ISPP
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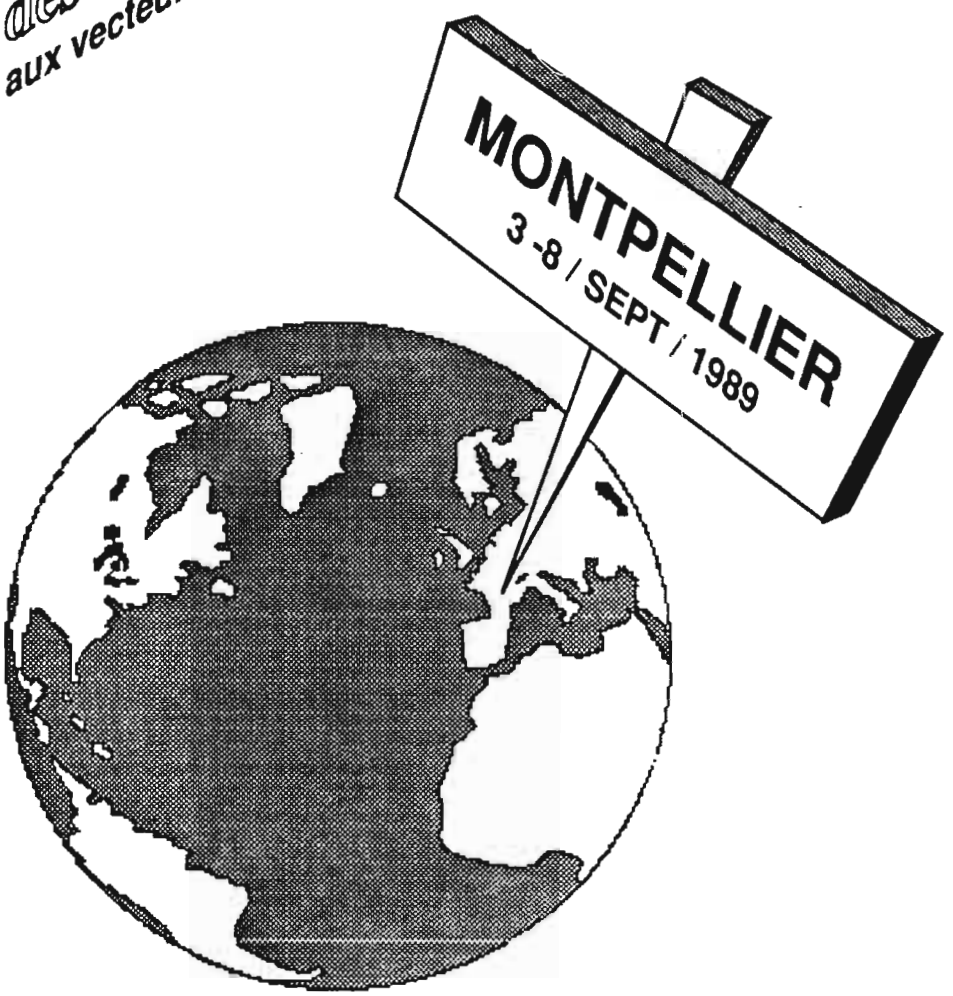
IVth International Plant Virus Epidemiology Workshop

Resistance to viruses and vectors - Temperate and tropical plants

IVème Colloque International Epidémiologie des Virus de Plantes

Résistance aux virus et aux vecteurs - Plantes tempérées et tropicales



AGROPOLIS

2nd Edition

CIRAD - CNRS - ENSAM - INRA - ORSTOM

These workshops are under the auspices of the Plant Virus Disease Epidemiology Committee of the International Society of Plant Pathology. Previous workshops were held in Oxford, England 28-30 July 1981, Carowa, Australia 25-27 August 1983 and Orlando, Florida 6-8 August 1986. The Committee has been chaired by Michael THRESH since its conception.

The following sponsored this workshop :

ISPP - SFP

INTERNATIONAL SOCIETY OF PLANT PATHOLOGY

SOCIETE FRANÇAISE DE PHYTOPATHOLOGIE

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PROGRAMME

DIMANCHE 3 SEPT. — **Enregistrement et installation des posters**
SUNDAY SEPT. 3 — ***Registration and poster installation***

16H Enregistrement - Mise en place des posters
Registration - Poster installation

19H Vin d'Accueil / *"Vin d'Accueil"*

JOUR 1 : LUNDI 4 SEPT. — **Concepts et approches de la résistance**
DAY 1 : MONDAY SEPT. 4 — ***Resistance Concepts and Approaches***

La première journée est consacrée à des exposés magistraux sur les divers aspects de la résistance aux virus et aux vecteurs, synthèse de travaux anciens mais aussi nouveaux concepts et nouvelles techniques à prendre en considération par les épidémiologistes.

The first day is devoted to invited papers on different aspects of resistance to viruses and vectors, including new concepts and new techniques that should be considered by epidemiologists.

08.00 Enregistrement - Mise en place des posters (suite).

08.45 Paroles de bienvenue de M. le Président du Conseil Régional.

09.00 **Session d'ouverture** : Allocution de M. le Député-Maire de Montpellier.
Introduction de M. le Professeur VAGO parlant au nom de tous les co-organisateurs.

09.30 **Session 1 : Concepts et Approches Classiques de la Résistance**
Session 1 : Classical Concepts and Approaches of Resistance

Introduction du président de séance / *Introduction by the chairman :*

		I.W. BUDDENHAGEN	**(USA)**
10.10	Méthodologie / *Methodology*	H. LECOQ	(FRANCE)
10.50	Pause café / *Coffee break*		
11.30	Résistance aux vecteurs / *Vector resistance*	S. M. GRAY	(USA)
12.10	Epidémiologie / *Epidemiology*	L. MADDEN	(USA)
13.00	Déjeuner / *Lunch*		

14.30 **Session 2 : Concepts et Approches Modernes de la Résistance**
Session 2 : Modern Concepts and Approaches of Resistance

Introduction du président de séance / *Introduction of the chairman :*

		B.D. HARRISON	**(UK)**
15.10	Protection croisée / *Cross Protection*	D. GONSALVES	(USA)
15.50	Plantes transgéniques / *Transgenic plants*	R. BEACHY	(USA)
16.30	Pause thé / *Tea break*		
17.00	Biochimie / *Biochemistry*	B. FRITIG	(FRANCE)
17.40	Ribozymes / *Ribozymes*	P. WATERHOUSE	(AUSTRALIA)
18.30	Fin du jour 1 / *End of day 1*		

19.30 Repas à la ferme / Country dinner

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PROGRAMME

JOUR 2 : MARDI 5 SEPT. — **Composantes de la résistance**
DAY 2 : TUESDAY SEPT. 5 — ***Components of resistance***

Le second jour est basé sur les résultats des participants qui seront tous exposés sous forme de posters et discutés en assemblée plénière, et devront porter sur la mise en évidence des composantes de la résistance.

The second day is devoted to offered posters, which will be presented and discussed in plenary session, dealing with the evidence of resistance components.

09.00 **Session 3 : Résistance aux virus**
Session 3 : Virus resistance

Président / *Chairman* : S. COHEN (ISRAEL)

09.30 **Session 4 : Tolérance**
Session 4 : Tolerance

Président / *Chairman* : J.M. THRESH (UK)

10.00 **Session 5 : Résistance aux vecteurs**
Session 5 : Vector resistance

Président / *Chairman* : A.T. JONES (UK)

10.30 Visite des posters / *Poster visit*

11.00 Pause café / *Coffee break*

13.00 Déjeuner / *Lunch*

14.30 Discussion des sessions 3 et 4 / *Discussion of sessions 3 and 4*

16.30 Pause thé / *Tea break*

17.00 Discussion de la session 5 / *Discussion of session 5*

18.00 Fin du jour 2 / *End of day 2*

19.30 **Session nocturne**
Evening session

Président / *Chairman* : H. LECOQ & J.B.QUIOT (FRANCE)

19.40 Géostatistiques / *Geostatistics* — **R. LECOUSTRE (FRANCE)**

20.20 Modélisation de la croissance des plantes / *Simulation of plant growth* — **P. DE REFFYE (FRANCE)**

21.10 La Télédétection / *Teledetection* — **G. GUYOT (FRANCE)**

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JOUR 3 : MERCREDI 6 SEPT. **Excursion à l'INRA d'Avignon**
DAY 3 : WEDNESDAY SEPT. 6 ***Visit of INRA in Avignon***

08.00 Transport en bus de Montpellier à Avignon
Bus transport from Montpellier to Avignon

Visite de parcelles d'essais virologiques et génétiques à l'INRA d'Avignon.
Visit of virological and genetical trials at the INRA in Avignon

Visite d'une cave à LISTEL */Visit of a wine cellar in Listel*

JOUR 4 : JEUDI 7 SEPT. **Résistance induite**
DAY 4 : THURSDAY SEPT. 7 ***Induced resistance***

Le matin du jour 4 porte sur l'acquisition de résistances; les résultats seront tous exposés sous forme de posters et discutés en assemblée plénière. L'après-midi sera dévolue aux stratégies de sélection qui prennent en compte les résistances aux virus ou aux vecteurs acquises de façon classique ou par biologie moléculaire. Cette session sera présentée par des communications orales de personnes invitées.

The morning sessions of day 4 are devoted to posters reporting induced resistance. The afternoon session concerns breeding strategies, taking in account virus and vector induced resistance, would it be by conventionnal or molecular approaches. This session will be presented by invited speakers.

09.00 **Session 6 : Protection croisée**
Session 6 : Cross protection

Président / *Chairman* : **J. DUNEZ** **(FRANCE)**

09.30 **Session 7 : Plantes transgéniques**
Session 7 : Transgenic plants

Président / *Chairman* : **C. FAUQUET** **(FRANCE)**

10.00 Visite des posters / *Poster visit*

11.00 Pause café / *Coffee break*

12.00 Discussion des sessions 6 et 7 / *Discussion of sessions 6 and 7*

13.00 Déjeuner / *Lunch*

14.30 **Session 8 : Stratégies de sélection**
Session 8 : Breeding strategies

Président / *Chairman* : **A.J. PUTTER** **(FAO)**

14.40	Sélection / *Breeding*	M. PITRAT	(FRANCE)
15.10	Variabilité virale / *Viral variability*	J.-B. QUIOT	(FRANCE)
15.40	Utilisation de gènes / *Gene deployment*	R.S. FRASER	(UK)
16.10	Pause thé / *Tea break*		
16.40	Lutte intégrée / *Integrated control*	Y. ROBERT	(FRANCE)
17.10	Plantes transgéniques / *Transgenic plants*	X. DELANNAY	(USA)
17.40	Conclusions / *Conclusions*	J.M. THRESH	(UK)
18.10	Fin du jour 4 / *End of day 4*		

21.00 Banquet au Château de Grammont / *Banquet in the Castel of Grammont*

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PROGRAMME

JOUR 5 : VENDREDI 8 SEPT. **Epidémiologie générale**
DAY 5 : FRIDAY SEPT. 8 ***General epidemiology***

Une journée "Epidémiologie générale" permettra aux participants qui n'émargent pas au thème du colloque de pouvoir s'exprimer. Les modalités fixées sont identiques à celles des jours 2 et 4.

