from *Candida Antarctica*. Hydroxytyrosol esters have shown promising results when they were tested in biological models particularly in lipid systems (Gordon et al. 2001). Findings have revealed that both esterified compounds show a biological antioxidant activity compared with that of the parent compound suggesting that both Hydroxytyrosol esters could be metabolized at intestinal level by cellular esterases, to yield Hydroxytyrosol, which is the effective antioxidant compound and oleic acid in the case of Hydroxytyrosol oleate (Manna et al. 2006). Such fatty acid benefits are well-known and it is recognized by its anti-atherogenic activity through the reduction of cholesterol rate in the blood (Nicolosi et al. 2004). Moreover, oleic acid consumption was associated with lower cancer risk (Woutersen et al. 1999).

### 2. Material and Methods

- *C. antarctica* SP 435 lipase was purchased from Novo Industries (Denmark). Oleic acid and ethyl oleate were from Fluka. Standard Hydroxytyrosol was purified in our laboratory.
- Olive leaves extract was prepared following the protocol previously reported by Bouaziz and Sayadi (2006).
- Hydroxytyrosol esters were synthesized in presence or absence of solvent. Esterification reactions were performed in a double jacket batch reactor with 160 rpm stirring rate and 37 °C working temperature. Reaction solvent was previously dried for 24 hours with 200 g/l molecular sieves. Hydroxytyrosol esters synthesis was started by the addition of 5 g/l immobilized *Candida antarctica* lipase (Fig. 1).

- The method used for evaluating the free radical scavenging activity was derived from that described by Brand-Williams(1995).
- Cytotoxicity and antioxidant assays in human HeLa cells were performed as previously reported by Bouaziz et al. (2008).

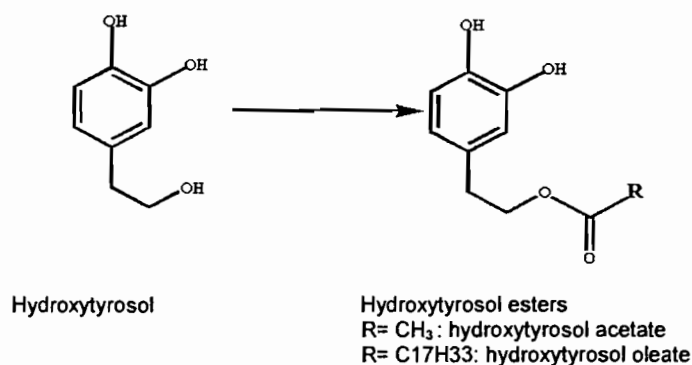


**Figure 1:** Lipase-catalyzed regioselective Hydroxytyrosol esterification and transesterification.

### 3. Results and Methods

#### 3.1. Synthesis of Hydroxytyrosol acetate

Hydroxytyrosol acetate was synthesized following the transesterification between Hydroxytyrosol, present in olive leaves extract, and ethyl acetate. After seven hours of reaction 98% of the Hydroxytyrosol previously present in the medium was found to be transformed to a monoester of acetate (Fig. 2). Mass spectrometry analysis was in accordance with the fact that our product was a monoester having a molecular weight  $m/z$  197.

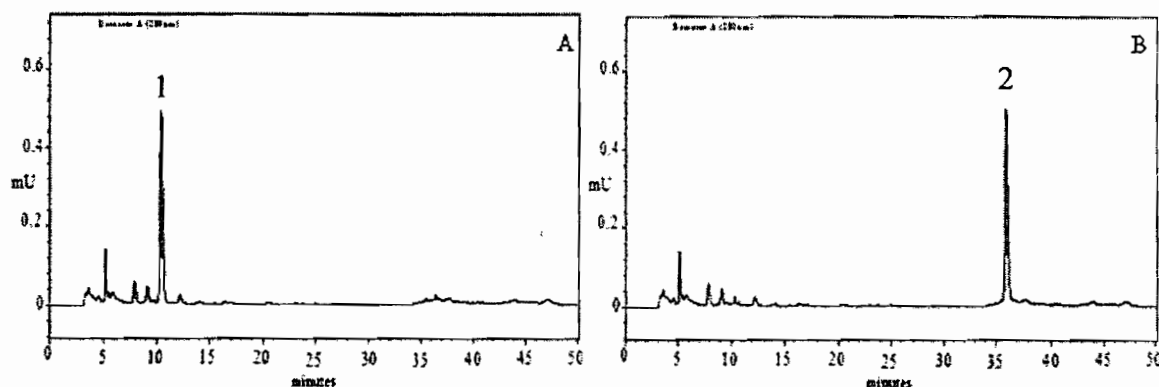


**Figure 2:** HPLC chromatograms (280 nm) of olive leaves extract before (A) and after enzymatic esterification (B); 1: hydroxytyrosol, 2: Hydroxytyrosol acetate.

#### 3.2. Production of Hydroxytyrosol oleate in organic medium

In a preliminary experience, Hydroxytyrosol oleate was synthesized in 2-methyl 2-butanol with 1:4 molar ratio. *Candida antarctica* lipase and molecular sieves concentrations were fixed at 10 g/l and 200 g/l respectively. Hydroxytyrosol oleate synthesized under these conditions was purified and used for structural characterisation. Mass spectrometry analysis showed a molecular weight of  $m/z$  441 corresponding to hydroxytyrosol oleate ionised with a sodium ion (Na<sup>+</sup>). NMR characterisation data proved the regioselectivity of Novozyme 435 toward the primary hydroxyl group.

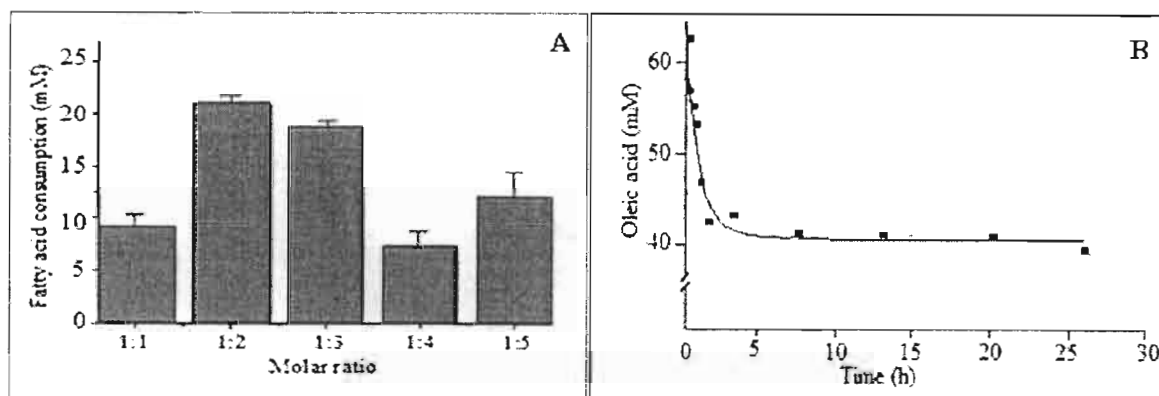


**Figure 3:** Synthesis of hydroxytyrosol oleate in organic medium (2-methyl 2-butanol); **A:** Effect of molar ratio hydroxytyrosol/oleic acid on the final oleic acid consumption, **B:** oleic acid consumption kinetic at molar ratio 1/2.

The effect of Hydroxytyrosol/oleic acid molar ratio on oleic acid consumption was examined. The highest oleic acid conversion was observed at a 1:2 molar ratio where Hydroxytyrosol oleate synthesis reached 69% of initial Hydroxytyrosol molar concentration (Fig. 3-A). The oleic acid consumption kinetic revealed that during the first five hours oleic acid was exponentially decreasing to reach a steady state (Fig. 3-B). The released water coupled with the esterification reaction could explain the establishment of this equilibrium.

### 3.3. Cytotoxic effects of hydroxytyrosol derivatives

Hydroxytyrosol and its enzymatically synthesized esters exhibited cytotoxicity against HeLa cells in a dose-dependent manner. These compounds displayed different cytotoxic effects with  $IC_{50}$  values of 0.5, 0.43 and 0.35 mM for hydroxytyrosol and acetate and oleate esters, respectively (Fig. 4). A synergistic effect is also proposed after hydrolysis of the hydroxytyrosol esters which yield the phenolic molecule and an acyl group. In the case of hydroxytyrosol oleate the released oleyl group could be a good candidate for having the lowest observed  $IC_{50}$ , as it was previously shown to have cytotoxic effects on human cancer cells in vitro (Cury-Boaventura et al. 2003).

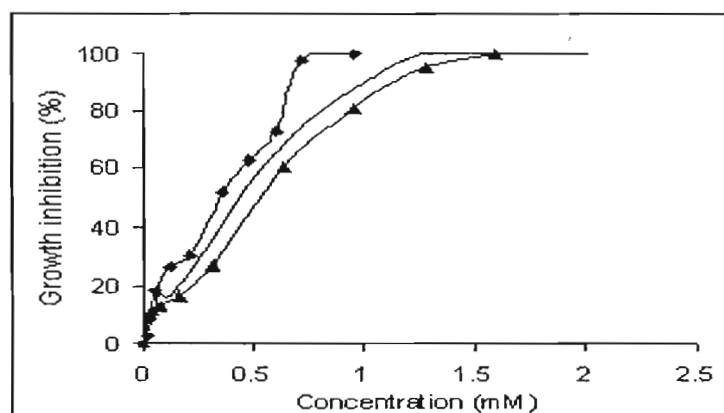


**Figure 4:** Cytotoxic effects on human cervical (HeLa) cells. hydroxytyrosol (▲); hydroxytyrosol acetate (—) and hydroxytyrosol oleate (◆).

### 3.4. Biological antioxidant activity in human cervical cells

The oxidative stress induction in HeLa cells resulted in eight fold increase in the oxidative stress ratio explained by the enhanced MDA production as a biomarker of lipid peroxidation (Fig. 5). The pre-incubation of HeLa cells with hydroxytyrosol or esterified derivatives at 25 or 100  $\mu$ M concentrations led to a significant oxidative stress fold decrease at both conditions. Hydroxytyrosol oleate at 100  $\mu$ M was found to be more effective than hydroxytyrosol and the acetylated derivative.

