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Effect of soil conditions on the fruiting of the Perigord Truffle (*Tuber melanosporum*)
Incidences des conditions de milieu sur la production de la truffe noire du Périgord

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The Perigord truffle (*Tuber melanosporum*) is the fruitbody of a subterranean ectomycorrhizal fungus of the *Ascomycetes* family. About 1.000 tonnes of this sought-after fungus was produced in France at the start of the century, but the production has dropped continually. We conducted a detailed analysis of pilot truffle orchards in different calcareous soils to find out the real reasons for the decline in particular the very low productivity of the new plantations. Analysis of the spatial variabilities in the soil physical and microbiological structures of truffle soils in relationships with the production of truffles were revealed. There is thus only minor genetic diversification within the various truffle populations of the same truffle bed and the soil colonization by the fungus would thus appear to be linked to changes in the microbiological and biological structures of the topsoil. The development of fruitbodies were very dependent of drainage conditions and macro-biological activity. The soil fauna appeared to be a decisive factor both in the structuring of the soil around the ascocarp, in the regulation of microbial activity and increasing the permeability of the sub soil favourable to truffle development.

Keywords : Truffle (*Tuber melanosporum*), calcareous soils, genetic diversity
Mots clés : truffe (*Tuber melanosporum*), sol calcaire, diversité génétique

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1- Focus of the present research

The biological cycle of the truffle is not yet known and it has not been grown in a controlled culture medium. Truffles are grown only in truffle orchards.

Research into truffle-growing in France and elsewhere has focused on the symbiotic relationships between the root system of the tree and the mycelium of the fungus. It has resulted in the development of techniques for controlled mycorrhization (Inra-Anvar patent) and artificial truffle beds are planted using mycorrhized seedlings. The fact that truffles are obtained 4 years after planting in favourable soils appeared to indicate that the mycorrhized tree was the decisive factor for the success of the plantations. "*Plant trees and harvest truffles*" has become a traditional slogan and, although not been demonstrated experimentally, the fruiting of this fungus appeared to be directly connected with the root system of the host tree. We conducted a series of in-field studies on different scales, to improve our understanding of the tree-fungus and fungus-soil relationships.

2-Methodology

The precise location of truffle ascocarps was noted in various different pilot truffle beds about 1 hectare in area and production recorded during the first few years of fruiting.

A detailed soil survey of the beds was conducted, complemented by geophysical surveys to analyze the variability of the subsoil structures. The different truffle populations were identified using molecular techniques. The organic status and the microbiology of the topsoil were characterized, to identify fungal competition.

3- Effect of the subsoil structures on the onset of fruiting**.**

The truffle is a fungus which develops only in calcareous soil. In all the lithosystems analyzed, we noted that the onset of fruiting and the quantity produced were related to soil type. Unproductive soil and soil producing few truffles had either a clayey, relatively impermeable subsoil, a shallow hard layer or a horizon showing signs of hydromorphy (Bg or Bfe) (Callot and Jaillard, 1996). Good truffle-producing soil was deep, draining soil with a permeable subsoil, but often with a level of calcium carbonate accumulation (Bca) below 50/60 cm depth. The example presented in Figure 1 shows that fruiting increased differently according to the soil units identified in the bed, with fluctuations linked to climatic factors. Two categories can be clearly distinguished: soils where the onset of fruiting was late (units **a**, **c2** and **c1)** and good truffle-producing soils (units **d2**, **b**, **d1)**.