This session "General Epidemiology" will provide an opportunity to present epidemiological results not related to the main theme of the meeting. The format of presentation will be the same as days 2 and 4.

09.00 **Session 9 : Virus transmis par aphides**
Session 9 : Aphid transmitted viruses

Virus non-persistants
Non-persistent viruses
Présidents / *Chairmen* : B. RACCAH (ISRAEL)
M. IRWIN (USA)
Virus persistants
Persistent viruses
Président / *Chairman* : R.T. PLUMB (UK)

10.00 Visite des posters / *Poster visit*

11.00 Pause café / *Coffee break*

12.00 Discussion de la session 9 / *Discussion of session 9*

13.00 Déjeuner / *Lunch*

14.30 **Session 10 : Virus transmis par d'autres vecteurs**
Session 10 : Viruses transmitted by other vectors

Aleurodes
Whiteflies
Président / *Chairman* : D. FARGETTE (FRANCE)
Autres vecteurs
Other vectors
Président / *Chairman* : M. CONTI (ITALY)

15.30 Visite des posters / *Poster visit*

16.00 Pause thé / *Tea break*

17.00 Discussion de la session 10 / *Discussion of session 10*

18.00 Conclusions générales de la conférence / *General Conclusions of the Workshop*
Activités futures organisées par l'ISPP / *Future activities organized by ISPP*
Comité d'Epidémiologie Virale / *Plant Virus Epidemiology Committee*
J.M. THRESH (UK)

18.30 Fin du jour 5 / *End of day 5*

SAMEDI 9 SEPT.
SATURDAY SEPT. 9

08.00 Visite de la quarantaine canne à sucre et
du laboratoire de culture *in vitro* de banane du CIRAD
Visit of the sugarcane quarantine service and
of the banana tissue culture laboratory in the CIRAD centre

10.00 Visite des laboratoires et des parcelles expérimentales de l' ENSA - INRA Montpellier
Visit of the laboratories and field trials of ENSA - INRA Montpellier

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Fin du IVème CIEVP / End of IVth IPVEW

CIRAD CNRS ENSA-M INRA ORSTOM

These expanded abstracts in this Proceedings were requested by the Program Committee so that more information would be available to participants which might stimulate discussion. The authors have generously shared unpublished information. **Please obtain permission from authors prior to citing any of these abstracts.**

The Program Committee hopes that the format will lead to new ideas. The invited speakers and discussion leaders were asked to be provocative by not only giving their views on major accomplishments but also giving voids in knowledge and roadblocks to achieving these objectives. Offered papers were grouped into topics so that discussion around a general topic could include material in posters.

O P E N I N G A D R E S S

OPENING ADRESS

C. VAGO, Academy of Sciences of France

I wish to welcome you most warmly to this conference. The fact that you are so numerous and diverse shows the strong international impact of the problems raised by viral plant diseases at the end of this century and consequently the great current value of research in this field. For some time now this interest has taken the form of various encounters dealing with epidemiologic aspects of plant viroses in different countries and particularly as a series of international conferences, specially devoted to this precise field under the care of the International Society of Plant Pathology. The present meeting constitutes the fourth of these conferences. In this context I should like to pay tribute to the organizers of the preceeding meetings which were held respectively in England, Australia and the United States.

The first task of a series of conferences of a discipline occuring at regular intervals is to give account of the development of the field concerned. The series should reflect the international situation of each moment concerned as regards attainments, main principles, new techniques and prospects. Previous meetings have contributed to this task by dealing concurrently with the major contemporary problems of viral epidemiology and their approach with new means of epidemiological analysis of populations and the study of virus-plant relations in biochemistry and cellular biology.

Todays' conference will play a part which will prove both exciting and difficult. Indeed, during the last few years preceeding this meeting several great, economically important problems of the extension of viral diseases of crops have been defined ; regulation mechanisms - notably of resistance and vectoring - have been envisaged from different angles ; and the efficiency of the epidemiological approach to problems using the principles and methodology of molecular biolology and genetics was confirmed on a large scale.

. . .

Thus, taking into account such contributions, the overall picture of the present state of our discipline exhibits several characteristic orientations which may motivate those of this meeting.

One invariably important aspect is the evidencing of viral infections and epiphyties and investigation of the circulation of the viroses involved. Indeed, these date define on the one hand the economic and social acuteness of the problems raised and, on the other hand the elements on which all the other studies are based. Numerous observations on these subjects, in different countries, will be presented in the course of the present colloquium : You will note an increasing tendency for analysis by computer systems and radioactive virus labeling.

These remarks are also valid for the field of vectors and more especially for the two aspects which appear to predominate at present, namely the dynamics of the vectorial populations and the analysis of the mechanisms of virus adaptation to the vectors, notably with regard to the different variations of the state of persistence. In this latter field I should like to emphasize the increasingly frequent use - above all recently - of molecular biology analysis, notably as regards the mechanism of specific insertion of the viruses in the vector.

Finally, I should like to insist on recent and particularly important changes which have been noted in the field of plant resistance to viral infections. This sector, whose growing significance was not overlooked in previous meetings, has recently become even more important due to perspectives which are constantly being confirmed for several reasons. On the one hand resistance is increasingly recognized as a basic factor of the dynamics of viral epiphytics in the natural or cultivated environment and at the same time the understanding of its mechanisms has greatly progressed, due to the even more generalized intervention of molecular biology in this sector. Finally, as direct consequences of this fact, biotechnological possibilities for antiviral crop protection can be envisaged through molecular genetics. In this context I must admit that I am also glad to preside this conference, as it is particularly in line with what I forecast right at the beginning of molecular biology regarding the considerable potentialities of this latter in ecopathology : at that time this did not seem likely as biological and methodological approaches of the two fields appeared as essentially opposed.

The organizers of the conference have paid attention to the signs of development and the prospects of the different orientations mentioned above. Consequently they have designed a program to show the meanings of the latter in plant and comparative pathology while reserving parts in each of the topics for their consequences in protection of crops and environment.

However the great changes, which I have mentioned, in the field of antiviral resistance of plants, and the prospects which follow from this, have meant that this topic has been granted a special position in this conference. I should be pleased if this occasion might lead to a discussion to define the most suitable directions for this field and initiate internationally co-ordinated collaboration and actions.

Finally, the present meeting also seems to illustrate the fact that research organizations are increasingly interested by our discipline. Indeed, it has been organized under the care of the International Society of Plant pathology and the French Society of Phytopathology and also by joint decision and in cooperation with important Organizations with vocations as different as National institute of agronomic research (INRA), National Center of scientific research (CNRS), National higher school of agriculture (ENSAM), Research institute for development in cooperation (ORSTOM), International cooperation center in agricultural development and research (CIRAD) and the AGROPOLIS-complex for research and higher education in agriculture.

I wish to voice the feelings of us all by expressing my warmest thanks to the Consultative Committee and its coordinator, to the Organizing Committee, and above all to the person foremost in conceiving and realizing this Colloquium, Dr. Fauquet. We also appreciate the place reserved for this meeting by the City and the District of Montpellier, the Regional Council of Languedoc-Roussillon, and the Herault County Council within the framework of their scientific activities.

In opening this conference I should above all like to wish you a rewarding work. However at the same time I hope that your stay in Montpellier and your visits to laboratories beyond the Rhone will leave happy memories of the summer-end atmosphere of the Languedocian and Provençal coast of the Mediterranean sea.

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CONCEPTS ET APPROCHES DE LA RESISTANCE/

RESISTANCE CONCEPTS AND APPROACHES

SESSION 1

CONCEPTS ET APPROCHES CLASSIQUES DE LA RESISTANCE / CLASSICAL CONCEPTS AND APPROACHES OF RESISTANCE

THE DEVELOPMENT OF NEW CROP VARIETIES IN RELATION TO VIRUS DISEASES AND THEIR EPIDEMIOLOGY

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Virologists consider virus diseases to be very important. From them they derive their livelihood. Sometimes they are important, often they are not very. But, indeed, viruses and virus diseases are fascinating, and challenging to study. Virologists take as a given the reality of existing varieties and existing fields and they study the interaction of vectors, virus sources, environmental factors, crop variety and time , and call it epidemiology. This is only natural. The one factor of the system that is really manipulatable is the crop variety. It is the crop variety whose resistance levels, to vectors and viruses, determines the result of any study of epidemiology.

As this workshop begins, I wish to emphasize the intimate relationship between crop varietal resistance and the actual epidemiological reality of viral diseases in farmers' fields.

I wish to emphasize that the person who actually controls the importance and the epidemiology of viral diseases (or any other diseases) is the plant breeder who actually breeds and selects the individual plant or plants which are to become the new variety. In the case of hybrid crop varieties, two parental lines are selected, among many thousands, to create millions of identical progeny.

New varieties are being developed all the time. The world-wide activity in crop breeding in public institutions and private companies is enormous. The development of new varieties is both complex and diverse. So, generalities have many exceptions, but the key question to explore here is "How much effort is made in a given breeding program to involve a consideration for virus resistance, as thousands of crosses are made and hundreds of thousands or millions of plants are judged in segregating generations for their suitability as a new variety. Are the viruses that the crop is subjected to considered at all? One of them? Two? Five?

The plant breeder is seeking high yield and quality as primary goals, as measured by observations and data from very small plots. He may protect those plots with pesticides to enable better assessment of "genetic yield potential". If some plant pathologist has convinced him a pathogen is important he may inoculate progenies with the pathogen (fairly easy for mechanically-transmitted viruses - much more difficult for those not). But thousands or even millions of plants must be inoculated and evaluated. This takes effort, money, time, and careful supervision. One cannot do it for many pathogens.

A breeder must balance limited resources applied to many objectives simultaneously. The more characters, the less will be the genetic gain for any single one. If the breeder believes that viruses are not that important generally on his crop, or that viruses can be controlled sufficiently with good crop husbandry, he may not wish to add virus resistance as a breeding objective. Thus, his new varieties will be virus susceptible, and vulnerable to the extent that any given field offers opportunity for viral invasion and spread.

One constantly sees new papers on virus resistance. Virologists and breeders often write papers on resistance, genetics of resistance, etc. But detecting resistance in a wild accession, in a landrace, or even in a variety is one thing; being able to actually utilize this resistance within a program targeted at developing a new variety, is quite another. Then, having that variety actually grown, whereby it replaces a susceptible variety and actually operates to reduce a potential epidemic or the damaging effects of an epidemic in farmers' fields, is yet quite another. Each of the three activities is quite distinct and different people and even different organizations may be involved.

Promoters, salesmen and institutions are constantly pushing for new varieties to be grown in areas distant from where they were bred and tested originally. These areas, in different regions or on different continents may have very different agroecosystems or different pathosystems, resulting in unsuspected viral vulnerability, or even in susceptibility to unexpected viruses. It is the new variety itself, with its differing genotype, which offers new opportunities for the local viruses and vectors, which then come to our notice because of devastating and unexpected effects. Their epidemiology may be studied - but their very existence, for us, is due to development and deployment of a variety with some definite and new level of susceptibility.