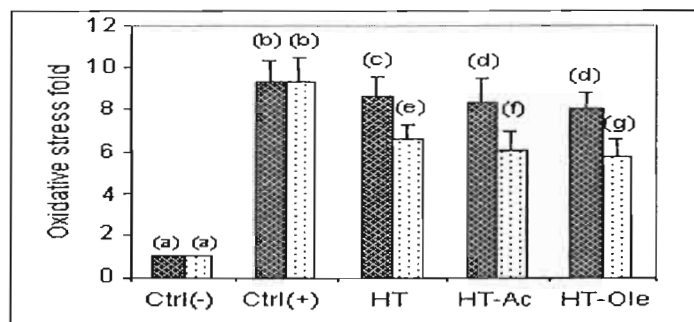


**Figure 5:** Effect of Hydroxytyrosol and acylated derivatives on the oxidative stress induction within HeLa cells. (■): 25  $\mu$ M; (□): 100  $\mu$ M. Results are expressed as oxidative stress fold compared to the negative control (Ctrl(-)). Statistical significance at  $p < 0.05$  was calculated relatively to the positive control (Ctrl(+)). Data with different scripts are statistically different.

Protective effect of hydroxytyrosol against induced oxidative stress in HeLa cells was reported by Bouaziz et al. (2008). These authors have used hydroxytyrosol at 65 and 130  $\mu$ M, respectively. As far as hydroxytyrosol esters activities are considered, few works have been recently described. Both hydroxytyrosol acetate and hydroxytyrosol oleate inhibited the t-BHP induced oxidative stress in rat brain cells and data are more relevant for hydroxytyrosol oleate used at 5 mM (Trujillo et al. 2006). Hence, both hydroxytyrosol acetate and hydroxytyrosol oleate are suggested to be enzymatically metabolized to release free hydroxytyrosol. Then, acylation of hydroxytyrosol through esterification reactions is considered as a promising method for the preparation of lipophilic and more stable derivatives of hydroxytyrosol and probably many other valuable molecules.

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## Production des laccases de *Trametes trogii* sur margines

CHAKROUN Hanen Sami SAYADI & Abdelhafidh DHOUB

### Résumé

Les laccases ont connu un essor croissant comme enzymes industrielles potentielles dans différentes applications. Il est donc évident de pouvoir produire ces enzymes sur des milieux de culture à très faible coût. Ce travail vise à valoriser les margines comme milieu de base pour produire les laccases par *Trametes trogii* et utilisation de ces enzymes en bioremédiation comme le traitement des eaux usées des industries textiles. Une forte concentration en laccase ( $25120 \text{ U l}^{-1}$ ) est obtenue en réacteur agité sur un milieu de culture composé de margine/eau (80/20) supplémentés avec l'urée ( $2 \text{ g l}^{-1}$ ) ce qui correspond à des valeurs initiales de DBO<sub>5</sub>, DCO et phénols totaux de 18.4, 46 et  $4.6 \text{ g l}^{-1}$  respectivement. Les Laccases obtenues et partiellement purifiées permettent une réduction de 85% des colorants bleu tubantin GLL 300 et noir tubantin VSF 600, et une réduction de 45% du colorant bleu solophenyl après 6 h d'incubation. L'utilisation des margines pour la production de laccases est un procédé à faible coût et permet de résoudre en partie le problème de pollution pose par cet effluent agro-industriel problématique. Les laccases produites pourraient être appliquées dans la décoloration des eaux usées textiles.

**Mots clés :** Laccases, *Trametes trogii*, margines, bioremédiation, colorant .



## Screening of *Lentinula edodes* strains able to degrade polyphenols in Olive Wastewater

Lakhtar Hicham Philippoussis Antonis<sup>2</sup>, Labrousse Yoan, Ismaili-Alaoui Mustapha<sup>3</sup>, Roussos Sevastianos

### Abstract

The olive waste (both solid and liquid) generated from olive industry such as Olive mill wastewater, olive cakes, leafs etc. plays a considerable role in environmental pollution. The uncontrolled disposal of this biomass is becoming a serious environmental problem. As a means of recycling, these wastes can be used as manure, but the presence of aromatic compounds, such as polyphenols makes it recalcitrant as very less species of natural microflora are able to degrade these polyphenolic substances. Sometime, these wastes when used in anaerobic treatment plants doesn't show up good results because, the anaerobic microbes were sensitive to the high concentration of polyphenols which inhibited their growth, subsequently resulting in less products. Although, many of the micro-organisms were not reported to degrade the polyphenols such as tannins, certain filamentous fungi belonging to the species *Aspergillus*, certain edible mushrooms such as *Pleurotus*, shiitake etc. are able to produce enzymes resulting in the degradation or biotransformation of these molecules, utilising it as sole carbon source. The Shiitake mushroom (*Lentinula edodes*), falls under the category of the second most popular edible mushroom available in the global market. Its quality as an edible mushroom is not only attributed to its nutritional value or its flavor, but also to its possible applications in medical and food industry. Also this mushroom is reported to be able to synthesize certain substances with antitumor activity, antibacterial such the lentinan. The objective of the present work, was to screen shiitake strains available in the culture collection from different areas (like China, Europe, Belgian, American, Japan, Taiwan), and to select one, able to degrade or transform polyphenols existing in olive waste. Solid-state fermentation was carried out using olive waste as substrate to check the capability of the strains to degrade polyphenols. The degradation of the polyphenols will be analysed along with enzyme activity assays and results will be discussed.

**Key words:** Olive cake, solid state fermentation, edible mushroom, polyphenols degradation, *Lentinula edodes*.

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## Pilot-plant results of the electro-Fenton treatment of olive mill wastewaters followed anaerobic digestion

Khoulfi Sonia Aloui Fathi et Sayadi Sami

### Résumé

Un nouveau procédé a été développé à l'échelle pilote pour le traitement des margines. Ce procédé est basé sur la combinaison de trois technologies : l'électro-Fenton, la digestion anaérobie et l'ultrafiltration. L'application de l'électro-Fenton en mode semi-continue a permis un important abattement de la DCO totale (50%) et des composés phénoliques (95%). Ce prétraitement a amélioré significativement la méthanisation des margines dans un digesteur de type filtre anaérobie (300 l). Dans ce bioréacteur, un abattement de la DCO de 75% a été atteint avec un temps de rétention hydraulique de 4,5 jours et en appliquant une charge organique de  $10 \text{ g l}^{-1} \text{ j}^{-1}$ . L'utilisation de la technologie membranaire, l'ultrafiltration, comme post-traitement a détoxifié complètement l'effluent anaérobie et a éliminé les polyphénols résiduels de hautes masses moléculaires.

### Abstract

A novel process has been developed at pilot scale for the treatment of olive mill wastewater (OMW), which combines electro-Fenton, anaerobic digestion and ultrafiltration. Application of electro-Fenton procedure in semi-continuous mode permitted high removal efficiencies of COD (50%) and monophenolic compounds (95%). This pre-treatment was found to enhance the anaerobic activity of an up-flow anaerobic filter (300 l) significantly. In this bioreactor, COD removal efficiency of 75% was reached at a hydraulic retention time of 4.5 days and an organic loading rate of  $10 \text{ g COD l}^{-1} \text{ d}^{-1}$ . The use of ultrafiltration technology as a post-treatment completely detoxified the anaerobic effluent and removed its high molecular mass polyphenols.

**Key words:** Anaerobic digestion, electro-Fenton, olive mill wastewater, polyphenols, toxicity

## Effects of short term application of Olive mill wastewater on sandy and clay soils under fenugreek

Ines Friha and Sami Sayadi\*

### Abstract

Olive mill wastewater (OMW) is a by-product of olive oil extraction. Since OMW contains compounds potentially useful for plants, an attractive application is its use as a fertiliser to recycle the organic matter and fertilise the soil. The goal of this work was to test the effect of OMW ( $80 \text{ m}^3 \cdot \text{ha}^{-1}$ ) as an organic fertiliser on two types of soil: sandy and clay soil in pots under fenugreek. Phenolic content of the treated soils was followed by High Performance Liquid Chromatography (HPLC). The obtained results showed that OMW exerts beneficial effects on both soils, especially potassium, whereas, negative effects were observed on plant growth. Phenolic compounds, hydroxytyrosol and tyrosol, have disappeared from both soils after two months from OMW application.

**Keywords:** Olive mill wastewater, organic fertiliser, sandy and clay soil, fenugreek, polyphenols.

### 1. Introduction

Olive mill wastes represent an important environmental problem in Mediterranean areas where they are generated in huge quantities in short periods of time. Their high phenol, lipid and organic acid concentrations turn them into phytotoxic materials. Several studies have proven the negative effects of these wastes on soil microbial populations (Paredes et al., 1987), on aquatic ecosystems (DellaGreca et al., 2001) and even in air medium (Rana et al., 2003). But these wastes also contain valuable resources such as a large proportion of organic matter and a wide range of nutrients that could be recycled.

Many researchers have applied OMW directly on soil and have tested its effect as an organic fertilizer showing positive and negative effects. Beneficial effects are related to its high nutrients concentration, especially K, and its potential for mobilizing soil ions, whereas, negative effects are associated with its high mineral salt content, low pH and the presence of phytotoxic compounds, especially polyphenols (Paredes et al., 1999). OMW may reduce the mobility of certain organic compounds in soil. Cox et al. (1997) suggested its use to attenuate leaching of toxic organic chemical such as herbicides (clopyralid and metamitron) in cultivated lands.

Another characteristic of OMW is its high antimicrobial capacity, which can be used for soil sanitation against certain pathogens. Thus, Kotsou et al. (2004) verified the OMW suppressiveness against the plant pathogen *Rhizoctonia solani*. Therefore, its addition prior to planting could be a good prevention method.

### 2. Material and methods

- Two types of soil were chosen according to their difference in texture, one is a sandy soil (93.71%) and the other is a clay soil (56 %). Both were characterised before and after the experiment (granulometry, pH, total limestone, cation exchange capacity, exchangeable bases, assimilable phosphorus, total nitrogen, total carbon and organic matter, phenolic content).
- OMW, generated by a two-phase system, was collected from a storage pond and stored at 4 °C. EC, pH, dry matter, moisture, organic and mineral matters, and phenolic content were determined.

- The choice of the plant (Fenugreek) was done according to its ability to grow rapidly and especially its role in atmospheric nitrogen fixation thus enriching the soil in nitrogen as the OMW is low in nitrogen. The following plant analyses were done: non destructive parameters (growth stage, vegetation height, crown diameter, size of cotyledonary leaves, fresh and dry weight of the plant), calcination of the powder plant and determination of mineral elements P, K, Na, and Ca).

### 3. Results and discussion

#### 3.1. OMW effects on mineral and organic matters of the tested soils

The results obtained showed that the incorporation of OMW in both soils resulted in an improvement in phosphorus content (Fig.1a).

Although OMW brings to the soil significant amounts of exchangeable potassium, the levels found at the end of the experiment were close to those at the beginning of the experiment (Fig.1b), this could be explained by the fact that the plant has drawn the total exchangeable potassium provided by the OMW.