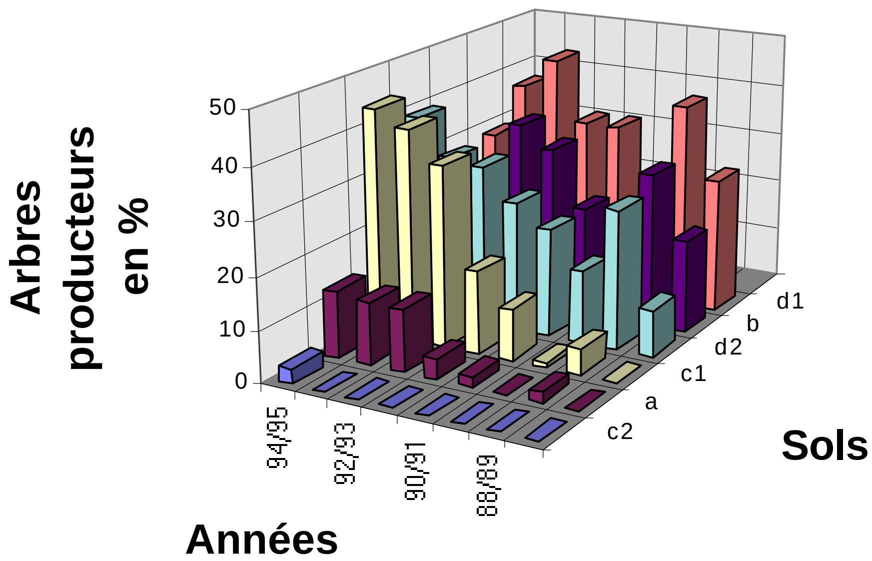

Figure 1- Onset of fruiting and increase in fruiting relative to soil type and year. Example of a truffle bed of live oaks planted on calcareous sandstone, fruiting 10 years after planting**.**

Deep draining soil with a horizon containing a diffused accumulation of calcium carbonate below 50 cm depth is good truffle soil. Most wild truffle beds, however, are found in shallow, stony soil.

4 - Increases in fruiting within the same soil unit.

To understand the adaptation of the truffle to such different soils, we analyzed fruiting in truffle beds established in shallow soil (Rendolls with petrocalcaric horizons) located on hard dolomitic limestone. The geophysical surveys conducted in these soils using electrical resistivity techniques clearly indicated that soil units producing truffles always had a fissured subsoil. We also noted a close correlation between the fissure lines and the alignment of some truffle-producing trees (Lacruz and Largeron, 1996), see Figure 2.