Cassava mosaic in Africa is a good case in point. The virus is African; the cassava host, American. No one knew cassava was vulnerable to this African virus (whose original host is still not known), but through natural selection and breeding in Africa, cassava varieties with "enough" resistance now exist. They still get cassava mosaic and studies of disease epidemiology have been carried out showing that spread occurs at certain rates in certain places. But when much new diverse germplasm from America is introduced, it is so susceptible, and the epidemics are so severe, that no one has a chance to show the epidemic potential of cassava mosaic on these new varieties because no one would even consider growing such susceptible material.

Just changing planting dates in a single location may change viral susceptibility from an irrelevant, unimportant factor to one of major importance, and result in devastating epidemics. Maize streak virus in Africa illustrates this, with late-planted maize suffering major yield losses compared with early plantings, in complex interactions of incidence, phenology and severity. Even more remarkable are chickpeas in California, where their susceptibility to many viruses was not even known until planting dates were shifted from April to December resulting in viral epidemics and complete yield loss for some varieties (Bosque-Perez & Buddenhagen, this conference). Other chickpea varieties, with different levels of resistance suffered less yield loss due to differences in epidemic progression on them.

The crops which will remain fruitful for virologists for a long time are the fruit and vegetable crops where high product quality and price and, additionally for some, their long cycle and difficult breeding, inhibit an emphasis on resistance breeding. Pesticides are used instead of resistance (Buddenhagen, 1983a). Where virus resistance has been sought and used there is sufficient virologist input so that the resistance obtained is usually strain-specific and it doesn't work for other strains.

What does breeding for virus resistance, as one of many breeding objectives, actually entail? What does it have to do directly with virus disease epidemiology?

First, it is important to realize that nearly all such programs are concerned with an individual plant reaction to a virus, not to a population response. Plants are selected for resistance, following a viral challenge, based on 1) absence of symptoms (i.e. immunity or escape) or on mildness of symptoms (i.e. tolerance). Tolerance for systemic diseases I define as less symptoms when infected, than for other plants similarly challenged and also infected. (Buddenhagen, 1983b).

If the selection is accurately done on the new variety at first,for immunity, then the key point is, how strain-specific is it? If it is not strain-specific then there will be no disease, no epidemic and no study of epidemiology. How strain specific it will be depends upon the lack or presence of diverse strains in the virus challenge the breeder or virologist uses to detect immunity. It also depends on the diversity and flexibility of strains in nature which would invade the deployed crop with its "resistance". If this diversity is great, and much greater than the original challenge viruses, then deployment of that resistance will soon reveal viral susceptibility and an epidemic will ensue, revealing a certain complex relationship among vector/viral strain/new host genotype and environment. The result is unpredictable and offers scope for epidemiological studies again, after-the-fact.

If, on the other hand, the breeder selects for tolerance, he expects his variety to suffer less from the disease. But this is not due to expectation of differing disease epidemiological potential but only to a less serious yield reduction of each and every individual plant in the field should they become infected. Therefore, by selecting for tolerance, one is not making any assumptions about changed epidemiology. Again, this can be studied after-the-fact and one can determine if that type of resistance, for each variety and virus, confers a changed epidemiology.

If the disease is a dead-end-disease in the crop, and all spread is from outside invaders, one would expect little change in field epidemiology. If, however, spread is largely within-field, then expectations could be that the tolerance itself might affect vector acquisition and transmission due to indirect effects from changed rates of viral replication and systemicity.

Where breeding was successfully done for tolerance to maize streak virus, the result was also a marked reduction in epidemic disease potential - i.e., virus incidence was much reduced. I have termed this characteristic of a variety, "tolremicity", and recognize it as a key component of resistance to systemic pathogens. I now suspect that obtaining and deploying tolerance will generally result for viruses in a changed epidemiology - i.e., a reduction in disease incidence populationwise, with time, as compared with varieties not having such tolerance. This is a fruitful area for research with other viral pathosystems.

What about the stability and durability of viral tolerance? This, again, should depend on the diversity of strains in the original and continued challenge, where intolerance is identified. In the case of MSV, the tolerance (and tolremicity) works across Africa, in an area four times the size of Europe. The original challenge was purposely very diverse, from many grasses and insect individuals (Soto et al., 1982). Without realizing it at the time, it included a completely different virus (maize mottle/chlorotic stunt) for which tolerance was automatically selected, also.

On durability; if selection is based on individual plant tolerance and the highest levels of tolerance are continually sought, one is automatically selecting for minor host genes which affect the host/viral interaction favourably for the host. I believe this will preclude strain selection, since many host genes are involved in the plant response, probably operating on many different intra-and inter-cellular viral/host interactions. Time will tell. Again, a fruitful area for research. And a good model system.

The final type of resistance which one could seek is that of "tolremicity". If neither tolerance nor immunity can be found, the case of last resort is to try to identify plants which, enmass, will act tolremically. This is not easy due to the mechanics of the plant breeding operation itself. In reality, however, it is a direct attack on epidemiological potential of the disease. Here we may be involved primarily with vector host relations. (We will hear about vector resistance later in this session).

Where epidemiological studies (or even simple observations) reveal that different varieties growing in the same place at the same time get less/more disease, in terms of incidence only (ignoring severity) one is detecting different levels of tolremicity. The question then is, was this accidental in terms of varietal development or did the process in some way, undetected and undirected, result in selecting for tolremicity? I think, in some cases, yes, by selecting for yield alone. If viruses were sufficiently severe and abundant in breeders plots, part of the better yield could reflect tolremicity. But since varieties may differ greatly in tolremicity, this must mean that usually it is not operating in varietal selection, even inadvertently. It is an accident.

I know of two cases where selection for tolremicity was actually sought in the breeding and selection of new varieties, and both appear successful. One was on tomatoes for resistance to curly top virus in Washington State, and one on chickpeas, in California for resistance to many different viruses (Bosque-Perez and Buddenhagen, this conference). The method in each case was to select on a family basis in F_3 and subsequent generations under high natural virus incidence on an index intercalating incidence with yield and and final product quality.

In conclusion, there is much to do to reduce virus effects in farmers fields by understanding the complexities of viral sources, viral/host evolution if any, vector sources, vector/host evolution and biology, crop breeding and the strategies and methods used to develop the new varieties which are deployed on a massive scale and which provide new opportunities for complex pathosystems to grow or to diminish, and to evolve.

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METHODS FOR THE DETECTION OF RESISTANCE TO VIRUSES IN PLANTS AND FOR THEIR USE IN BREEDING PROGRAMS.

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The reasons for breeding for resistance to viruses in cultivated plants will be briefly introduced: practical, economical and ecological. Then different aspects related to the identification and the use of resistance will be discussed.

Where to look for resistance to viruses?
It is necessary to have access to germplasm collections which represent the natural variability of the plant under study, or of related species. These "gene banks" may be institutional, or may result from individual botanical prospectings. They may also now, include plants transformed through genetic engineering.

How to reveal resistance characters?
They are different alternatives to challenge plant genotypes to viruses: in the field under natural epidemic conditions, or in the laboratory in a controlled environment. In this later case the choice of the virus strain used for screening is critical (one or several, standard or atypical strains...), as well as the inoculation procedure and the incubation conditions.

The types of resistance are very diverse. Resistance characters may interfere with the virus at different levels of the virus biology: inoculation, multiplication, migration within the plant, acquisition by a vector....Some of these mechanisms confer a complete resistance while others will provide only partial resistances or lower susceptibility.

How to use resistances?
To be used in a breeding program, it is necessary to define for every resistance mechanism, simple, reproducible (sometimes indirect) tests which enable the plant breeder to differentiate easily (1) susceptible from

resistant plants (2) plants with different types of resistance. This is particularly important when cumulating different resistance mechanisms to a single virus within the same cultivar.

The evolution of the virological techniques bring new possibilities: this point will be discussed by comparing the techniques used in breeding for resistance in papers published in 1978 and 10 years later: are the technical innovations of plant virology available to plant breeders?

The conclusion will bring the question of how to evaluate the field efficiency of partial resistances, in order to judge their interest for a breeding program. The potential of modelling for predicting the effectiveness of partial resistances will be introduced.

Host Plant Resistance to Vector Transmission of Plant Viruses

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Host plant resistance is the most effective, and in many cases the only, strategy for the control of plant virus diseases. A dependence of most plant viruses upon specific vectors for interplant dispersal increases the diversity of resistance mechanisms available to control virus diseases. Resistance mechanisms are not limited to those affecting virus-host interactions, and may include those that disrupt vector-host or vector-virus interactions. The overall strategy for exploiting these latter two types of resistance mechanisms is not to reduce the direct effects of the virus or its vector on the host plant, but to reduce the efficiency by which the virus is transmitted among potential host plants. The resistance becomes, in effect, a "resistance to virus transmission". The purpose of this type of resistance is to delay or slow the epidemic and, therefore, reduce the overall effect of the disease on crop growth and yield.

Host plant resistance that affects virus transmission is difficult to recognize; it is often first identified as a reduction of virus incidence in the field. There are numerous reports of host resistance affecting transmission and the resulting epidemiological benefits; however, in many instances, the benefits appear to be transient. The usefulness and success of this resistance strategy depends on our knowledge of the mechanism(s) of resistance and its affects on the virus-vector-host interactions. In recent years numerous mechanisms of host resistance that reduce virus transmission have been described and shown to affect many different virus-vector-host interactions. A knowledge of resistance mechanisms and their epidemiological consequences will allow optimal deployment of existing resistance mechanisms and the development of new strategies to disrupt virus-vector-host interactions.

Virus-Host Interactions. These interactions are covered in depth in other sessions of this conference. However, host resistance that alters, but does not prevent, infection often affects transmission efficiency and is worth a brief mention here.

Incomplete resistance to a virus that results in a lower virus titer or an altered virus distribution in the plant can reduce the acquisition efficiency and thus the number of viruliferous vectors moving within or emigrating from a crop. These types of resistance can reduce virus incidence if secondary spread is the main contributor to epidemic development within the resistant crop. Alternatively, virus incidence in other crops may be reduced if the resistant crop is the major source of virus and vectors.

Virus-host interactions may affect inoculation efficiency if infection is inoculum dose dependent. Several plant genotypes reported to be resistant to vector inoculation are also difficult to mechanically inoculate. The resistance to mechanical infection is often overcome by increasing the concentration of virus in the inoculum; however, the resistance may be effective in the field. The relatively low doses of virus inoculum from natural vectors may not be enough to overcome the resistance.

Virus-host interactions may also alter vector behavior by altering the physiology and/or morphology of the plant. Several viruses affect the color of the infected host, therefore altering the attractiveness of the plant by the vector. In many instances the color change serves to attract vectors (e.g. yellows-type viruses and aphid vectors). An understanding of virus symptom expression may allow for the development of symptom types that instead of attracting, repels virus vectors from alighting and feeding on infected plants (e.g. silvery leaves in cucurbits).

Vector-Host Interactions. Virus transmission efficiency is partially dependent upon the ability of the vector to feed on (and to some extent colonize) the plant and does not directly involve the virus. Host resistance to the vector has been associated with reduced virus incidence with two broad categories described: (1) Antixenosis, mechanisms of resistance that adversely affect vector behavior, (2) Antibiosis, mechanisms of resistance that are directed at a reduction in vector populations, i.e. an inability or reduced ability of the vector to survive and reproduce on a host plant. Antibiosis and antixenosis are often expressed concurrently in a single plant and their effects on vectors or virus transmission may not be mutually exclusive.