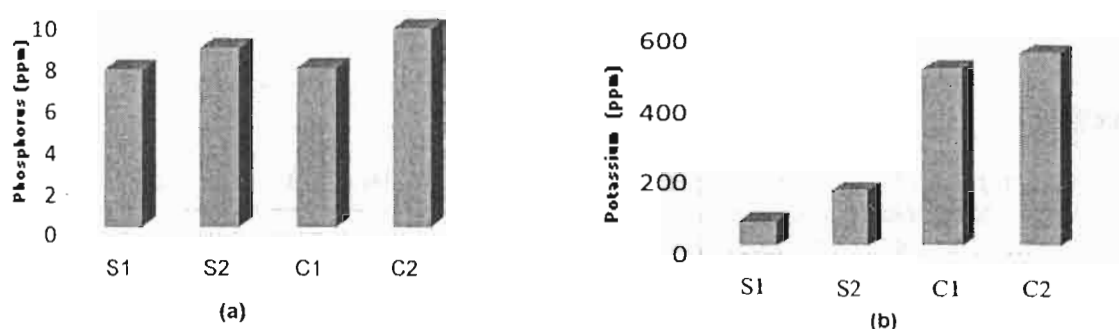


**Figure1:** Effect of OMW on phosphorus and potassium content of soils.

The levels of total organic carbon (TOC) recorded in both soils at the end of the experiment showed that the adding OMW induced an improvement in the two soils compared to the starting rates.

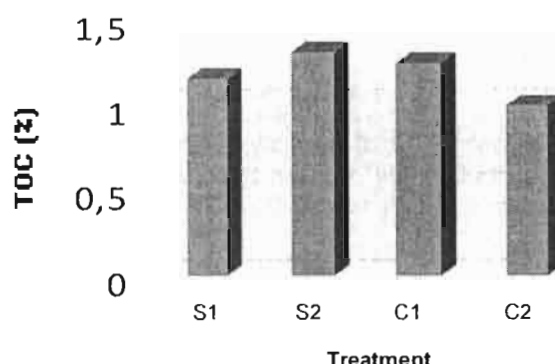


**Figure 2:** Effect of OMW on TOC content of soils.

**S1:** Sandy soil untreated ; **S2:** Sandy soil treated with OMW ; **C1:** Clay soil untreated ; **C2:** Clay soil treated with OMW

#### 3.2. OMW effects on plant growth

The monitoring of the pace of leaf appearance shows that this parameter has been affected by the addition of OMW, but only in sandy soil. For the clay soil, the pace of branching was not affected by the addition of OMW. However, in sandy soil, whether treated or not with OMW, we note the complete absence of branching, the plant continues to grow vertically without branching. Regarding the

vegetation height of the plant, OMW has slowed the evolution of this parameter compared to plants of untreated soils.

### 3.3. OMW effects on mineral nutrition of Fenugreek

The mineral nutrition, the fresh and dry weight of the plant showed that there is a combined effect of soil texture and OMW. Indeed, OMW promoted the absorption of phosphorus and potassium for plants grown on sandy substrate only.

### 3.4. Phenolic content evolution of OMW in soils

The monitoring by HPLC of the evolution of the phenolic content of the treated soils, revealed that the dose of OMW incorporated in soils is low, which explains that at the end of the experiment all the polyphenols identified in the two soils at a certain time cycle of the plant have disappeared.

## 4. Conclusion

OMW application to soils has modified their physico-chemical composition and has slowed the growth of the plants. Polyphenols have disappeared from treated soils after two months from OMW application.

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## Recovery of high added values compounds from Olive Mill Wastewaters

M. Bouaziz, I. Feki, S. Sayadi

### Abstract

Liquid-liquid extraction was used in order to recover phenolic compounds from olive mill wastewater (OMWW), a polluting by-product of olive oil production process. The identification of phenolic compounds was based on separation by high-performance liquid chromatography equipped with a diode array detector followed by liquid chromatography-mass spectrometry analysis. HPLC analysis showed that hydroxytyrosol is the most abundant biophenol in ethyl acetate extracts from OMWW. Many other biophenols (tyrosol, caffeic acid, ferulic acid, p-coumaric acid, 3,4-dihydroxyphenylacetic acid, p-Hydroxyphenyl acetic acid) were quantified then their radical scavenging activity was measured by the (2, 2-diphenyl-1-picrylhydrazyl) (DPPH) assay. Hydroxytyrosol had the highest antioxidant activity.

**Keywords:** Olive mill wastewater, polyphenols, hydroxytyrosol, Antioxidant activity.

## Production de molécules à haute valeur ajoutée à partir des margines

### Résumé

L'extraction liquide-liquide a été utilisée afin d'isoler les composés phénoliques de margine, un polluant des sous-produit des huileries d'olive. L'identification des composés phénoliques a été basée sur la séparation par HPLC équipé d'un détecteur DAD suivi d'une analyse LC-MS. L'analyse par HPLC a montré que l'hydroxytyrosol est le composé phénolique le plus abondant dans l'extrait. D'autres biophénols (tyrosol, l'acide caféique, acide férulique, acide p-coumarique, acide 3,4-dihydroxyphénylacétique, p-hydroxyphényl acide acétique) ont été identifiés et quantifiés. L'activité antioxydante a été mesurée par la méthode (2, 2-diphényl-1- picrylhydrazyl) (DPPH) et a montré que l'hydroxytyrosol possède activité antioxydante la plus puissante.

**Mots clés :** Margine, Phénols, Hydroxytyrosol, Extraction, Activité biologique

### 1. Introduction

Olive mill wastewater (OMW) is a liquid effluent which has a high polluting organic load, due to a high content of organic substances, including sugars, tannins, polyphenols and lipids. Most frequently, OMW are pumped and discharged into evaporation ponds or directly dumped in rivers or spread on soil. This becomes a major environmental problem in the main olive producing countries of the Mediterranean region. It is known that phenolic compounds are major contributors to the toxicity and the antibacterial activity of OMW. This limits its microbial degradability. However, these phenolic compounds, which are present in general in olives, olive oil, and olive by-products are endowed with several biological activities such as antioxidant property (Allouche et al., 2004; Fki et al., 2005; De Marco et al., 2007; Obeid et al., 2008).

### 2. Methods and Materials

1. Extraction of phenolic compounds from Olive mill wastewaters (OMWW). Liquid-liquid extraction with ethyl acetate was carried out on olive mill wastewater samples as described by Allouche et al. (2004).

2. OMWW sample. Olive mill wastewaters were obtained from a discontinuous olive oil processing plant located in Sfax.

3. Reverse phase HPLC and LC-MS conditions. A reverse phase HPLC and LC-MS techniques were developed to identify and quantify phenolic compounds. Analysis conditions were as described before by Bouaziz et al. (2005).

4. DPPH radical-scavenging effect of some phenolic compounds. The DPPH radical-scavenging effect was evaluated according to Bouaziz et al. (2005).

### 3. Results and Discussion

#### 3.1. Identification, quantification and fractionation of biophenols extracted from OMWW.

Identification of phenolic compounds in the OMWW extract was preliminary performed by HPLC-UV, comparing the relative retention times and UV spectra with those of standard solutions. The HPLC profile showed several peaks corresponding to different biophenols (Table 1), among which ten compounds were identified by LC-MS: phenyl acids (vanillic acid, caffeic acid, ferulic acid), phenyl alcohols (hydroxytyrosol, tyrosol), secoiridoids derivatives (verbascoside and oleuropein), flavonoids (luteolin, luteolin-7-glucoside). These results are in accordance with those reported by De Marco et al., 2007 and Obeid et al. (2008).

**Table 1:** Phenolic compounds identified in olive mill waste water extract.

| N° | Compound             | T <sub>R</sub> (min) | UV $\lambda_{max}$ (nm)      | MW  | Main fragments by ESI-MS <sup>2</sup> |
|----|----------------------|----------------------|------------------------------|-----|---------------------------------------|
| 1  | Hydroxytyrosol       | 1.9                  | 220, 277                     | 154 | 153, 123                              |
| 2  | Tyrosol              | 2.63                 | 218, 272                     | 138 | 137                                   |
| 3  | Vanillic acid        | 3.21                 | 257, 289                     | 168 | 167                                   |
| 4  | Caffeic acid         | 3.34                 | 235, 323                     | 180 | 179                                   |
| 5  | Luteolin 7-glucoside | 5.1                  | 255, 267 <sup>sh</sup> , 350 | 448 | 285, 327, 267                         |
| 6  | p-coumaric acid      | 5.16                 | 273                          | 164 | 163                                   |
| 7  | Ferulic acid         | 5.6                  | 233, 320                     | 194 | 193                                   |
| 8  | verbascoside         | 5.9                  | 217, 305 <sup>sh</sup> , 329 | 624 | 461, 315, 161                         |
| 9  | Oleuropein           | 8.55                 | 228, 278                     | 540 | 377, 275, 153                         |
| 10 | Luteolin             | 10.94                | 251, 348                     | 286 | 285                                   |

MW: Molecular weight, T<sub>R</sub>: Retention times (min), <sup>sh</sup>: shoulder

Concentration of the major phenolic compounds in the extracts was calculated comparing HPLC peak areas with those of tyrosol as an external standard (Table 2). Hydroxytyrosol and tyrosol were the major compounds detected (Table 2). Their concentrations in the extract were 1225 and 345 mg/l, respectively. p-Hydroxyphenyl acetic acid, caffeic acid and p-coumaric acid were present at lower concentrations. 3,4-dihydroxyphenylacetic acid and ferulic acid were also detected at the same concentration, 70 mg/l (Table 2).

#### 3.2. DPPH radical-scavenging effect of some phenolic compounds.

The DPPH radical scavenging effect of some major phenolic compounds is shown in Table 2. Among compounds obtained from OMWW, ortho-dihydroxylated aromatic components exhibited higher scavenging activity on DPPH, particularly hydroxytyrosol and 3,4- dihydroxyphenyl acetic acid, which exhibited IC<sub>50</sub> values of 0.57 and 0.64 µg/ ml, respectively. However, tyrosol and p-hydroxyphenylacetic acid, possessing only one hydroxyl function, showed no activity (IC<sub>50</sub> = 10.9 and 12 µg/ml). In particular, the antioxidant activity of hydroxytyrosol and 3,4-dihydroxyphenyl acetic acid, as well as, caffeic acid were higher than those of BHT.