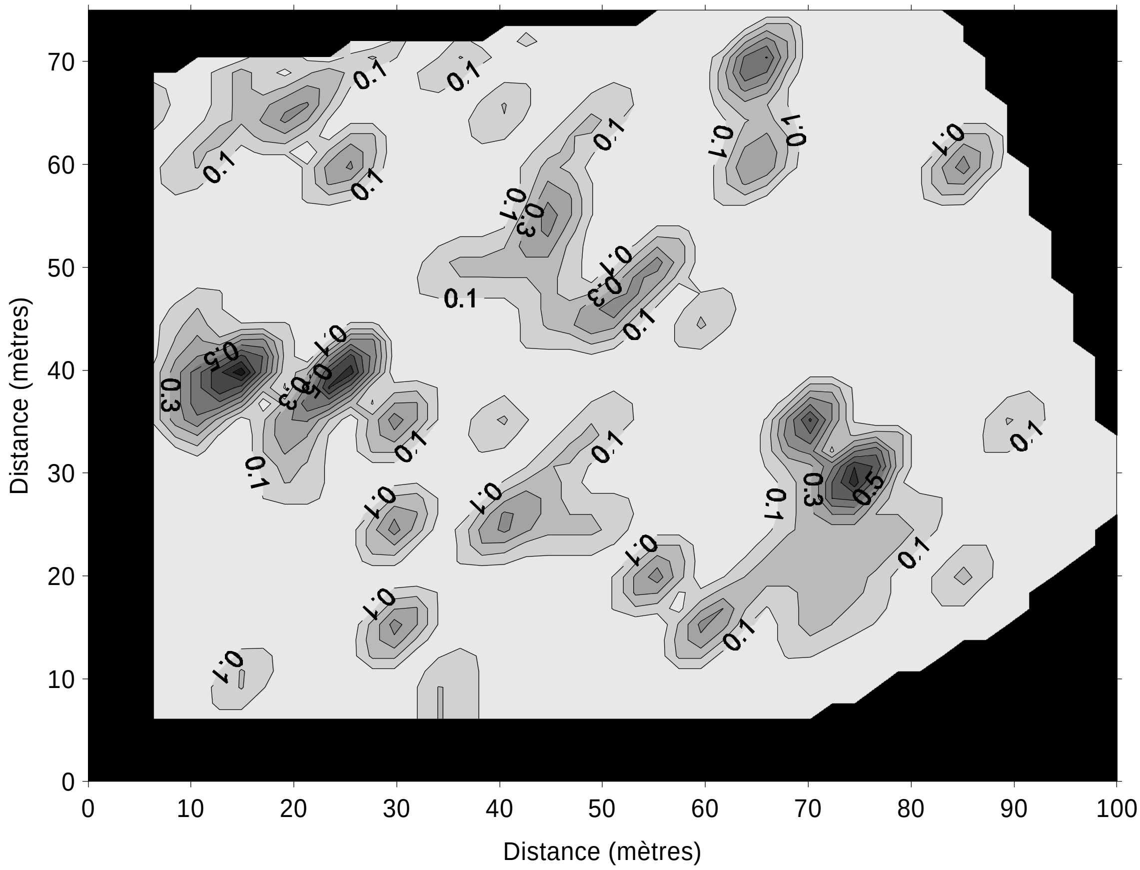

Figure 2- Map showing frequency of fruiting in a truffle bed on dolomitic limestone. Fruiting began 4 years after planting. Trees giving a good level of fruiting (contour 0.5) produced truffles 4 years out of 5 and appear as the sources of fruiting within the truffle bed. It is also apparent that the sources of fruiting have a particular alignment and are closely correlated with the fissure lines. The resistivity curves showing the fissuring of the substrate are presented on the poster.

The trees producing a good level of fruiting were most often located in the vicinity of the fissure lines, which are preferred lines of drainage into which the roots penetrate. Deep rooting provides the tree with an adequate water-supply during the summer, the period during which the truffles developed. The trees producing a good level of fruiting were usually the sources from which production develops. The example presented in Figure 2 illustrates the production dynamics.

Growth in ascocarp production

The growth in ascocarp production was monitored in two productive soil units with a fissured calcareous substrate (Table 1). In the example given, the soil of unit **a** is shallow while that of unit **b** is deep. One can therefore logically conclude that the deepest soil is also the most productive, in terms both of truffle-producing sites (patches devoid of vegetation) and of numbers of ascocarps. The average weight of the truffle ascocarps, however, is not related to soil depth. It is determined by other parameters not analyzed in this poster.

	Total				**Unit a**				**Unit b**			
Year	90	91	92	93	90	91	92	93	90	91	92	93
Host trees	398	398	398	398	124	124	124	124	110	110	110	110
Productive bare patches	15	18	32	41	5	6	13	9	10	12	19	32
Ascocarps harvested (no. of)	47	57	135	161	11	22	47	26	36	35	88	135
Average weight (g)	110	80	89	87	140	95	104	57	81	64	74	118

Table 1- Comparison of fruiting in two productive soil units with a calcareous substrate. The **a** soils are shallow, fersiallitic rendisols; the **b** soils are deep, carbonated fersialsols.

Soil analysis showed that fruiting was related to soil type, but fruiting in each soil unit always began at particular drainage sites, often associated with deep fissuring of the substrate. Studies of the variability of topsoil water content revealed no significant differences between productive and non-productive sites (Dejean, 1997). The centrifugal spread of the fruiting can thus be explained either by the progressive colonization of a truffle ecotype particularly well adapted to the soil or by the progressive evolution of the organic and biological status of the topsoil. These two hypotheses are discussed in the following paragraphs.