Antixenosis types of resistance usually interfere with the ability of vectors to recognize the plant as a host and initiate feeding. Plant chemicals (e.g. root and trichome exudates) or physical barriers (e.g. pubescence, specialized trichomes, epicuticular wax) may act to repel vectors or prevent initial feeding attempts. The degree of resistance to virus transmission is dependent upon the time the vector is able to probe or feed upon the plant, and the acquisition or inoculation times required for successful transmission. Viruses acquired or inoculated during brief probes may be transmitted, but a reduction in the number of successful probes decreases the probability of transmission. Complete control of transmission of viruses requiring lengthy acquisition and inoculation times is possible if the vector is prohibited from establishing a feeding relationship on the host. If these resistance mechanisms are not successful at preventing transmission, they may be useful in reducing the longevity and reproductive ability of the vector (antibiosis).

Antibiosis resistance mechanisms affect the ability of the vector to survive and reproduce once it has successfully identified a plant as a host. The mechanisms are diverse and not well understood for most vector-host combinations. Resistance mechanisms of host plants to aphid vectors are the best studied and most appear to be associated with either reduced ingestion of plant material or an inability of the vector to locate preferred feeding sites. In addition, the role of toxic secondary plant products has also been suggested as a possible aphid resistance mechanism.

Pesticides may also be considered a form of antibiosis in that they reduce vector populations. Although the use of pesticide applications for controlling virus epidemics has not been universally effective, there are success stories. Major drawbacks are required frequency of applications, inconsistency of coverage and concentration of active compound needed. The effectiveness of toxic compounds, both natural and synthetic, is dependent upon the presence of the compounds in target tissues at high concentrations. Natural pesticidal activity that would be effective against vectors is rarely identified, but these types of activities might be genetically engineered into plants. Tissue specific expression of foreign genes

whose products have pesticidal activity could provide superior vector population control and overcome the economic and environmental drawbacks of applied compounds.

Virus - Vector Interactions. Historically, disruption of virus-vector interactions has not been considered as a potential control strategy. This situation is understandable due to our lack of knowledge of the mechanisms of interactions of viruses with their vectors. It is known that taxonomic and mechanistic specificity exist between virus and vector. A single taxonomic group of viruses is transmitted specifically, with few exceptions, by a single taxonomic group of vectors with the mechanism of transmission remaining consistent within the virus group (i.e. non-circulative, circulative nonpropagative or circulative propagative). Resistance mechanisms that target virus-vector interactions may be effective against a taxonomic group of viruses or vectors and not specific for one virus-vector-host combination.

Recent advances in virus and vector biology have identified that virus-encoded proteins (structural and nonstructural) regulate transmission not only at the vector taxa level but they may also determine vector specificity within that taxa. Also, substantial evidence exists that viral proteins interact with specific attachment sites or receptors on or within the vector. An understanding of these specific transmission mechanisms should lead to the development of methods to disrupt or inhibit virus-vector interactions. While it is impractical to attempt to modify the virus or vector populations, it may be possible to modify the host plant to disrupt virus-vector interactions.

As our understanding of virus-vector-host interactions increases so does our development of novel ways to interfere with these processes. The use of incomplete host plant resistance mechanisms to reduce or control the incidence of plant virus diseases is gaining acceptance and is successfully being applied in many crops. However, there are still many problems to overcome, most notably in the detection of and exploitation of resistance mechanisms. Many resistance mechanisms are discovered serendipitously during a search for other agronomic traits. Small scale screening methods may not detect subtle forms of resistance or they may not be recognized by plant breeders who do not have the resources to test for them. The techniques are available to exploit these types of resistance mechanisms, but the degree of effectiveness will be correlated with the degree of interdisciplinary effort given to understand the complex virus-vector-host relationships that drive virus disease epidemics.

SESSION 2

CONCEPTS ET APPROCHES MODERNES DE LA RESISTANCE/

MODERN CONCEPTS AND APPROACHES OF RESISTANCE

PROSPECTS FOR SATELLITE-MEDIATED TRANSGENIC VIRUS RESISTANCE

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The development of methods of inserting defined nucleotide sequences in the nuclear genome of plants has opened the door to a range of new approaches to making plants resistant to viruses. One approach would involve the introduction of virus resistance genes from other plant species, although as yet no such genes have been identified at the molecular level and cloned. However, several other approaches, which make use of virus-related nucleotide sequences, already show various degrees of promise. One such method uses satellite nucleic acid, which is a molecule with a characteristic nucleotide sequence, and although not an essential part of the viral genome, or a sub-genomic fragment, is replicated only in virus-infected cells. Such satellite RNA is found in some isolates of viruses in a range of taxonomic groups and is of two main types: larger molecules of 1 to 3 kb which encode at least one polypeptide that is produced in plants, and smaller molecules, mostly of less than 500 nucleotides, and which typically do not encode any recognised polypeptide (Murant & Mayo, 1982). Satellites of this second type occur in some cultures of cucumber mosaic cucumovirus (CMV) and tobacco ringspot nepovirus (TRSV), and their presence can either attenuate (benign satellites) or intensify (virulent satellites) disease symptoms (Schneider, 1971; Mossop & Francki, 1979; Waterworth et al., 1979).

PROPERTIES OF SATELLITE-TRANSFORMED PLANTS

The rationale for the production of satellite-mediated transgenic virus resistance involves the transformation of plants with a DNA copy of a benign satellite RNA. When this was done with a suitable 335-nucleotide satellite RNA of CMV, using the Agrobacterium Ti plasmid system, the regenerated transformed tobacco plants looked completely normal. However, they contained small amounts of RNA transcripts containing the satellite sequence and, when the plants were infected with a satellite-free culture of CMV, large amounts of unit-length satellite RNA were produced (Baulcombe et al., 1986). Moreover some of the satellite RNA became packaged in CMV-like particles and was then transmitted together with the virus when sap from infected transgenic plants was used to inoculate non-transformed plants. In addition, CMV replication in systemically infected leaves of the transformed plants was strongly repressed, few symptoms developed and the plants grew nearly as well as virus-free ones (Harrison et al., 1987). The plants were also protected from damage by the closely related tomato aspermy virus but not from the effects of other viruses. The transformed plants were not resistant to infection by aphids carrying satellite-free CMV but once infected they were relatively poor sources of virus for vector aphids, and

the few plants to which CMV was transmitted by the aphids developed only mild symptoms because the satellite RNA was transmitted too (Harrison et al., 1988). Thus it is expected that crops of the transgenic plants would develop few symptoms when infected and that CMV would spread from plant to plant in such crops to a much smaller extent than in control crops.

Comparable work with the TRSV satellite RNA has given similar results to those described above except that the infection apparently did not spread systemically in all the transgenic plants inoculated with satellite-free TRSV (Gerlach et al., 1987). Another difference is that whereas multimeric RNA transcripts were produced in non-inoculated plants transformed with a multimeric DNA copy of CMV satellite RNA (Baulcombe et al., 1986), unit-length satellite RNA was produced in comparable plants transformed with a multimeric DNA copy of TRSV satellite RNA (Gerlach et al., 1987). The conversion of TRSV satellite RNA to unit-length molecules results from the autocatalytic processing of multimeric molecules that can be observed in experiments in vitro (Prody et al., 1986).

COMPARISON WITH OTHER SYSTEMS

Transgenic plants containing a variety of other kinds of DNA intended to confer virus resistance have also been produced in work in several countries. Among these approaches, that using the coat protein (CP) gene of the virus to which resistance is required (Powell Abel et al., 1986) shows the most promise. CP-mediated transgenic resistance differs from satellite-mediated resistance in several ways. It is largely a resistance to infection that can be overcome by more potent inocula which then induce normal disease symptoms, its strength depends on the level of gene expression, and it is largely ineffective against RNA inocula (Nelson et al., 1987). In contrast, satellite-mediated transgenic resistance is unaffected by inoculum potency, is insensitive to the level of gene expression, is effective against RNA inocula, restricts virus replication and confers tolerance of infection. However, whereas relatively few satellite RNA systems are available for use, CP-mediated resistance seems to be widely applicable.

In comparison with these two approaches, transformation with antisense RNA has proved relatively ineffective (Cuozzo et al., 1988) and further progress would seem to depend on introducing a virus-induced amplification step to counteract the tendency of unsequestered viral nucleic acid to replicate and swamp the antisense molecules. The use of ribozymes (Haseloff & Gerlach, 1988) is another interesting possibility that may be of use in plants if a way can be devised for it to operate effectively at physiologically relevant temperatures.

PROSPECTS FOR FIELD USE OF SATELLITE-MEDIATED TRANSGENIC RESISTANCE

In China large-scale experiments over several years involving the mechanical inoculation of tomato and pepper seedlings in seed-beds with CMV cultures containing a benign satellite RNA have shown that this practice gives consistent yield increases in regions where epidemics of satellite-free CMV are common (Tien et al., 1987). These results encourage the hope that transgenic satellite-mediated resistance will be even more effective. In the Chinese work, no tolerance-breaking CMV strain has been reported nor any evidence found that the benign satellite RNA used will mutate to a damaging form or will damage

crops of other kinds. Similarly, in our tests in the glasshouse with different satellite-free strains of CMV, the virus symptoms in satellite-transformed plants have been attenuated in all instances. However, despite these promising results, experiments to test rigorously the conjectural environmental hazards of growing satellite-transformed plants are essential, especially because benign and damaging forms of satellite RNA can differ in only a few nucleotides (Gordon & Symons, 1983). A further safety precaution therefore would be to modify the satellite so as to make mutation to a damaging form still more unlikely.Perhaps the most sensible use of transgenic virus resistance is to combine it with other kinds of resistance or to complement it with other control measures. In this connection, our first experiments with transgenic plants having a combination of satellite-mediated and CP-mediated resistances to CMV have given very promising results.

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Cross Protection

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In this presentation, cross protection is defined as: "The use of a mild virus isolate to protect plants against economic damge caused by infection with a severe challenge strain(s) of the same virus." This term is also referred to as classical cross protection. This definition places emphasis on the action of the mild strain to limit economic damage and does not imply knowledge of the mechanism of cross protection. Furthermore, classical cross protection is distinguished from the term 'engineered' cross protection which refers to resistance or tolerance of transgenic plants expressing the coat protein gene of a plant virus to infection by isolates of the same virus. In this talk, we will primarily concern ourselves with classical cross protection. The cases of cross protection with tomato mosaic, citrus triteza, papaya ringspot, and zucchini yellow mosaic viruses will be covered. Several points will be emphasized: 1) selection and evaluation of mild strains, 2) cross protection effectiveness of the mild strain in geographic areas where differences in virus strains, disease pressure, and vector efficiency might occur, and 3) the integration of cross protection in practical crop management systems. The above points will be illustrated with viruses infecting long-, medium-, and short-term crops. These are citrus tristeza (long term), papaya ringspot (medium term), and zucchini yellow mosaic (short term).