**Table 2:** Quantification and antioxidant activity of major phenolic compounds identified in OMWW extract.

| Compound                        | IC <sub>50</sub> (µg/l) | Concentration in the extract (mg/l) |
|---------------------------------|-------------------------|-------------------------------------|
| Hydroxytyrosol                  | 0.57                    | 1225.6                              |
| 3,4-dihydroxyphenyl acetic acid | 0.64                    | 70.2                                |
| Tyrosol                         | 10.9                    | 345                                 |
| p-hydroxyphenyl acetic acid     | 12.2                    | 198.9                               |
| Caffeic acid                    | 0.87                    | 256.7                               |
| p-coumaric acid                 | 9.54                    | 169                                 |
| Ferulic acid                    | 1.21                    | 70.2                                |
| BHT                             | 0.82                    | ----                                |

#### 4. Conclusion

In this study, in addition to the extraction and identification of OMWW biophenols, fractionation of phenolic extracts gave promising results. Hydroxytyrosol, may potentially be used as alternative natural antioxidants to stabilize edible oils, while at the same time appeasing a major concern of consumers over the use of synthetic antioxidants in food products.

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## Characterization of dried mixture of olive cake with olive mill wastewater: elemental, spectroscopic analyses (FTIR and $^{13}\text{C}$ -NMR) and polyphénols dynamics

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### Abstract

Morocco is a country which produces olive oil extensively and this industry within the country is currently under huge expansion. The olive mill wastewater OMW which is being dumped in the environment results in serious environmental pollution while the olive cake does have a proper disposal. In the present work, a new integrated process is described to eliminate the OMW produced by a tri-phase system using the technique of pressing. This process consists of mixing olive cake with the OMW produced and then to dry the mixture in a greenhouse to get a final moisture content of 10% (w/w). The dried mixture was characterized by elemental analysis and spectroscopic methods (Fourier transform Infra-Red: FTIR and Nuclear Magnetic Resonance:  $^{13}\text{C}$ -NMR), in order to compare it with the crude olive cake and predict its future biotechnological valorization in the way existing as composting, lombricomposting and edible mushroom culture. The extractable polyphenols dynamics were determined by HPLC and compared to those present in OMW. The stability of the dried mixture was checked after one year of storage under the standard conditions. The results obtained will be discussed in detail.

**Keywords:** OMW, olive cake, solar drying, greenhouse, NMR, FTIR, Elemental analysis, polyphenols, HPLC, dynamics.

### 1. Introduction

Mediterranean basin concentrates 97% of the world olive oil production (Ismaili-Alaoui, 2006). The three-phase which generates two wastes (olive mill wastewater and olive cake) remains the dominating technology in the south of the Mediterranean basin. With a production of 50,000 tonnes of olive oil per year, Morocco is presently located at the sixth rank of the world olive oil producers. Fifty percent of this production still comes from traditional mills commonly called "Maasra" which use the classic "three-phase system" with the technique of pressing (Serghini et al., 2007).

Due to its residual oil content (5-10 % w/w) which depends on the extraction technology and also to its other components, olive pit (42-54% w/w), skin (10-11% w/w) and pulp (21-33% w/w) (Lopez-Villatta, 1998), OC has been the subject of many valorization studies (Masghouni and Hassairi, 2000; Haddadin et al., 2002). In a country such as Morocco, its main use is still limited to the recovery of oil by extraction with hexane for the production of soap (Serghini et al., 2007). The de-oiled OC which has excellent calorific properties is usually reused directly as combustible by the OC oil extraction units to dry OC to 6-10% (w/w) humidity before the extraction step. As a consequence, OC should not be considered as waste and it is actually rarely dumped into the environment.

Contrary to OC, OMW which contains high concentrations of toxic phenolic compounds (Di Serio et al., 2008) and organic matter (100-200 g COD/l) is extremely hazardous for the environment especially the water bodies. OMW drying is actually a slow process since the olive oil and some mucilage cover the surface and make it impermeable to water (Jimenez and Lao, 2004). As so, OMW drying could be improved by increasing the surface of exchange with the atmosphere through the addition of a high surface absorbing solid matrix. The OC readily available from the same olive mill could be a good candidate for this use. Thus, the amount of wastes, generated by olive mill, could be controlled, based on the assumption that the resulting product could be valorised in the same way as crude OC.

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The objectives of the present study were to determine under which conditions all the OMW generated by a mill could be absorbed by the OC produced by the same mill and if the resulting OC-OMW mixture could be dried to 10% (w/w) humidity in order to obtain a solid matrix which could be valorized in the same way as crude OC. To achieve these objectives we (1) performed a mass balance of the different products generated by a traditional maasra mill (2) determined the OMW retention capacity by the mill OC, (3) performed several drying experiments in open sun and in a greenhouse under natural convection and (4) determined the final quality of the dried material and compared it to that of dried crude OC.

## 2. Materials and methods

### 2.1. Origin of samples

OC and OMW were obtained from an olive oil mill plant located in Beni-Mellal in the centre of Morocco. This mill used a three phase system with press and operated mostly with the "picholine marocaine" which is the main olive variety cultivated in Morocco for production of both olive oil and table olives. As so this mill can be considered as representative of the ones used in the country.

### 2.2. Description of the greenhouse dryer

The greenhouse dryer, used in the study was located at Hassan II Agronomic and Veterinary Institute, in Rabat, Morocco. It was built on a concrete floor and oriented in the north-south direction. It had a parabolic cross-section and a size of 7 (length), 3 (width) and 2.5 m (height) giving on the ground a surface of 21 m<sup>2</sup>. It was covered with polycarbonate but the facade was made of transparent extern stratum. The air ventilation was obtained by some openings at different levels of the facade. One set of iron shelves with iron trays was used to place the products to be dried. The trays were located on three different levels (0.5, 1 and 1.5 m from the ground) on the shelves. The free space between them was sufficient for loading and unloading the products. The trays were made of a wire netting to allow air circulation. A second set of identical shelves and trays were used for the outdoor drying. The structure of the greenhouse and of the shelves and trays is shown in Fig. 1.

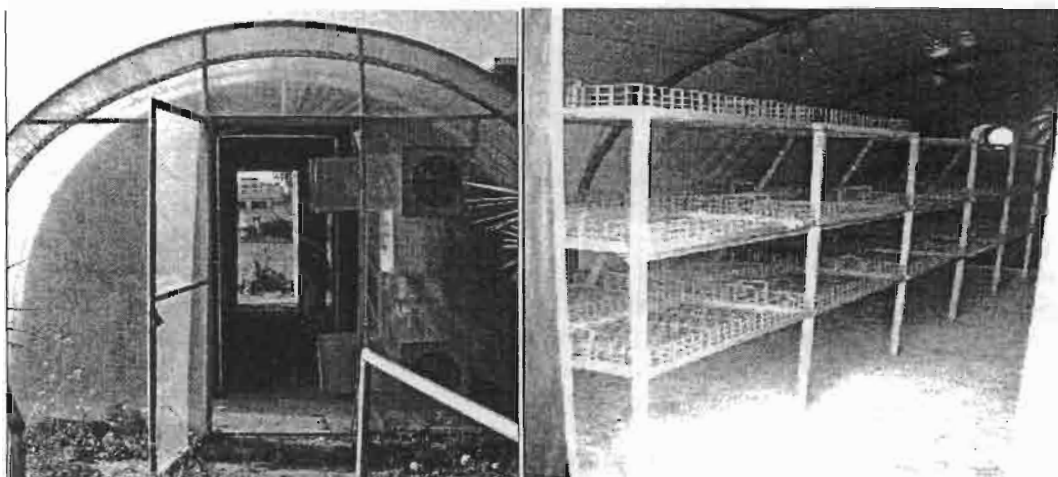


**Figure 1:** General view of the greenhouse solar dryer (left) and of its interior with the shelves (right). Only the lower and upper trays were used for loading the material to be dried.

### 2.3. Drying experiments

Outdoor and greenhouse drying were performed under identical conditions in order to be comparable. Saturated OC with OMW (no free liquid OMW present) were distributed uniformly in thin layer on the trays the bottoms of which were covered by plastic mesh. The solids on the trays were turned manually once every hour. The drying process was pursued until reaching the target moisture. For that, the whole solid present on each trays were weighted periodically at 1 h intervals using a digital balance and their moisture content was determined. Solar radiation and temperature inside and outside the greenhouse were determined periodically. In order to avoid the interference of rain on the

outdoor drying process, it was planned to cover the shelves with a plastic sheet during rainfall events. Finally this was unnecessary since no rain occurred during all the experimental period (February 2008).

#### **2.4. Chemical characterization of dried crude olive cake and OC-OMW mixture**

OC and the new product (OC-OMW mixture) both dried at 10% (w/w) humidity in the same conditions were characterized for their content in carbon, nitrogen (proteins), fats and total phenols. Carbon and nitrogen concentrations were determined by a combustion-gas chromatography technique (Flash EA 1212 Elemental Analyzer, France). The instrument was calibrated with aspartic acid standard. The amount of proteins was estimated from the nitrogen content using the general factor of 6.25 (Weinberg et al., 2008). Fats were obtained by Soxhlet extraction with hexane as solvent according to established procedures (Cordova et al., 1998). Phenol content was assessed as follows: 10 mL of 60% (v/v) ethanol-water were added to 1 g of milled solid sample. The mixture was homogenized, incubated for 1 h at 4°C and centrifuged at 4500 rpm for 10 minutes. The extraction was repeated three times. The supernatant was then filtered under vacuum. The non phenolic fraction still presents in the filtered liquid (e.g. fats) was eliminated by extraction with 10 ml of n-hexane, repeated three times. The total phenolic content of the remaining aqueous phase was quantified by the method of Folin and Ciocalteu (Bärlocher et al., 2005). The blue reactive complex formed was dosed spectrophotometrically at 760 nm. Caffeic acid being used as standard for the calibration, the phenolic content of the samples was expressed as caffeic acid equivalent.

#### **2.5. NMR analysis of dried crude olive cake and OC-OMW mixture**

Dried crude OC and the mixture OC-OMW were used directly in NMR experiments without special preparation. Owing to their size, the samples were gently ground (IKA Labortechnik, Germany) in order to fill the rotor. Solid-state <sup>13</sup>C CPMAS NMR spectra, of dried crude OC and dried mixture OC-OMW, were obtained on a Bruker Avance-400 MHz spectrometer (Bruker, Bremen, Germany) operating at a <sup>13</sup>C resonance frequency of 100.7 MHz. Samples were placed in a 7-mm zirconium rotor and spun at the magic-angle at 6 kHz. All measurements were made at room temperature. The <sup>13</sup>C chemical shifts were referenced to tetramethylsilane and calibrated with the glycine carbonyl signal, set at 172.5 ppm.