5 -Genetic diversity of the *Tuber melanosporum* ascocarps

Random Amplified Polymorphic DNA (RAPD) analysis was performed on 159 truffles from the one plot, shown in Figure 2, and harvested over 3 consecutive years from underneath different trees (Bertault, 1997). After extraction, the DNA of the ascocarps was amplified with a series of random sequence primers. The amplification fingerprint obtained proved to be very similar in terms of both the size and the number of the amplified DNA fragments, irrespective of the RAPD primer used (Figure 3). The result was confirmed using microsatellite markers, which showed no diversity among the sampled ascocarps (Berthomieu, 1996; Bertault, 1997). The results show that, on the scale of the truffle bed, the *Tuber melanosporum* population is made up of genetically very closely-related individuals and are related to a single clone.

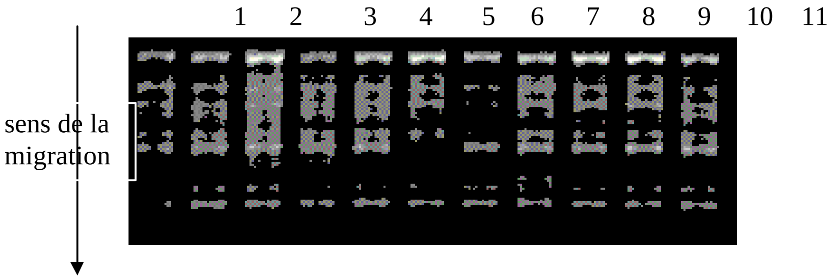

Figure 3 Electrophoresis gel showing the RAPD fingerprints obtained with OPF-11 primer for 11 *Tuber melanosporum* ascocarps harvested from underneath different trees in the truffle bed shown in Figure 2. Note the marked similarity of the 11 ascocarps.

There is thus only minor genetic diversification within the various truffle populations of the same truffle bed, despite the different origins of the mycorrhized seedlings. Soil colonization by the fungus from good truffle-producing sites would thus appear to be linked to changes in the microbiological and biological structures of the topsoil.

6 -Organic status and microbiology of the soil of the truffle bed.

To determine the organic status of the soil of the truffle bed, we conducted a compartmental analysis of the organic matter in the different granulometric classes of the topsoil of sites producing *Tuber melanosporum* and of those which did not, for the same soil type. Truffle-producing sites usually had a low organic matter content in the coarse fraction and a high percentage of stable organic matter in the fine fraction, unlike the non-productive sites. The organic matter content nevertheless differed considerably according to the soil in question and no significant thresholds were apparent. More precise data was obtained from the microbial biomasses, and we noted that the soil favoured truffle development when the microbial biomass was under 1.7% of the total organic carbon. These data were complemented by enzymatic activity tests, which showed that the soil favoured truffle development when the FDA hydrolase activity was 0.2 or less. Respiratory activities were also recorded for 3.3% or less of total carbon in the *Tuber melanosporum* sites over 28 days of standard incubation.

These microbiological characterizations showed that the truffles developed in soil with a low microbial activity. When we studied the microstructures of the truffle/soil interface (Callot and Guyon, 1990), we also noted that soil fauna activity and in particular earthworm activity was very intense in the top-soil of truffle beds.

The soil fauna activity helped to reduce the overall organic matter content of the soil and regulated microbial and enzymatic activity. But the role of the soil fauna was not restricted tomixing the organic and mineral functions: it was also responsible for the aeration and decompaction of the soil around the truffle ascocarp, essential to its harmonious development.

Conclusion

Analysis of the spatial variabilities in the soil physical and microbiological structures of pilot truffle beds revealed that the developmental conditions for the fruiting of this subterranean fungus were very dependent on the soil conditions, which determined both the onset and the intensity of fruiting. This very great dependence on soil conditions results from the fact that the fruitbody is autonomous right from its initial stages of development (Barry et al., 1996). The tree appears to have only an indirect role, as carbon producer and support for the mycelium. The soil fauna appeared to be a decisive factor both in the structuring of the soil and in the regulation of microbial activity. There was always a very high level of fauna activity in soils with high truffle bed potential. Reduction in soil biological activity usually led to compaction of the subsoil, and the latter became relatively impermeable, leading to saturation and/or prolonged drying phases in the topsoil which were unfavourable to truffle development.

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