PLANT TRANSFORMATION FOR COAT PROTEIN MEDIATED PROTECTION AGAINST VIRUS INFECTION

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The technical advances in plant genetic engineering during the last 10 years has made it possible to introduce foreign DNA into a wide variety of plant cells, some of which have been regenerated into plants. We have used these advances to introduce gene sequences derived from plant viruses in order to study the effects of these genes on plant resistance or susceptibility.

In 1986 Powell Abel et al. (*Science* 232, 738-743) described the results of experiments in which transgenic tobacco plants that expressed the coat protein (CP) gene of TMV were substantially more resistant to infection by TMV than were control plants. Resistance is manifested in several ways, including a reduction in the number of sites of infection and a reduced rate of systemic spread of the virus if infection is established (Nelson et al., *Virology* 158, 126-132, 1987). In subsequent experiments it was found that transgenic tomato plants that express the CP gene from TMV were resistant not only to TMV but to some strains of tomato mosaic virus (Nelson et al., *Bio/Technology* 6, 403-409, 1988). This report also described the results of the first field trial of virus resistance.

While there are certain similarities between CP mediated protection (or resistance) to cross protection, there are some features that clearly demonstrate that the two types of protection differ from each other. For example, cross protection is most effective against closely related strains of virus, while TMV CP mediated protection protects tobacco plants against closely related strains as well as other tobamoviruses (Nejidat and Beachy, manuscript in preparation). Like cross protection, CP mediated protection can be largely overcome by high levels of virus inoculum. In my laboratory we are continuing research to better understand the cellular and molecular bases of CP mediated protection, and to extend resistance to other virus groups and plant species. The results of the work in progress will be reported at this meeting.

Since the first report of virus resistance in transgenic plants, a number of different research groups have reported similar results to produce resistance against a variety of different viruses. Thus far resistance has been described against potato virus X (Hemenway et al., *EMBO J.* 7, 1273-1280, 1988) alfalfa mosaic virus (Tumer et al., *EMBO J.* 6, 1181-1188, 1987; Loesch-Fries et al., *EMBO J.* 6, 1845-1851; van Dun et al., *Virology* 159, 299-305, 1987) cucumber mosaic virus (Cuozzo et al., *Bio/Technology* 6, 549-557) tobacco streak virus (van Dun et al., *Virology* 164, 383-389, 1988), tobacco rattle virus (van Dun and Bol, *Virology* 167,649-652, 1988) and tobacco etch virus and potato virus Y (Stark and Beachy, manuscript submitted). As the list of successful uses of transformation for virus resistance grows, the degree of understanding of differences between examples also grows. The implications of the differences in terms of field application will be discussed at this meeting.

The technology used in CP mediated protection, although complicated by the tissue culture aspect of such a project, is conceptually simple. Under the appropriate set of circumstances CP mediated protection could provide new sources of germplasm for plant breeders and plant pathologists where more classical sources of resistance are missing.

BIOCHEMICAL ASPECTS OF THE RESISTANCE OF PLANTS TO VIRUSES

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THE HYPERSENSITIVE REACTION AS THE MODEL SYSTEM OF RESISTANCE

The model system that will be considered here is the hypersensitive response since it is the most efficient mechanism of natural defense against various pathogens. It has two main characteristics : necrosis at and around each point at which the leaf tissue was infected ; and localization of the parasite to the region of each initiated infection. The cells surrounding the necrotic area undergo marked metabolic changes which are believed to cause the resistance observed.

The resistance results from an active defense mechanism that is induced by the invading pathogen itself. This will be illustrated in the case of a virus infection using the example of two almost isogenic lines of *Nicotiana tabacum* micro-inoculated with the U1 strain of tobacco mosaic virus (TMV). The micro-inoculation procedure yielded infection sites at predetermined locations on the leaves of the two host cultivars (Konate and Fritig 1984). At various intervals after the micro-inoculations the individual infection sites were assayed for virus content by ELISA or for incorporation of ^{3}H-uridine into the viral RNA after pulse-labelling by injection around the micro-inoculated areas at the end of the infection period (Konate and Fritig 1983). Analysis of more than 3000 individual infection sites showed that, up to the time (33-36 hours after inoculation) visible necrosis appeared, the rates of virus multiplication did not differ significantly between the two hosts. Hypersensitive resistance to virus infection does not preexist but is induced by the infection itself. Hypersensitive resistance appears at about the same time as the necrotic symptoms and its efficiency increases with time after infection (Konate 1984). Around the necrotic lesions there is a ring of cells about 1.0-1.5 mm in width containing detectable virus but in which virus multiplication is inhibited.

METABOLIC CHANGES DURING THE HYPERSENSITIVE REACTION TO VIRUSES

Even though the triggering of the hypersensitivity reaction results from a very specific gene-for-gene recognition between plant and pathogen, what the plant does thereafter to defend itself appears to be specific only to the host. It will be shown that most of the metabolic changes that have been reported as associated to active defense in incompatible plant-fungi and plant-bacteria interactions have also been found during the hypersensitive reaction of plants to viruses (Fritig et al. 1987). The alterations include cell wall thickening resulting from production and deposition of various macromolecules, and the production of defense enzymes and proteins. Among the cell wall macromolecules are lignins and other phenolic polymers, polysaccharides such as callose, and proteins such as the hydroxyproline rich glycoproteins. Defense enzymes fall into two classes : enzymes that catalyze the production of various metabolites participating in resistance (ethylene, phytoalexins, aromatic compounds, oxidized metabolites) ; and direct defense enzymes (hydrolases such as chitinases and glucanases). The defense proteins include inhibitors of microbial proteases or polygalacturonases and "pathogenesis-related" (PR) proteins, the latter being rather abundant proteins.

INHIBITORS OF VIRUS REPLICATION ASSOCIATED WITH RESISTANCE RESPONSES

As will be discussed below, these major defense-associated proteins do not appear to be specifically anti-viral. However, anti-viral components appear to be produced during the hypersensitive response. Some might be secondary metabolites, for instance aromatic metabolites (Fritig et al. 1987). Some others might be of a proteinaceous nature. It was found by the group of G. Loebenstein (Volcani Center, Bet Dagan, Israel) that protoplasts from tobacco, carrying the NN gene responsible for resistance (local lesions) to tobacco mosaic virus (TMV), release after their inoculation with TMV a compound that inhibits several plant viruses, the effect being dose responsive (Loebenstein and Gera 1981). This substance termed inhibitor of virus replication (IVR) inhibited TMV in protoplasts, TMV, potato virus X (PVX), potato virus Y (PVY), and cucumber mosaic virus (CMV) in leaf disks from different hosts, indicating that IVR is neither host nor virus-specific (Gera and Loebenstein 1983). IVR also inhibited TMV replication in intact leaves when applied by spraying to tobacco and tomato plants and CMV in cucumber. Subsequently, IVR was also obtained from the intercellular spaces of TMV-infected tobacco NN plants and from induced-resistant tissue (Spiegel et al. 1989). The amount of IVR recovered from 10^8 protoplasts or 100 g of leaf tissue was approximately 1 µg ; and biological activity could be detected by applying 10-20 ng of IVR. It will be interesting to know more about the biochemistry of this type of protein(s), its mechanism of action on virus replication, its occurrence in other plant-virus interactions or even in other plant-pathogen interactions.

THE MAJOR DEFENSE-RELATED PROTEINS INDUCED DURING THE HYPERSENSITIVE REACTION

The PR-proteins represent the major changes in protein production during the hypersensitive response to viruses as well as to other pathogens (Van Loon 1985 ; Fritig et al. 1987 ; Lamb et al. 1989). These PR-proteins have characteristic properties : they are selectively extractable at low pH, highly resistant to exogenous and endogenous proteases, easily resolved by electrophoresis on polyacrylamide gels under native conditions.

We have isolated up to now 23 defense-related proteins from tobacco reacting hypersensitively to TMV. These include the ten acidic PR-proteins already detected by L.C. Van Loon (1982) and named PR-1a, 1b, 1c, 2, N, O, P, Q, R and S in order of decreasing mobility on native basic gels. These 10 latter proteins belong to 4 distinct serological groups : the PR-1 group with PR-1a, 1b, 1c whose biological function is not known and which have one basic counterpart ; PR-2, N and O are in fact 1,3-ß-glucanases (Kauffmann et al. 1987) that are serologically related to another acidic glucanase and to a basic glucanase, these latter glucanases exhibiting typical behaviours of PR-proteins ; PR-P and Q are chitinases serologically related to two other chitinases with again a behaviour typical of PR-proteins even though they exhibit basic isoelectric points (Legrand et al. 1987) ; PR-R and S (MW ~24 kD) are of a yet unknown function but appear to have a serologically-related basic counterpart. An activity of proteinase inhibition is induced in TMV-infected leaves in a manner similar to PR-proteins but does not correspond to any known PR-protein or to any of 4 new PR-proteins that migrate in native gels like PR-R and S but have significantly lower MW (13-14.5 kD).

Among the 23 defense-proteins are 10 glycanhydrolases (4 chitinases and 6 1,3-ß-glucanases) with an endo-catalytic activity. Thus, they may play a central role in the release of oligosaccharides from the walls of some plant pathogens or of the plant cells themselves, some of these oligosaccharides being known as elicitors of defense reactions (Darvill and Albersheim 1984 ; Ryan 1988).

EFFECTS OF OLIGOSACCHARIDE SIGNALS ON PLANT-VIRUS INTERACTIONS

In collaboration with P. Albersheim and A. Darvill (CCRC Russel Laboratories, Agricultural Research Center, Athens, Georgia, USA) we tried an approach using oligosaccharidic fractions

known as elicitors of defense reactions in order to tentatively prestimulate typical defense pathways and examine if the pretreatment would result in any interference with a subsequent virus infection. This approach led to very surprising results. A glucan preparation obtained from the mycelial walls of the fungus *Phytophthora megasperma* f.sp. *glycinea* and known as an active elicitor of phytoalexins in soybean (Darvill and Albersheim 1984) was shown to be a very efficient inducer of resistance against viruses in tobacco (Kopp et al. 1989). The glucan preparation protected against mechanically transmitted viral infections on the upper and lower leaf surfaces. Whether the glucan preparation was applied by injection, inoculation, or spraying, it protected the plants if applied before, at the same time as, or not later than 8 hours after virus inoculation. At concentrations ranging from 0.1 to 10 µg/ml, the glucan preparation induced protection ranging from 50 to 100 % against both symptom production (necrotic local lesions, necrotic rings, or systemic mosaic) and virus accumulation in all *Nicotiana*-virus combinations examined. However, no significant protection against some of the same viruses was observed in bean or turnip. The host plants successfully protected included *N. tabacum* (9 different cultivars), *N. sylvestris*, *N. glutinosa*, and *N. clevelandii*. The viruses belonged to several taxonomic groups including tobacco mosaic virus, alfalfa mosaic virus, and tomato black ring virus. The glucan preparation did not act directly on the virus and did not interfere with virus disassembly ; rather, it appeared to induce changes in the host plant that prevented infections from being initiated or recently established infections from enlarging.