Deconvolution of the NMR spectra was performed using the DmFit software (Massiot et al., 2002). This software adjusts the spectra to obtain the linewidth and the peak positions (in ppm) and to integrate each peak so as to obtain the percentage of each contribution. The aromaticity of dried mixture OC-OMW and crude OC was calculated by expressing aromatic C (110–165 ppm) as a percentage of aliphatic C (0–110 ppm) + aromatic C (Wikberg and Liisa Maunu, 2004). The lignin content was calculated according to the model of Gilardi et al. (1995), which takes into account the total lignin carbon. These authors calibrated lignin content from aromatic carbons plus aliphatic carbons of lignin. The cellulose crystallinity ( $X_c$ ) was expressed by the ratio between the signal at 84 ppm, assigned to the C-4 of amorphous cellulose, and the signal at 89 ppm, assigned to the C-4 of crystalline cellulose (Newman and Hemmingson, 1990).

#### **2.6. Statistical treatment of results**

Data of drying, chemical analysis and NMR spectra were subjected to analysis of variance (ANOVA). The error square was obtained from the analysis of variance and used to calculate the least significant difference (LSD,  $P < 0.05$ ). Variations between data are significant when the difference between is more than the LSD.

### **3. Results and discussion**

#### **3.1. Drying of the mixture of OMW-Olive cake**

After a set of trials, it was found that the best way to perform the two step absorption-drying was the one shown in Table 1.

**Table 1:** Optimized procedure for the two-step drying process of OMW absorbed on OC.

| Substrate             | Olive cake (OC)  |                     | OMW   |         | Mixture before 1 <sup>st</sup> drying |         | Mixture after 1 <sup>st</sup> drying |         | OMW   |         | Mixture before 2 <sup>st</sup> drying |         | Mixture after 2 <sup>st</sup> drying |         |
|-----------------------|------------------|---------------------|-------|---------|---------------------------------------|---------|--------------------------------------|---------|-------|---------|---------------------------------------|---------|--------------------------------------|---------|
| Processtime (Hours)   |                  |                     |       |         | 8                                     |         |                                      |         |       |         | 35                                    |         |                                      |         |
| Drying cycle          | Quantité (Q.) Kg | Humidité (H. %)* Kg | Q. Kg | H. % Kg | Q. Kg                                 | H. % Kg | Q. Kg                                | H. % Kg | Q. Kg | H. % Kg | Q. Kg                                 | M. % Kg | Q. Kg                                | M. % Kg |
| 1 <sup>st</sup> cycle | 100              |                     | 62    |         | 162                                   |         | 122                                  |         |       |         |                                       |         |                                      |         |
|                       |                  | 35                  |       | 90      |                                       | 56      |                                      | 40      |       |         |                                       |         |                                      |         |
| 2 <sup>nd</sup> cycle |                  |                     |       |         |                                       |         | 122                                  |         | 25    |         | 147                                   |         | 82                                   |         |
|                       |                  |                     |       |         |                                       |         |                                      | 40      |       | 90      |                                       | 50      |                                      | 10      |
| Input(kg)             | 100              |                     | 62    |         |                                       |         |                                      |         | 25    |         |                                       |         |                                      |         |
| Output (kg)           |                  |                     |       |         |                                       |         |                                      |         |       |         |                                       |         | 82                                   | 10      |

The first step consisted to saturate OC with OMW (resulting product with 56% moisture, w/w) and then to limit the drying of the mixture up to a final moisture of 40% (w/w). This level of humidity was found as a good compromise to achieve three objectives. It allowed the evaporation of enough water (42 L from 166 kg of OC-OMW mixture) to give to the partial dried OC-OMW mixture the capacity to absorb all the OMW remaining to absorb (25 L). It permeated to avoid the problems generated by a more intensive drying which could have affected the absorption capacity, because OC became more water repellent upon drying due to the shrink of the water absorbing solid material that it contains resulting in a loss of porosity of this material, porosity which is the driving motor of absorption (Liu and Lee, 2006). It also allowed a fast drying for this first step since the drying from a humidity of 56% to 40% (w/w) concerned mostly to free water which is easy to be removed (Ekechukwu, 1999). Quantitatively, the total mass of by-products (OC and OMW) generated by a maasra mill (78 kg / 100 kg of olives processes) can be reduced by 56.15% (final amount of 35-36 kg / 100 kg of olives processes) thanks to such drying.

### 3.2. Chemical characterization of dried crude OC and OC-OMW mixture

Chemical analysis results for the dried crude OC and the dried OC-OMW mixture are presented in table 2. Statistical analysis ( $P=0.05$ ) showed significant differences in pH, C, N, C/N and total phenols, but no significant difference in fat content. Incorporation of OMW into OC allowed to increase by 24% of the protein content in the mixture compared to dried crude OC which is considered as a poor material for animal feed (Martin Garcia et al., 2003). However, this was counterbalanced by total phenol content which was 5 times higher than that in the dried crude OC since phenols are known for their anti-digestive properties (Grabber, 2009). On the other hand, this converted the OC-OMW mixture into a good raw material candidate for the extraction of antioxidants which are used in the food, cosmetic and pharmaceutical fields (Erbay and Icier, 2009). The fat content and dryness of the mixture indicates also that it could be used directly by the traditional oil recovery units in place of OC.

**Table 2:** Global chemical composition of the OC-OMW mixture and crude OC both dried at 10% moisture\*

| Component        | OC-OMW dried mixture | Dried crude OC |
|------------------|----------------------|----------------|
| pH               | 5.1 ± 0.1            | 4.9 ± 0.2      |
| C (%)            | 52.49 ± 0.01         | 51.11 ± 0.18   |
| N (%)            | 1.06 ± 0.01          | 0.85 ± 0.09    |
| C/N              | 49.51 ± 0.64         | 59.77 ± 2.00   |
| Protein (%)      | 6.62 ± 0.08          | 5.34 ± 0.56    |
| Olive oil (%)    | 10.00 ± 1.02         | 10.00 ± 0.54   |
| Total Phenol (%) | 4.1 ± 0.81           | 1.20 ± 0.15    |

\*All % in w/w, the values after ± represent standard deviation

### 3.3. NMR analysis of OMW-olive cake

The  $^{13}\text{C}$  NMR spectra of dried crude OC and dried OMW-OC mixture are presented in Figure 2. The peak assignments and interpretations (Table 3) are based on previous NMR studies of organic matter (Albrecht et al., 2008; Baddi et al., 2004; Farnet et al., 2009).

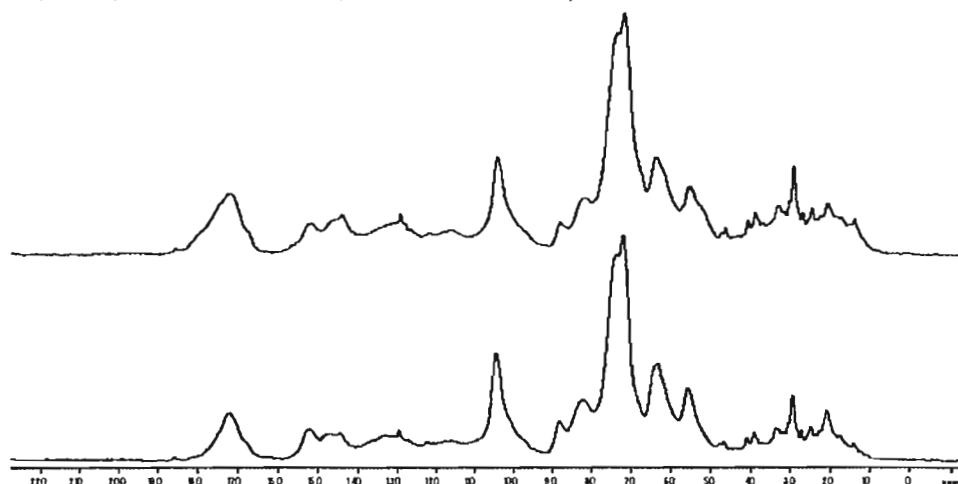


**Figure 2:** Solid-state  $^{13}\text{C}$  NMR spectra of, (a) dried OC-OMW mixture and (b) dried crude OC.

**Table 3:**  $^{13}\text{C}$ -NMR spectral assignment of C functional groups.

| Chemical shift (ppm) | Assignment         | Compounds included                         |
|----------------------|--------------------|--------------------------------------------|
| 0-45                 | Alkyl C            | Lipids, Amino-acids,                       |
| 45-60                | N-Alkyl/methoxyl C | Amino acids                                |
| 60-110               | O-Alkyl C          | Celluloses, hemicellulose, polysaccharides |
| 110-145              | Aryl C             | Polyphenols, Lignin (aromatic C)           |
| 145-165              | O-Aryl C           | Phenols, lignin (phenolic C)               |
| 165-190              | Carboxyl C         | Organic acids, amide and ester C           |

Deconvolution of spectra allowed integration of 27 peaks. Figure 3 showed relative integration values for the major C-types from the dried substrates. A comparison of the spectra of dried OC-OMW mixture and crude OC showed that the major difference are in the Alkyl C (0-45 ppm), O-alkyl C (60-110 ppm) and Carboxyl C (165-190 ppm). The addition of OMW to OC led to an apparent low content of carbohydrate in the dried mixture. The high intensity of O-alkyl C in the dried mixture was consistent with total phenol content which was 5 times higher than in OC. The dried mixture showed more intense peaks at 175 ppm, because of the carboxyl group which can result from the degradation of OMW upon drying, especially, during the first stage of drying. The intensities of the peaks at 84 and 89 ppm showed a very low degree of crystallinity of cellulose both for the dried OC-OMW mixture and OC and the lignin content was not affected by the additions of OMW (Table 4).

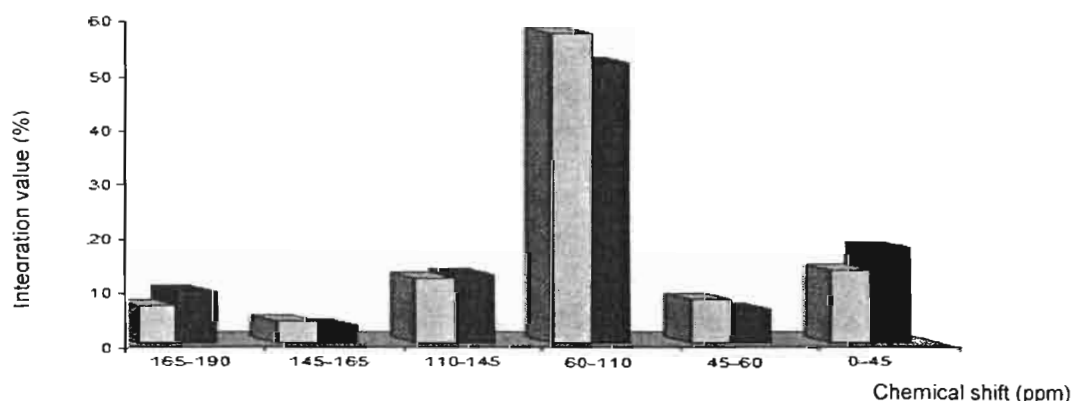


**Figure 3:** Integration values for the major C-types in the  $^{13}\text{C}$  NMR spectra of dried OC-OMW mixture (■) and dried OC (▒).