Preliminary experiments aiming to find a relationship between the degree of protection and prestimulated defense reactions led also to surprising results (Rouster et al, unpublished results) : the glucan-mediated protection did not depend on induction of the known pathogenesis-related proteins (including glycanhydrolases) or on enzymes of the phenylpropanoid pathway. We believe the induced protection results from a mechanism that has yet to be described, and that does perhaps not involve typical defense mechanisms. Other oligosaccharides from various sources containing 3-, 6-, and 3,6-linked glucosyl residues, or only 3-linked glucosyl residues protected similarly *Nicotianae* from virus infections, though with less efficiency than the *Phytophthora megasperma* glucan preparation. Other oligosaccharides known as elicitors (Ryan 1988) have similar protecting activity against virus infections, but with apparently broader host specificities (Rouster et al, unpublished results). Biochemical studies are under way to tentatively correlate, here too, these effects with typical defense reactions.

CONCLUSION

The hypersensitive reaction is believed to be triggered by the very specific recognition between the pathogen and the host. However, active defense of plants is associated with metabolic alterations that are apparently only host specific and confer a universal type of resistance. It is noteworthy that some of the tobacco PR-proteins, which were first detected in viral infections and were thought to be related to antiviral defense, are chitinases and glucanases and are therefore defense enzymes directed against insects and fungi. It follows that controlling the production of regulatory molecules such as elicitors could perhaps confer resistance to various pathogens. Hypersensitivity to viruses might be a simplified model in which to identify elicitors of host origin. These elicitors could then be used to induce metabolic alterations, and the elicitors that proved most effective in inducing resistance against challenge infection by various pathogens could be selected. The gene(s) of the corresponding hydrolase(s) releasing the most efficient elicitor(s) would be useful for plant genetic engineering. If adequate regulatory sequences were used, the expression of these genes could be targeted to the sites of preferential attack by pathogens.

The finding that various oligosaccharides known as elicitors of defense reactions can protect plants against virus infections is intriguing. More has to be discovered about the generality of these oligosaccharide-induced protections, their host specificities, their efficiency against modes of transmission of virus infection other than mechanical, their relationship with defense or stress reactions, before it can be concluded that oligosaccharides may be of practical use in protecting plants against virus infections.

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Ribozymes and Mutant RNAs.

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Certain naturally occurring RNA molecules possess the property of self-catalysed cleavage. One class of this reaction is shared by a group of small RNAs which replicate in plants, either alone (viroid RNAs) or dependent on a helper virus (satellite RNAs). Using *in vitro* mutagenesis, we have mapped a 52 nucleotide domain required for self-catalysed cleavage of the satellite RNA of tobacco ringspot virus. This has allowed definition of an RNA segment with endoribonuclease activity, and comparison with naturally occurring self-cleavage sites has led to the design of the new RNA enzyme activities.

The model consists of three elements (Fig. 1). First, a region (A) containing conserved sequences adjacent to the site of cleavage is brought in close proximity to a second region (B) of highly conserved sequence and secondary structure. Third, flanking regions (C) of base paired RNA helix stabilize this interaction.

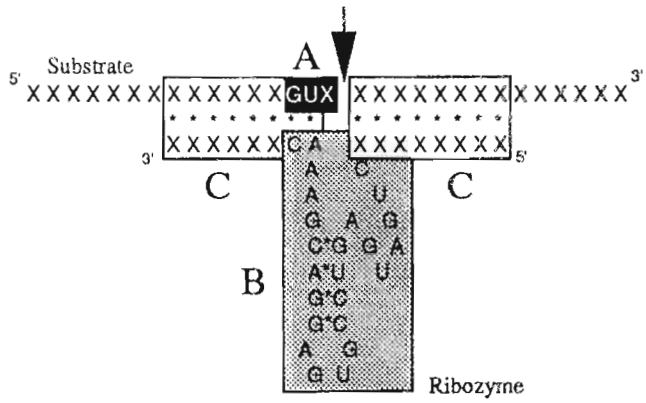

Fig. 1. Model for design of ribozymes.

Using this model, suitable sites could be chosen from within essentially any RNA and used to construct highly sequence specific endoribonuclease activities. Initially, this was tested and proven successful by the design and synthesis of several RNA enzymes targeted against sites within the chloramphenicol acetyl-transferase (CAT) mRNA sequence (Fig. 2).

A major potential application of these highly sequence specific endoribonuclease is the cleavage, and thereby inactivation of gene transcripts *in vivo*. The effective inactivation of RNA transcripts *in vivo* would be similar to inactivation of the corresponding gene. Thus a specific mutant phenotype may be produced in genetically unaltered organisms or cells. Such an antigene activity could provide the basis for a number of

gene and viral therapies or analyses.

Early characterization of RNA enzyme design following the model shown above showed that, at temperatures similar to those expected under physiological conditions, the annealing of RNA enzyme and

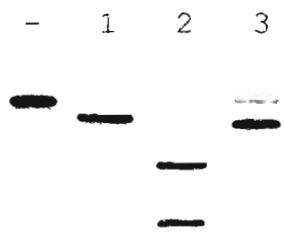

Fig 2. Cleavage of CAT gene transcript. ^{32}P labelled CAT RNA after incubation alone (-) or with one of three ribozymes (1,2 and 3). Band in lane (-) shows full length transcript. Lanes 1-3 show that in each case only two fragments were produced after incubation with a given ribozyme. Their nucleotide sizes were consistent with the predicted sites for cleavage.

substrate was the rate-limiting step in cleavage. However, if the extent of complementarity between the enzyme and substrate (C) was extended, rates of cleavage at lower temperature were markedly increased. To capitalize on this effect, an *in vitro* mutagenesis technique has been used to introduce multiple catalytic domains into CAT anti-sense RNA. This provides for the required extended complementarity between enzyme and substrate sequences as well as a second potential benefit of an increase in rates of cleavage due to the number of catalytic domans. Transient assay experiments using anti-sense/ribozyme constructions in mammalian cos cells and tobacco protoplasts have shown inhibition of CAT activity.

Antisense/ribozyme constructions against tobacco mosaic virus, barley yellow dwarf virus and citrus exocortis viroid have been integrated into the genomes of plant cell suspension cultures or whole plants, and are currently being challenged with the appropriate pathogen.

COMPOSANTES DE LA RESISTANCE/ RESISTANCE COMPONENTS

SESSION 3

SESSION 4

RESISTANCE AUX VIRUS / VIRUS RESISTANCE

DIFFERENCES BETWEEN LILY SPECIES AND CULTIVARS IN THEIR RESISTANCE TO VARIOUS ISOLATES OF TULIP BREAKING VIRUS

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Mechanical inoculation of lilies with various isolates of tulip breaking virus (TBV) did not always lead to a succesful infection, although the cultivar was known to be susceptible. The Asiatic lily cultivar Enchantment became infected to high percentages with TBV isolates from the same and three other Asiatic lily cultivars. In contrast, no or little infection was obtained with either two isolates from tulip or one from an unnamed Oriental lily hybrid. The isolate from the unnamed Oriental hybrid, however, gave rise to a high infection percentage in the Oriental hybrid cv. Journey's End. This cultivar did not become infected after mechanical inoculation with an isolate of tulip and with one from the Asiatic lily cv. Enchantment. Some experiments were repeated by inoculation with aphids resulting in similar infection percentages.

Lilium formosanum became infected by all TBV isolates tested (about 20). These isolates originated from Asiatic lilies, Oriental hybrids, Lilium speciosum cvs., L. tigrinum cvs., L. regale and tulip cultivars. Isolates from, a.o., 'Enchantment' and the unnamed Oriental hybrid gave rise to high infection percentages in tulip cultivars.

These and other experiments led to the following conclusions:

- It is not enough to use one TBV isolate in testing a range of lily species and cultivars for their degree of resistance.
- Growing lily cultivars of the same group close to each other leads to greater risks concerning the spread of TBV than growing lily cultivars from different groups close to each other.

IN SITU STUDY OF CUCUMBER MOSAIC VIRUS MIGRATION IN SUSCEPTIBLE AND RESISTANT PLANTS OF PEPPER USING INDIRECT IMMUNOFLUORESCENCE MICROSCOPY.

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INTRODUCTION

Incidence of cucumber mosaic virus is increasing in many pepper production areas. One of the most efficient factor of resistance in pepper was reported by Pochard (1977) in *C. baccatum* and *C.annuum* varieties: it involves the restriction of the systemic spread of the virus to some parts of the plants whereas the other parts did not display any symptoms. This resistance factor named "resistance to the migration of the virus" has been improved through recurrent selection and it is now introduced into bell pepper varieties (Pochard & Daubèze, 1989). Using these varieties, we have attempted to determine the pathways of virus migration through the plant and the barrier sites impeding systemic infection. This paper presents the histological aspects of the virus dectection in resistant and susceptible plants. Serological detection and effect of environmental factors on resistance are presented elsewhere (Nono Womdim *et al* 1989).

MATERIAL AND METHODS

Four inbred lines of pepper were used : PEN 3-4, *C. baccatum*, genitor of resistance to the CMV ; Milord and Ivan, two *C. annuum* varieties partially resistant ; and the susceptible control, Yolo Wonder.

Inoculation was performed with the CMV strain "Mes" according to the method of Pochard (1977) : plants grown in glasshouse were cut below the 5th developped leaf. Four days later, the upper surface of the 3rd leaf lamina was mechanically inoculated.

At different times after inoculation, epidermal strips from the lower surface of leaves, free hand sections from petioles, from primary and axillary stems, and from roots were removed. These fresh tissues were processed for Azur A staining of viral inclusion bodies (Christie and Edwardson 1986) or for indirect immunofluorescence staining using a polyclonal antiserum anti-CMV prepared by Dr. H.Lot in the laboratory, and protein A - TRITC (Hiebert *et al* 1984, Dufour *et al* 1989).

RESULTS

Virus spread in the inoculated leaf lamina

Two days after inoculation, viral inclusion bodies were detected in the lower epidermis of this leaf stained with Azur A. Immunofluorescence staining also revealed the presence of viral antigens in groups of cells which were dispersed over the surface of the leaf.

From the 2nd to the 7th day, the number of cells with inclusion bodies or displaying a bright fluorescence increased progressively and similarly for every variety. Seven to ten days after inoculation, every strip removed from the lower part of the inoculated leaf were found infected.

Virus spread into the petiole of the inoculated leaf

As soon as two days after inoculation in the susceptible variety Yolo Wonder, sections of the petiole of the inoculated leaf stained for immunofluorescence microscopy showed a bright fluorescence indicating that vascular, cortical and epidermal tissues were already infected.

In the resistant varieties, the spread of the virus to the petiole was delayed and occured only 5 days after inoculation in Milord and Pen 3-4. In Ivan, only half of the plants had infected petiole at this time. Moreover the virus was firstly restricted to the phloem bundle of the petiole and spread only later (7-10 days) to the cortical cells around the phloem vessels.

Virus spread to the other organs of the plant

In the susceptible variety Yolo Wonder, the virus spread from the petiole into the primary stem 3 days after inoculation. Sections of stem removed below and above the inoculated leaf where simultaneously infected. In these sections, the virus infected all the tissues : cortical layers, phloem bundles and young xylem vessels, medullary cells. This infection reached the primary root and the axillary stems only 4 days after inoculation.