**Table 4:** Calculated parameters from NMR data of dried mixture OC-OMW and dried crude OC

| Parameters               | OC-OMW dried mixture | Dried crude OC |
|--------------------------|----------------------|----------------|
| Aromaticity %            | 17.42±0.26*          | 16.78±0.33     |
| Lignin%                  | 25.23±1.24           | 25.81±1.31     |
| Carbohydrate%            | 47.11±1.02           | 53.00±2.32     |
| Crystallinity (89/84ppm) | 0.15±0.01            | 0.15±0.02      |

\*The values after ± represent standard deviation

#### 4. Conclusions

The absorption of all the OMW produced by an olive mill on the OC generated by the same mill was found to be possible. It was carried out in two steps with alternate drying. The experimental results showed that the drying of the OC-OMW mixture in greenhouse was very effective, and that the drying period to achieve the same level of moisture was shortened relatively to the open sun drying. The homogeneity of air in the greenhouse under natural convection eliminated any difference in drying rate at different levels of trays. The chemical and NMR analysis of the dried OC-OMW mixture showed that the new product was sufficiently similar to OC to be at least valorised in the same way with the advantage to avoid the dumping of OMW in the environment.

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## Le contenu total phenolique et leur activite antioxydante dans les feuilles et les drupes des cultivars d'olive grecs

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### Résumé

Les produits de l'olive, huile d'olive et olive de table, jouent un rôle socio-économique important en Grèce. Dans le présent travail, des cultivars d'olive grecs ont été étudiés dans le but de déterminer la quantité totale de leur contenu phénolique, ainsi que leur activité antioxydante, dans les feuilles et les drupes (vertes et noires). Le contenu total phénolique, dans des jeunes feuilles, a varié entre 1,01 mg GAE / gr (Adramytini) et 2,06 mg GAE / gr (Konservolia), tandis que les valeurs correspondantes pour l'activité antioxydante ont varié entre 157,64 ppm (Konservolia) et 285,80 ppm (Adramytini). Dans les drupes vertes, le contenu total phénolique a varié entre 0,59 mg GAE / gr (Megaritiki) et 1,98 mg GAE / gr (Mastoidis), tandis que les valeurs correspondantes pour l'activité antioxydante ont varié entre 119,66 ppm (Lianolia Kerkyras) et 496,45 ppm (Megaritiki). Le contenu total phénolique dans les drupes noires a varié entre 0,54 mg GAE / gr (Gaidourelia) et 0,99 mg GAE / gr (Lianolia Kerkyras), tandis que les valeurs correspondantes pour l'activité antioxydante ont varié entre 284,23 ppm (Lianolia Kerkyras) et 886,36 ppm (Chalkidiki).

## Total phenolic content and antioxidant activity in leaves and drupes of greek olive varieties

### Abstract

The olive products, olive oil and table olives, play an important socioeconomic role in Greece. In the present work, Greek olive cultivars were studied in order to determine their total amount of phenolic content as well as their antioxidant activity, both in leaves and drups (green and black). Total phenolic content, in young leaves, ranged between 1.01 mg GAE / gr (Adramytini) and 2.06 mg GAE / gr (Konservolia), while the corresponding values for the antioxidant activity ranged between 157.64 ppm (Konservolia) and 285.80 ppm (Adramytini). In green drups, total phenolic content ranged between 0.59 mg GAE / gr (Megaritiki) and 1.98 mg GAE / gr (Mastoidis) while the corresponding values for the antioxidant activity ranged between 119.66 ppm (Lianolia Kerkyras) and 496 Megaritiki). Total phenolic content in black drups ranged between 0.54 mg GAE / gr (Gaidourelia) and 0.99 mg GAE / gr (Lianolia Kerkyras), while the corresponding values for the antioxidant activity ranged between 284.23 ppm (Lianolia Kerkyras) and 886.36 ppm (Chalkidiki).

**Key words:** Olive, phenolic content, leaves, drups, antioxidant activity.

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## **Thème 7**

**Economie et marchés oléicoles: évolution, prospective et stratégies d'acteurs**

## Etude de l'efficacité technique des huileries en Tunisie

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### Résumé

Ce travail a pour objectif de mesurer et d'étudier l'efficacité technique et ses déterminants au niveau d'un ensemble d'enquêtes réalisées au près de 138 huileries localisés dans les différentes régions oléicoles de la Tunisie dans la région du Nord (gouvernorats de Bizerte, Tunis, Zaghouan, Ariana, Mannouba et Ben Arous), dans la région du Sahel (gouvernorat de Mahdia, Monastir et de Sousse) et dans la région du Sud (gouvernorat de Sfax). Pour cela, un modèle d'estimation simultané de frontière stochastique de production et des effets d'inefficacité technique est utilisé. La sélection entre les différentes formes fonctionnelles a permis de retenir la technologie de production Cobb-Douglas. L'analyse de ces enquêtes a permis de déceler que l'efficacité technique moyenne des huileries dans les trois régions d'étude est de l'ordre de 73,9%. Elle varie entre un minimum de 24,3% et un maximum de 93,6%. Les marges de progression de la production d'huile d'olive s'élèvent à 17,4% % en optimisant l'utilisation des facteurs de production. L'examen des déterminants de l'efficacité technique révèle que celle-ci est positivement affectée par la proportion de la main d'œuvre qualifiée, le type d'équipement utilisé, la présence d'un laboratoire d'analyse de l'huile d'olive dans l'entreprise et la variable formation continue du gérant/propriétaire. Par ailleurs, elle est affectée négativement par niveau d'étude du gérant / propriétaire et l'instauration d'un système de management de qualité vu le faible nombre d'huileries présentant ces variables.

**Mots clés:** Huileries, efficacité technique, production, modèle estimation.

### Measure and study the technical efficiency of tunisian olive oil mills

### Abstract

This work aims to measure and study the technical efficiency as well as its determinants according to series of surveys conducted in nearly 138 olive oil mills located in different olive growing regions of Tunisia in northern region (Bizerte, Tunis, Zaghouan, Ariana, Benarous and Manouba), in the Sahel region (Mahdia, Monastir, and Sousse) and in the southern region (Sfax). Therefore, a model of simultaneous estimation of stochastic production frontier and technical inefficiency effects in used. The selection between the different functional forms resulted in the selection of production technology Cobb-Douglas. The analysis of these surveys identified that the average technical efficiency of oil mills in the three study areas in approximately 73,9%. It varies between a minimum of 24,3 % and a maximum of 93,6%. The margins of progress of the olive oil production amounted to 17,4% by optimizing these factors of production. Moreover, an examination of the determinants of technical efficiency shows that is positively affected by the proportion of skilled labor, the type of equipment used, the presence of a laboratory analysis of olive oil in the company and the training variable of the manager or owner and the establishment of a quality management system given the low number of olive oil mills with these variables.

**Key words:** oil unit, technical efficiency, factors of production, estimation model, Cobb-Douglas function.

### 1. Introduction

Dans le cadre d'un contexte concurrentiel concernant le positionnement de l'huile tunisienne sur le marché international et dans l'économie mondiale, l'état vise à maintenir les exportations d'huile d'olive en essayant d'améliorer la qualité et d'augmenter la quantité tout en mettant en œuvre une stratégie de développement durable de l'oléiculture. Le secteur d'huile d'olive contribue à 14% de la valeur totale la de la production agricole, représente 50% des exportations agricoles et 5% du taux global des exportations tunisienne (DGPA, 2008). C'est ainsi que les industries agroalimentaires (IAA), constituent l'un des maillons essentiels et indispensables de la chaîne oléicole. Le secteur de la

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transformation fait l'objet d'une véritable mutation, caractérisée par la création et la modernisation des huileries modernes et l'abandon des systèmes classiques, vétustes et peu performants. Près de 1.660 huileries sont recensés ayant une capacité théorique de trituration d'olives de plus de 33.000 tonnes par jour. Des études régionales et microéconomiques, constitue un diagnostic pertinent pour identifier et comprendre les principaux déterminants de l'efficacité technique des huileries cela d'une part. Ces mesures d'efficacité proposent d'autre part des solutions d'amélioration adaptées aux différentes régions et types d'unité de transformation étudiés.

technique de production. Ainsi, ces études utilisaient des variantes de modèles qui permettent d'estimer simultanément la frontière stochastique de production et les effets de l'inefficacité technique (Kumbhakar et al., 1991; Reifschneider et Stevensen, 1991; Huang et Liu, 1994; Battese et Coelli, 1995).

C'est dans ce cadre qu'intervient ce travail de recherche qui a pour objectif de d'évaluer l'efficacité technique et ses déterminants au niveau d'un ensemble d'enquêtes réalisées au près de 129 huileries localisées dans les principales régions oléicoles de la Tunisie. Les résultats d'estimation de la frontière de production stochastique et des effets d'inefficacité technique seront obtenus par la procédure du maximum de vraisemblance en utilisant le logiciel FRONTIER-version 4.1 (Coelli, 1996).

## 2. Matériel et méthodes

### 2.1. Zone d'étude et Echantillonnage

Des enquêtes ont été menées dans les différentes régions oléicoles auprès d'un échantillon renfermant 129 huileries recensées dans les principales régions oléicoles.

- Nord : gouvernorats de Bizerte, Tunis, Zaghouan, Ariana, Mannouba et Ben Arous,
- Sud : gouvernorat de Sfax,
- Sahel : gouvernorats de Sousse, Monastir et Mahdia.

Cette enquête s'articule autour de trois grandes parties. La première traite les données générales sur les huileries. La seconde s'intéresse au capital de l'entreprise: superficie, capital humain, biens de l'entreprise, et source de fond de roulement. La dernière partie est consacrée à l'approvisionnement, commercialisation et technologie.

### 2.2. Spécification économétrique

La technologie ainsi que la composante relative aux effets d'inefficacité technique sont simulés selon les modèles suivants:

$$\ln Y_i = \beta_0 + \beta_1 \ln \text{TRAV}_i + \beta_2 \ln K_i + v_i - u_i \quad (1)$$

$$u_i = \delta_0 + \delta_1 (\text{PMOQ}) + \delta_2 (\text{EQUIP}) + \delta_3 (\text{NIVE}) + \delta_4 (\text{FORMAT}) + \delta_5 (\text{PLABOA}) + \delta_6 (\text{ISMQ}) + w_i \quad (2) \text{ Où :}$$

- $Y_i$  : Profit / bénéfice en DT,
- $\text{TRAV}$  : Travail effectué au niveau de l'huilerie  $i$  en valeur (DT) ;
- $K$  : Capital de l'entreprise en valeur (DT) ;
- $\text{PMOQ}$  : Proportion de la main d'œuvre qualifiée (en % de la main d'œuvre totale) ;
- $\text{EQUIP}$  : Type d'équipement utilisé,
- $\text{NIVE}$  : Niveau d'études du propriétaire/gérant ;
- $\text{FORMAT}$  : Formation continue du gérant/propriétaire ;
- $\text{PLABOA}$  : Présence d'un laboratoire d'analyse de l'huile d'olive dans l'entreprise ;
- $\text{ISMQ}$  : Instauration d'un système de management de qualité ;
- $v_i, u_i$  et  $w_i$  : Termes d'erreurs ;

## 3. Résultats

Le tableau 1 représente les résultats d'estimation de la frontière de production stochastique. Les coefficients estimés du travail et du capital de l'entreprise sont positifs et significatifs au seuil de 1%, donc il existe une relation positive entre le capital et le bénéfice/profit d'une part et le travail effectué

durant la campagne et le bénéfice/profit d'une autre part. L'élasticité de la production par rapport au capital est de 0,623, celle du travail est de 0,486 donc l'impact du facteur capital est le plus important (Tableau 2).

**Tableau 1:** Statistiques élémentaires.

| Variables                              | Moyenne | Minimum | Maximum   | Ecart-Type  |
|----------------------------------------|---------|---------|-----------|-------------|
| Valeur ajoutée (DT)                    | 286.042 | 6300    | 8.000.000 | 739 583,727 |
| Travail (UTH)                          | 1679    | 448     | 3408      | 641,788     |
| Capital de l'entreprise en valeur (DT) | 416.306 | 20.000  | 1.800.000 | 331 247,142 |

Source : Propre élaboration à partir des données de l'enquête

Les coefficients estimés des variables relatives à la proportion de la main d'œuvre qualifiée et à la présence d'un laboratoire d'analyse de l'huile d'olive, sont négatifs et significatifs au seuil de 1%, le coefficient estimé de la variable type d'équipement utilisé est négatif et significatif au seuil de 5%. De même le coefficient estimé de la variable formation continue du gérant/propriétaire est négatif et significative au seuil de 10%. Ceci témoigne de l'importance de ces variables dans l'amélioration de l'efficacité technique de ces huileries (Tableau 2).

**Tableau 2:** Paramètres estimés de la fonction de production stochastique et des effets d'inefficacité technique des huileries de trois régions en Tunisie.

| Variables                          | Paramètres estimés | t- Student |
|------------------------------------|--------------------|------------|
| $\beta_0$ (Constante)              | -0,575             | -0,68      |
| $\beta_1$ (Travail)                | 0,486*             | 4,68       |
| $\beta_2$ (Capital)                | 0,623*             | 7,38       |
| $\delta_0$ (Constante)             | 0,241              | 0,252      |
| $\delta_1$ (PMOQ)                  | -0,044*            | -6,76      |
| $\delta_2$ (EQUIP)                 | -0,610**           | -2,12      |
| $\delta_3$ (NIVE)                  | 0,523***           | 1,94       |
| $\delta_4$ (FORMAT)                | -0,537***          | -1,93      |
| $\delta_5$ (PLABOA)                | -0,356*            | -6,06      |
| $\delta_6$ (ISMQ)                  | 0,121*             | 3          |
| <b>Paramètres de la Variance :</b> |                    |            |
| $\sigma^2$                         | 0,761*             | 3,38       |
| $\gamma$                           | 0,409*             | 5,61       |

Source : Propre élaboration à partir des données de l'enquête.

\* indique la signification au seuil de 1% ; \*\* indique la signification au seuil de 5% ; \*\*\* indique la signification au seuil de 10%.

Le signe et la valeur des coefficients des variables relatives au niveau d'études du propriétaire/gérant et à l'instauration d'un système de management de qualité montrent que ces variables engendrent une diminution de l'efficacité technique.

Le paramètre de la variance estimé,  $\gamma = \sigma_u^2 / (\sigma_u^2 + \sigma_v^2)$ , est statistiquement significatif. Ceci implique que les effets d'inefficacité technique sont significatifs dans la détermination du niveau et de la viabilité de la production des huileries dans les trois régions.

Les résultats de la distribution moyenne des fréquences et de l'efficacité technique moyenne, sont notés dans le Tableau 3. La valeur moyenne de l'efficacité technique est de 73,9% variant entre un minimum de 24,3% et un maximum de 93,6%.

**Tableau 3:** Fréquences de distribution de l'efficacité technique de production pour un échantillon de 129 huileries des régions du Nord, du Sahel et du Sud en Tunisie pour la campagne 2008-2009.

| Efficacité Technique (%) | Nombre d'huileries | Pourcentage (%) |
|--------------------------|--------------------|-----------------|
| ET ≤ 20                  | 0                  | 0               |
| 20 < ET ≤ 30             | 3                  | 2               |
| 30 < ET ≤ 40             | 4                  | 3               |
| 40 < ET ≤ 50             | 8                  | 6               |
| 50 < ET ≤ 60             | 6                  | 5               |
| 60 < ET ≤ 70             | 13                 | 10              |
| 70 < ET ≤ 80             | 37                 | 28              |
| 80 < ET ≤ 90             | 48                 | 38              |
| ET > 90                  | 10                 | 8               |
| Efficacité moyenne       | 73,9%              |                 |
| Efficacité min.          | 24,3%              |                 |
| Efficacité max.          | 93,6%              |                 |

Source : Propre élaboration à partir des données de l'enquête.

L'efficacité technique estimée dans les différentes régions montre qu'au Nord, elle de 66,4% variant entre un minimum de 29,8% et un maximum de 88,8%. Dans la région du Sahel, elle est de l'ordre de 79,4% allant de 46,4% à 91,9%. Au sud, l'efficacité technique moyenne est de 76,2%, entre 24,3% et 93,6% (Tableau 4).

**Tableau 4:** Fréquences de distribution de l'efficacité technique de production pour chaque région : un échantillon de 43 huileries de la région du Nord, 40 huileries de la région du Sahel et 46 huileries de la région du Sud (gouvernorat de Sfax) pour la campagne 2008-2009.

| Efficacité Technique (%) | Région du Nord     |     | Région du Sahel    |      | Région du Sud      |     |
|--------------------------|--------------------|-----|--------------------|------|--------------------|-----|
|                          | Nombre d'huileries | (%) | Nombre d'huileries | (%)  | Nombre d'huileries | (%) |
| ET ≤ 50                  | 8                  | 18  | 1                  | 2,5  | 6                  | 13  |
| 50 < ET ≤ 70             | 13                 | 30  | 2                  | 5    | 4                  | 9   |
| 70 < ET ≤ 80             | 11                 | 26  | 15                 | 37,5 | 11                 | 24  |
| 80 < ET ≤ 90             | 11                 | 26  | 20                 | 50   | 17                 | 37  |
| ET > 90                  | 0                  | 0   | 2                  | 5    | 8                  | 17  |
| Efficacité moyenne       | 66,4%              |     | 79,4%              |      | 76,2%              |     |
| Efficacité min.          | 29,8%              |     | 46,4%              |      | 24,3%              |     |
| Efficacité max.          | 88,8%              |     | 91,9%              |      | 93,6%              |     |

Source : Propre élaboration à partir des données de l'enquête.

#### 4. Conclusion

Les facteurs de production travail et capital de l'entreprise contribuent positivement et significativement à l'amélioration de l'efficacité technique. Le facteur capital a un effet important sur la production d'huile d'olive par conséquent sur le bénéfice/profit. La main d'œuvre qualifiée, le type d'équipement utilisé, la présence d'un laboratoire d'analyse de l'huile d'olive dans l'entreprise et la variable formation continue du gérant/propriétaire ont un effet positif sur l'efficacité technique. Le niveau d'étude du gérant/propriétaire et l'instauration d'un système de management de qualité ont un effet négatif sur l'efficacité technique.

L'efficacité technique moyenne des huileries est de 73,9%. Les résultats montrent aussi un niveau d'efficacité technique important variant surtout de 80% à 90%. Les possibilités d'accroissement de la production d'huile d'olive tout en utilisant le même niveau de facteurs de production sont de 17,4%.

Ces efficacités peuvent être améliorées par une meilleure gestion du secteur et ceci par :

- L'incitation à l'augmentation de la proportion de la main d'œuvre qualifiée,
- la conception et la mise en œuvre d'un programme d'encadrement technique et des moyens de vulgarisation,
- l'extension de la démarche de marketing, d'innovation et de l'analyse de la concurrence qui reste encore trop timide, le plus souvent réservée aux grosses huileries,
- la valorisation de la matière exportée (appellation d'origine contrôlée).

En définitive, ce travail peut être une initiation pour formuler une étude plus approfondie pour analyser l'efficacité technique au niveau des huileries Tunisiennes. Le modèle sous forme fonctionnelle Cobb-Douglas utilisé pour l'estimation de la frontière stochastique pourrait être amélioré avec l'introduction d'autres variables pour assurer des résultats plus significatifs.

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## **Pieralisi great evolution of the technologies for the olive oil production**

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### **Abstract**

In the last years we have been witnesses of a great evolution of the technologies for the olive oil production, born to answer to environmental, economic and qualitative necessities.

At the end of the 1960s Pieralisi patented the first continuous system and, during the 90<sup>th</sup>, the system at 2 phases diffused quickly in all Worlds. This permits great water and energetic saving, smaller environmental effects for no water production and better oil quality.

Actually the last innovation is represented by a centrifuge of last generation planned by Pieralisi, that is still in phase of study, but at the moment has succeeded in conjugating to the advantages of the 2 phases, the production of new by-products with a great added value.