In the resistant varieties Milord and Pen 3-4, only the half of the plants observed with immunofluorescence microscopy displayed stem infections 10 days after inoculation. These stem infections were localised in one phloem bundle of the stem, on the side of the inoculated leaf. This phloem bundle was generally infected all along the stem, from the inoculated leaf down to the primary root. Such localised infections where generally associated with a systemic infection of the 3rd axillary shoot.

In the other plants of Milord and Pen 3-4, and in all the plants of Ivan, we did not detect any infection of the stem, roots or axillary shoots until 36 days after inoculation. Stem sections removed at the insertion site of the inoculated leaf revealed that the virus was stopped in cortical cells of the stem close to the insertion site of the petiole. In older plants showing necrotic streaks on the stem, the virus was detected in cortical cells surrounding the streak.

DISCUSSION AND CONCLUSION

Paths of virus migration

In the susceptible plants it proved difficult to determine the initial time and pathway of virus migration because this migration was very rapid and apparently in all directions and all tissues. However, it is possible that the virus genome had migrated previously under a specific form (Atabekov and Dorokov 1984) that was not detected by our coat protein antiserum.

The localised infections of phloem vessels in petioles and stems of partially resistant varieties underlines that these vascular tissues are the main way for the rapid transport of the virus through the plant.

Barriers against systemic infection in resistant plants

We did not detect differences between the varieties for the spread of the virus in the inoculated leaf lamina. Varietal resistance differences arised only later : 1/ infection of petiole was delayed and 2/ the virus was localised in vascular tissues of the stem or the virus did not infect the stem. This suggests that the resistance to the migration of CMV in these pepper varieties results from an inhibition of virus translocation into or outside the phloem vessels.

This barrier can be broken down, leading to virus spread and mosaic symptoms in the axillary shoots of partially resistant varieties, particularly under unfavorable cultivation conditions (Nono Womdim *et al* 1989). Thus, the other resistance mechanisms involved in the further breeding program

i.e. partial resistance to infection of the leaf and to multiplication of the virus in host tissues will improve the ability to localise the virus in the infected leaf. An evaluation of viral antigen concentration and the detection *in situ* of the transport-form of the virus would permit to progress in the description and the evaluation of these different resistance mechanisms.

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Role of Cultivar, Crop, Crop Cycle, and Initial Infection Level in Field Losses of Sugarcane from Sugarcane Mosaic

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ABSTRACT

Mosaic, caused by sugarcane mosaic virus (SCMV), is an important disease of Louisiana sugarcane. To determine the effect of yield of sugar per unit of land, field plots were planted using 0, 50, or 75% (100% in recent tests) SCMV-infected stalks and harvested each year of a three-year crop cycle (a combination of plant cane, first ratoon, and second ratoon crops). Eight cultivars, of which six are currently recommended for planting in Louisiana, were evaluated over two to eight crop cycles between 1978 and 1988. Among the diseased treatments, percent yield loss varied with cultivar, crop cycle, and initial infection level, but not with crop. The average total yield loss for the 3-year crop cycle among the diseased treatments ranged from 5% in CP 70-321 to 19% in CP 52-68. Yields of both initially healthy and diseased sugarcane declined in most first ratoon crops compared to the plant cane crop, and in all second ratoon crops for all cultivars. Within each cultivar, loss of yield across crops was proportional to the initial infection level. Although a large number of initially healthy plants of susceptible cultivars became infected naturally with SCMV, the initial level of SCMV infection remained important in the amount of yield loss observed, illustrating the importance of planting disease-free seed.

RESISTANCE OF PEPPER AGAINST THE MOVEMENT OF CUCUMBER MOSAIC VIRUS.

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Introduction

Cucumber mosaic virus (CMV) is one of the most damaging disease of pepper. To our knowledge, no complete resistance to CMV is known in the species of *Capsicum* and especially in *C. annuum*.

However, some pepper genotypes do show a partial resistance under natural and artificial infections.

Already, Pochard (1977) has reported the existence of different promising factors of resistance in *C. baccatum* and *C. annuum* varieties : one of them involves the restriction of the virus spread, resulting a sectorial infection of the plant. This factor of resistance to the migration or the systemic spread of the virus has been improved through a reccurent selection. It is now introduced in certain bell pepper varieties (Pochard and Daubeze, 1989).

Using some of these varieties, we have attempted to determine the possible pathways of the virus migration and the barrier sites impeding the systemic infection at the whole plant level (Dufour *and al*, 1989 ; Palloix *and al*, 1989).

In this paper we discuss the results so far obtained about some environmental and physiological factors that modulate the migration of CMV in certain resistant pepper varieties compared to a susceptible variety.

Materials and methods

Two inbred varieties of peppers (*Capsicum annuum* L.) "Milord" and "Vania" obtained from a recurrent selection at INRA-Avignon and a CMV susceptible variety Yolo wonder were used in all experiments. In most of the experiments, plants were grown under greenhouse in 10 cm diameter plastic pots during winter and summer time. For complementary experiments, plants of the same varieties were grown in a growth chamber at 25 °C for 12 hours day at about 7,000 Lux and 12 °C in obscurity.

In all these experiments, the third leaf of each plant at the six-leaf stage was mechanically inoculated with the "MES" strain of CMV.

In order to study the effect of the physiological development stage on the susceptibility of the different genotypes, 8 sets of plants were sown with intervalls of one week.

The inoculated leaf lamina, its petiole, primary root and apex extracts were analysed daily for the virus multiplication and migration by DAS-ELISA test (Clark and Adams, 1977).

Results

1- Virus spread throught the whole plant

Two days after inoculation, ELISA serological tests of sap extracts from the inoculated leaf lamina were strongly positive with all the varieties. In the susceptible variety "Yolo wonder", extracts

of the petiole of the inoculated leaf showed a positive reaction in ELISA tests as early as 2 days after inoculation. 3 days later the virus infected the primary root and the apex whatever the season (Table 1).

In "Milord" the virus was detected in the petiole from the 5th day following inoculation in most plants. During winter, 16 days after inoculation ELISA serological tests of extracts from primary root and apex were positive (Table 1.1). During summer, the plants of this variety were never systemically infected (Table 1.2).

In "Vania" the CMV migrated from the leaf lamina to the petiole 5 days after inoculation. Plants of this variety are not infected systemically in winter as well as in summer (Table 1).

2- Effect of temperature on the expression of resistance of plants against CMV.

The effect of temperature on the expression of the resistance and/or susceptibility of plants of the different varieties was evaluated by comparing 2 environmental conditions (Table 2) :

- in glasshouse during summer (temperature fluctuated between 21 °C and 32 °C) ;
- in growth chamber (12 h day at 25 °C and 12 °C in the dark).

With "Yolo wonder" ELISA tests showed that the virus can be detected in the root and the apex 5 days after inoculation, regardless the environmental temperature conditions.

In "Milord" variety, the virus was only detected in the root and the apex 13 days after inoculation in the growth chamber while at this date plants grown under glasshouse remain uninfected.

In "Vania" plants grown in growth chamber as well as in glasshouse, we have never detected the virus in apical parts of the plants until 20 days after inoculation.

3- Effect of physiological plant age on infection by CMV.

Generally it is agreed that the old plant is more resistant to infection than young plants. We have compared the different varieties by infecting sets of plants of different ages with CMV under glasshouse conditions (Table 3). Plants were considered as susceptible when systemic symptoms (mosaic) were seen on the shoots.

Yolo wonder plants regardless of their ages were found susceptible in all the studied cases (cotyledons - 12 true leaf stage).

Milord and Vania plants were found susceptible up to the 4th true leaf stage but not beyong this development stage.

Discussion and Conclusion

The multiplication of CMV in the inoculated leaf lamina of the 3 varieties regardless of the season was similar. However, the migration of the virus from the leaf lamina to the petiole was slowed in "Milord" and "Vania" varieties in comparison to the susceptible variety Yolo wonder.

These results show that "Milord" and "Vania" are not resistant to infection and multiplication of CMV but have different level of resistance to virus migration. The resistance of these varieties is more efficient beyong the six-leaf stage and under relatively high than low temperature (Table 2). It will be worth recalling that the strain "MES" like D-TL belongs to the group "Thermoresistant" (Marchoux, 1975) which can multiply at 22 °C as well as at 32 °C.

Nevertheless, it will be interesting to study the effect of photoperiodism and light quality, *in situ* and by mean of electron microscopic and immunofluorescent observations of ultrathin sections from infected tissues.

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TABLE 1 : Number of plants of three pepper varieties showing CMV infection as determined by DAS-ELISA tests of the inoculated leaf, primary root and apex samples at various times after inoculation during winter (table 1.1) and summer (table 1.2); 12 plants were tested at every sampling date.

table 1.1

VARIETY	DAY (a)	LEAF LAMINA (b)	PETIOLE (b)	PRIMARY ROOT	APEX
YOLO	2	12	9	0	0
WONDER	4	12	12	12	11
	7	12	12	0	0
MILORD	16	12	12	11	10
	7	12	12	0	0
VANIA	26	12	12	0	0

table 1.2

VARIETY	DAY (a)	LEAF LAMINA (b)	PETIOLE (b)	PRIMARY ROOT	APEX
YOLO	2	12	5	0	0
WONDER	5	12	12	12	12
	5	12	10	0	0
MILORD	26	12	12	0	0
	5	12	7	0	0
VANIA	26	12	12	0	0

(a) : days after inoculation.
(b) : lamina and petiole of the inoculated leaf.

TABLE 2 : Effect of temperature on pepper resistance to CMV.

VARIETY	DAY[a]	TEMPERATURE	PRIMARY ROOT	APEX
YOLO	5	[b] 12 - 25	[d] 5	5
WONDER		[c] 21 - 32	5	5
MILORD	13	12 - 25	4	5
		21 - 32	0	0
VANIA	20	12 - 25	0	0
		21 - 32	0	0

(a) : days after inoculation.
(b) : temperature variation in growth chamber.
(c) : temperature variation in greenhouse.
(d) : number of plants showing CMV infection as determined by DAS-ELISA tests (5 plants were tested at every sampling date).

TABLE 3 : Effect of physiological stage on pepper plants infection.

Variety	Age*	Number of leaves	Height (cm)	Observation
YOLO	14	2**	2	+
WONDER	21	2	4	+
	63	12	14	+
MILORD	14	2**	2	+
	28	4	5	+
	35	6	8	-
VANIA	14	2**	2	+
	28	4	6	+
	35	6	8	-

* : age in days after the sowing.
** : cotyledon
\+ : systemic infection.
\- : no systemic infection.

CRIBLAGE DE CLONES DE CANNE A SUCRE RESISTANT A LA MULTIPLICATION DU VIRUS DE LA MOSAIQUE (SCMV-D)

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La mosaïque de la canne à sucre est connue en Afrique depuis longtemps. Diverses souches en sont responsables : - SCMV-A en Afrique du Sud (Abbott & Stokes 1966), - SCMV-D en Egypte, au Kenya et en Afrique du Sud (Gillaspie & Mock, 1987), - SCMV-J en Afrique du Sud (Von Wechmar et Hahn, 1987). En Afrique du Nord, deux souches ont été identifiées au Maroc : SCMV-D (Fischer & Lockhart, 1974) et SCMV-J (Gillaspie et Mock, 1987).