**Key words:** Technologies – environmental – quality – innovation – by-products

## Mise en place d'une appellation d'origine contrôlée dans le secteur oléicole en Tunisie

Feki Mouna

### Résumé

A ce jour, la plus grande part de la production oléicole en Tunisie, est exportée en vrac vers les pays européens (principalement l'Italie), où elle est mélangée et vendue sous marques européennes. D'où un manque de valorisation pour la Tunisie. Une valorisation de l'huile d'olive tunisienne par un sigle officiel d'identification de la qualité et de l'origine devient alors nécessaire. Dans ce cadre, ce travail vise à étudier les conditions nécessaires pour accroître la valeur de la production d'huile d'olive tunisienne, en particulier dans la région « Bir el Mallouli » de la délégation Menzel Cheker du gouvernorat de Sfax, à travers un système d'appellation d'origine, comparable aux systèmes réussis en Europe. Le SIG nous a permis la délimitation de notre site « Bir el Mallouli » et de préciser ses caractéristiques, nécessaires à la préparation d'un cahier des charges de la production spécifique de ce site. Les analyses physicochimiques et organoleptiques des huiles issues des olives récoltées du site « Bir el Mallouli » nous a permis aussi de préciser les potentialités de cette zone et d'imposer la typicité du produit liée au terroir.

**Mots clés :** Valorisation, appellation d'origine, SIG, typicité, terroir.



## The Olive Industry in Malta

Farrugia Natasha

### Abstract

Olive cultivation and olive oil production in the Maltese Islands dates back to many years. Being situated in the heart of the Mediterranean, Malta offers suitable conditions for the cultivation of the olive tree. Following a historical episode, for many years no significant activity was reported within this sector. In recent years, interest in the olive sector has grown notably resulting in an increase in olive cultivation, and the production of local olive oil from various varieties. Great efforts for gaining knowledge and improvement with modern techniques in both cultivation and olive oil production have been the aims of the olive industry ever since. Research and trials, together with projects for the revival of old native varieties aim at promoting oleiculture in Malta and accomplishing a better knowledge and understanding of the revived olive sector.

**Key words:** Malta, Olives, Olive Oil, Revival.

The Mediterranean offers the perfect climatic conditions for the cultivation of the olive tree. Being situated right at the heart of the Mediterranean, the Maltese Islands offer an optimal environment for the growth of the Olive tree. Cultivation and olive oil production in the Maltese Islands dates back to many centuries although the precise origins are still unknown. Despite this fact however, history records reveal that during the 18th century, many olive trees, around 80,000 in number, were destroyed for the cultivation of other products namely cotton. Following this, for many years no significant activity was reported within this sector. In recent years, interest in the olive sector has started growing notably resulting in many new plantings, and great efforts for gaining knowledge and improvement with modern techniques in both cultivation and olive oil production. Ongoing research and trials together with the revival of old varieties aim at improving the olive industry in the Maltese Islands in order to accomplish a better knowledge and understanding of the revived olive sector.

### 1. Olive Cultivation and Distribution

The Maltese farming land is spread all over the Islands, and is found to be diffused in small parcels, many of which are separated by the local traditional boundary 'rubble' walls. An average parcel of farming land in Malta has a size of 0.15ha. The reason for this is mainly due to the division of land when it is inherited from father to sons and daughters. Due to this fact, large olive orchards are not common throughout the Maltese Islands. Typically olive groves vary in size, from as small as 0.1ha. Many farmers use olive trees to surround their land parcels serving as windbreakers for other products as well as for providing olives. Monovarietal plantations are uncommon in Malta due to size limitation. Normally olive groves in Malta have a planting density of about 300 to 400 trees per hectare especially the more recent plantations. The pruning methods vary between two predominant types that are the vase and the bush. The type of pruning is normally dictated by the microclimate especially with regards to the prevailing winds. In Malta the most problematic wind comes from the North West and is highly destructive. In exposed sites the trees are kept small and bush pruning on a low trunk is used to protect the tree and minimise damage. In less exposed sites trees grow larger and the vase pruning is used. New olive groves have been planted in recent years and until the year 2008, a total area of about 210 hectares was covered throughout Malta and Gozo. In the previous year, the total olive cultivated area was that of 150 hectares.

## 2. Olive Varieties Cultivated in Malta

Many olive varieties are found to be cultivated in Malta most of which are International ones, most commonly originating from Spain and Italy. The most common varieties amongst newly planted groves include Frantoio, Leccino, Carolea, Coratina, Pendolino and Cipressino for olive oil varieties and Uova di Piccione and Bella di Spagna for table varieties.

Apart from the International varieties, old trees found in the Maltese Islands show that the ancient varieties cultivated were native to our country. Studies recently carried out have shown that a variety known as 'Bidni', named after the village of Bidnija in which it was found, is indigenous to Malta and has been cultivated for thousands of years. Trees found growing in this area have been found to be between 1500 and 2000 years old and are remnants of larger ancient groves probably belonging to a Roman household since stone decantation basins have been found in sites very near to the groves. The 'Bidni' variety is a vigorous grower and of strong constitution. The leaves are typically broad and short and the fruit is very small with a comparatively small stone. The fruit is rich in oil however and also rich in flavour. It is relatively free from bitterness and therefore can be used both as a table olive and also for the production of olive oil. However, its small size limits its use as a table olive in favour of other fleshy varieties as the "Bella di Spagna" variety. The tree and its fruit are found to be quite resistant to disease, especially to the attack of the olive fly and therefore allows the fruit to be ripened for longer on the tree achieving higher amounts of oil. The fruit physiologically ripens around end October or early in November and turns to a dark violet at maturity. The fruit for crushing is picked by the end of September to beginning of October when the fruit is changing colour and the proportion of polyphenols and oil percentage is at its best. The old trees found growing in the Bidnija area are still in production today although not on a viable scale. It is also thought that these trees were supplying olives for the production of olive oil at the time when the Biblical event of St. Paul's shipwreck on Malta took place.

## 3. Olive Oil Production

Olive oil acquired its importance amongst the Maltese only recently along with the increase of olive oil production and awareness of its exquisite taste and health benefits. The olive oil Sector in Malta is a young developing one with a total annual production of around 30 tonnes. As a newly developing industry, many groves have been recently planted and are not yet in production, hence the amount of olive oil production is expected to increase by a substantial amount in the next few years.

The olive mills found in the Maltese Islands are modern stainless steel mills with a mechanical press and cold extraction. All the olive oil produced in Malta falls under the Extra Virgin category and is of the first press. No pomace oils are produced in Malta. Until the year 2009, there have been 6 registered oil mills, one of which has been certified as organic. The first olive mill to start operating in Malta commenced in 1998 and was followed by five others, two of which have commenced only in 2008. The Maltese olive oil producers are dependant on many olive cultivators. Due to the small size of farming parcels, an olive oil producer does not rely solely on his olive groves but purchases olives from individual farmers to maximise the production. Maltese olive mills also provide a pressing service for farmers who deliver olives and use the oil from their produce for their own family consumption. Olive oil extraction in Malta starts in September and normally continues until December, however most of the olives would have been pressed by the end of November.

## 4. Table olives

Most of the table olives consumed by the Maltese are imported from other countries. Cultivation of table olive varieties in Malta is mostly common to the part-time farmer who cultivates the land for personal consumption, whereas full-time farmers opt for oil varieties. A small amount of table olives delivered to the market whilst others for personal use are normally preserved in brine or in oil. Interest in table olives is also expanding with the plantation of new olive groves.

## **5. Ongoing Projects**

### **5.1. PRIMO Project**

The Project for the Revival of the Indigenous Maltese Olive (PRIMO) project is an initiative undertaken between a Maltese olive oil producer, Mr Sam Cremona, a local bank, Bank Of Valletta and the Ministry for Rural Affairs and the Environment, which was launched in January of 2006. The project aims at conserving local olive tree varieties and to disseminate these varieties amongst farmers in a programmed manner so as to be able to produce extra virgin olive oil from the varieties which are renowned for their quality. The project is also a conservation exercise that will permit the utilisation of such varieties on a commercial scale to complement the other international varieties which are presently more common. The project is essentially also environment sensitive in that the funding provided by the bank aims at increasing the land under olive trees in Malta and Gozo in a bid to improve environmental conditions such as preventing soil erosion and preserving terraced fields.

### **5.2. BIOLMED**

Malta, together with four other countries Italy, Greece and Spain, is participating in the BIOLMED network which is a project aimed at the improvement of Mediterranean organic olive-growing competitiveness through the sharing and the know-how transfer. This transnational Network for enhancing the Mediterranean organic olive-growing competitiveness has started on the 3rd of March 2009 and will finish on the 1st of March 2011. The project will create a permanent transnational network of BIOL quality circles based on some consolidated experiences at local level (Biol Prizes).

BIOL quality circles will have the aim to sustain the agriculture company development aimed at the organic olive oil quality improvement, to promote regional meetings, to support the organization of the Biol Exhibitions at local level and on sharing of regional initiatives through a network. The project, support the innovative information system, through the editing of quality production chain manuals and a catalogue on innovation, aims to share and transfer good practices to improve the production chain quality and its environmental impact, as well as to create and to spread company integrated management systems on organic product quality. In order to develop local markets, the project foresees the sharing and the realization of Group Purchasing Organisation and Farmers Markets, favouring the purchasing directly from small local producers. The whole project is particularly orientated to the sharing and transfer of project results, particularly contributing to the 4 BIOL prizes organizations.

## **6. Traditional Maltese Dishes with Olives and Olive Oil**

Olive oil and table olives are used in many Maltese traditional dishes. Olive oil is used to make the pastry of the traditional ftira which is similar to a pizza. The traditional maltese bread is also most commonly consumed with tomatoe paste, olive oil and sliced olives. A traditional maltese dip known as bigilla is made from beans, garlic and olive oil. Table olives are preserved with garlic and herbs to be served with many typical dishes. Being an Island, fish dishes are also common in Malta and the most common fish, the lampuka is served with a sauce that is made from tomatoes, onions, olives, capers and herbs.

## **Estimation d'une fonction de demande d'exportation d'huile d'olive en Tunisie; une analyse de co-intégration**

Mouna Maalej, Salma Zouari

### **Résumé**

L'objectif de ce travail est d'examiner les principaux déterminants des exportations de l'huile d'olive en Tunisie durant la période 1976-2008 moyennant une analyse basée sur la cointégration. Les résultats de l'estimation de la fonction de demande d'exportation pour la Tunisie montrent qu'à long terme l'Italie et la Grèce vont améliorer leur compétitivité de prix face à la Tunisie.

**Mots clés :** Fonction de demande d'exportation, huile d'olive, Tunisie, Union Européenne, cointégration.

### **Abstract**

The present study investigates the main determinants of Tunisian olive oil exports to the European Union during the period 1976-2008. To this end, an export function using the co-integration methodology is estimated. The variables with explanatory relevance appear to be the relative prices and, to a less extent, the exports of partners.

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