En Afrique Centrale, la maladie a été observée dans divers complexes sucriers. La souche SCMV-D a été identifiée au Cameroun (Gillaspie et al, 1978). Des symptômes de la maladie sont également très répandus au Congo, au Gabon et au Zaïre. Les complexes sucriers contaminés sont situés dans des zones équatoriales dans lesquelles la température est à faible amplitude et n'atteint pas des maxima très élevés. Au Cameroun, ces complexes sont situés en altitude (800 m) avec des températures relativement peu élevées. La maladie s'y est développée essentiellement sur la variété B 46/364 et à partir de là sur d'autres variétés comme Co 740 . Elle s'est également répandue dans les graminées cultivées (Maïs) ou sauvages. Le succès agronomique de la variété B 46/364, malgré la Mosaïque, pousse les producteurs à s'interresser à des clones issus de croisements avec un parent Barbade. Ces clones sont introduits de deux pays sans mosaïque, Barbade et Guadeloupe. Un premier tri éliminant les clones les plus sensibles à la multiplication du virus est donc nécessaire. Il a été entrepris en serre pendant ou à la sortie de la quarantaine établie sur le Centre CIRAD de Montpelier lors du transfert des clones de Barbade et de Guadeloupe vers les producteurs africains.

MATERIEL ET METHODES

Les clones sont importés des stations de création variétale de Barbade (W.I.S.C.BS.) et de Guadeloupe (CIRAD/IRAT), à raison d'une vingtaine d'yeux par variété. Quelques yeux sont plantés en serre de quarantaine pour contrôle de l'état sanitaire des cannes et sont destinés, deux ans après, à fournir du matériel garanti sain à la multiplication en culture *in vitro* et aux complexes sucriers et pays demandeurs. Une douzaine d'yeux par clone sont plantés en serre protégée des insectes pour l'étude de leur comportement vis-à-vis de la Mosaïque.

Les jeunes plants âgés de deux mois sont inoculés avec une suspension virale, par frottement de la première feuille bien déroulée et saupoudrée de carborundum (400 mesh). La souche virale a été récoltée au Cameroun (SCMV-D). Elle est maintenue en serre à Montpellier sur B 46/364 multipliée sur maïs, var. Golden Cross Bantam, ou sorgho, var. Tx 412.

Les extraits virosés sont préparés par broyage de feuilles de canne, maïs ou sorgho au broyeur Ultraturax avec du tampon phosphate 0,01 M pH 7,2, 0,2 % de mercaptoéthanol, à raison de 2 ml de tampon par gramme de feuilles (dilution 1/2. Pour des inoculations en série, la dilution 1/10 est utilisée.

Le virus est purifié après multiplication sur maïs, var. Golden Cross Bantam selon la technique de Baudin (1977). La détection immunoenzymatique ELISA a été mise au point par Devergne et al (1982).

Les clones à étudier sont introduits , en boutures ou en culture *in vitro*. Après germination ou sevrage, les plants sont repiqués dans des pots de 2 l sur un mélange de pouzzolane et de tourbe (dans un rapport de 4 pour 1) régulièrement alimenté par une solution équilibrée d'éléments nutritifs. Les pots sont placés en serre protégée des insectes, climatisée entre 12°C et 30°C. Environ 3 semaines après repiquage, les plants, généralement au stade 3 feuilles, sont inoculés avec des extraits de canne infectée par SCMV-D. Les premiers symptômes peuvent apparaître quelques jours après l'inoculation à la base de la nouvelle feuille qui se dégage du fuseau foliaire. Ils se manifestent par une mosaïque plus ou moins accentuée, l'évolution est notée chaque semaine.

Pour l'épreuve immunoenzymatique, un conjugué anti-SCMV-D a été préparé par marquage à la phosphatase alcaline (Sigma type VII) de γ-globulines obtenues après plusieurs relargages du sérum par le sulfate de sodium anhydre. Le couplage se fait en présence de glutaraldéhyde selon la technique d'Avrameas (1969). Le test immunoenzymatique proprement dit est réalisé en boîte de polystyrène NUNC. On adopte la méthode dite sandwich de Clark et Adam (1977) dans laquelle le virus se trouve doublement couplé aux anticorps adsorbés sur le support et aux anticorps marqués à l'enzyme (conjugué). Chaque incubation antigène-anticorps a lieu à 37°C pendant 3 heures. Chaque dépôt de réactifs est suivi de 3 lavages au tampon PBSD-Tween, la lecture à 405 nm est effectuée au lecteur Titertek-Multiskan.

RESULTATS

Observation des symptômes

Selon les plants et les conditions climatiques, les premiers symptômes apparaissent au bout d'un temps très variable, entre 10 jours et 1 à 2 mois. Ils se présentent d'abord sous forme de petites tâches allongées, de forme régulière et chlorotique. Les limbes des feuilles qui se déroulent ultérieurement sont entièrement mosaïqués. Sur maïs et sorgho, la maladie apparaît plus rapidement, en 8 jours environ.

Comparaison de la détection immunoenzymatique (ELISA) et de l'observation des symptômes

Dans une expérience portant sur 354 plants, avec 31 clones différents, 154/156 plants ayant manifesté des symptômes ont donné un test ELISA positif. Par contre 14 plants sans symptômes 3 mois après inoculation ont été détectés contaminés par un test ELISA positif.
La détection immunoenzymatique est plus précoce que l'apparition des symptômes (voir Tableau1)
De nombreux auteurs ont étudié les effets de l'environnement sur l'apparition des symptômes. Bailey et Fox (1980) ont observé que la date de plantation au champ joue sur le développement de la maladie ce qui a été rapporté à l'écologie du vecteur. En serre protégée des insectes à Montpellier, la date de plantation joue aussi sur l'apparition des symptômes : Deux lots de clones ont été observés la même année à Montpellier, l'un de Janvier à Avril, l'autre de Novembre à Avril (Tableau 2). Les plants introduits en Janvier ont eu une croissance plus rapide et plus homogène que ceux introduits en Novembre. La manifestation des symptômes a été plus rapide et la concordance entre détection immunoenzymatique et apparition des symptômes est totale, alors que sur les plants introduits en Novembre, la plupart des plants virosés d'après la détection ELISA n'ont pas montré de symptômes.

n° de plant	durée d'apparition des symptômes en jours	ELISA à 8 semaines (56 jours)
1	9	+
2	33	+
3		-
4	38	+
5	109	+
6	33	+
7	74	+
8	47	+
9	47	+
10	62	+
11	47	+
12	99	+

Tableau 1 : Durée d'apparition des symptômes sur des plants du clone sensible G.P. 82/550

	plantation fin janvier	plantation novembre
stade 3 feuilles	3-6 jours	4-10 jours
apparition des symptômes après inoculation	15 jours	5-12 jours
résultats		
plantes avec symptômes	94 plants	15 plants
détection ELISA positif	94 plants	113 plants
plantes sans symptômes	48 plants	110 plants
plantes ELISA négatif	48 plants	27 plants

Tableau 2 : Effet de la période d'observation sur la multiplication du SCMV-D dans des clones de canne à sucre

Multiplication du virus dans différents clones

Malgré la variabilité d'apparition des symptômes d'un plant à l'autre dans un même clone, les observations sur l'ensemble des clones donnent des résultats nets. Trois types de comportements peuvent être observés :

la quasi totalité des plants d'un même clone permet la multiplication du virus avec symptômes et détection sérologique positive au bout de 2 mois,

- des clones qui n'extériorisent aucun symptôme avec détection immunoenzymatique négative,
- des clones dont quelques plants montrent des symptômes ou une détection immunoenzymatique positive, d'autres restant sans multiplication de virus.

A partir d'un croisement polycross, les clones testés à Montpellier peuvent être sensibles ou résistants quelque soit le comportement du parent. Aucune corrélation n'a pu être encore établie entre le comportement des parents et la réaction des hybrides obtenus.

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SCREENING OF SUGARCANE CLONES WHICH ARE RESISTANT TO THE MULTIPLICATION OF MOSAIC VIRUS (SCMV-D)

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In Africa sugarcane crops are contaminated by various Mosaic strains. The regions most affected are East Africa from Egypt to South Africa. In French-speaking Africa, the disease is present in regions which have a relatively cool season, either in the equatorial zone (Gabon, Zaire) or in high altitude zones (Cameroon, Madagascar).

The new clones introduced for varietal selection are mostly imported from non-infected countries (Barbados, Guadeloupe). Their reaction to Mosaic infection is observed in an insect-proof glasshouse in Montpellier. One leaf per one-month old plant is inoculated by rubbing with a D strain of SCMV, which commes from Cameroon. The multiplication of the virus is observed when the symptoms appear, and with ELISA, using a specific serum on approximately twelve plants per clone.

Some plants show a highly positive serological reaction, whereas the leaves maintain a healthy aspect, although in the same clone other plants exhibit very marked symptoms. The response of the different plants among the twelve plants of a clone ir fairly homogeneous : either multiplication in most plants, or on the contrary disappearance of the virus. On the other hand, among the progeny of the same crossing, we can observe host clones of the virus, and others in which multiplication does not occur. After eight crop years of observing clones, no correlation has been established between the behaviour of the parents and that of the clones which result from their crossing.

RESISTANCE TO BEET WESTERN YELLOWS VIRUS IN *LACTUCA* SPECIES

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Beet Western Yellows Virus (BWYV), a member of the Luteovirus group causes severe losses in many lettuce growing areas. The disease was known for many years in Great-Britain as "June Yellows" and more recently outbreaks were recorded in the summer crops in France and Southern Europe. High light intensity and the susceptibility of the cos type and of the bolting resistant butterhead varieties were among the reasons for that situation. Faced with these severe outbreaks an urgent solution was required, therefore, we have started, in 1983, a search for resistance in lettuce cultivars and wild *Lactuca* species.

The symptoms caused by BWYV are interveinal yellowing on the outer and sometimes middle head leaves; they appears 12 to 25 days after inoculation depending on plant age and light intensity. The BWYV-FL1 strain which was isolated in 1982 in Provence, infects a large host range including oilseed rape, cabbage, radish, pea, fodder and table beet, but sugar beet is infected with more difficulty. Other isolates which were collected from lettuce in France and Spain or which were obtained from Great-Britain were compared for their host range in the same conditions. It was found that they share mainly the same properties (H.LOT, unpublished results). ELISA tests were carried out using standard DAS procedure and antibodies against BWYV-FL1.

Viruliferous *Myzus persicae* were maintained on virus infected *Physalis floridana* in a growth chamber (20°C, 16 h daylenght). Mass inoculations were made using two small leaf pieces of *P. floridana* carrying aphids (10 to 30 adults and larvae) which were placed on each plant to be infected for 48 h. The aphids were then killed with insecticide.

TOLERANCE IN LETTUCE CULTIVARS

In summer 1983 and 84, about fifty commercial cultivars and lines of all lettuce types were tested in field trials for their reaction after artificial inoculation of BWYV-FL1. All appeared to be susceptible. However, in each type, several cultivars have shown an apparent lower reaction to the virus. European crisphead ("Batavia") type was always more tolerant, "Iceberg" and particularly cos types appeared always more susceptible; in butterhead type, the bolting resistant "varieties ("Kagraner" type) were the most susceptible but gradation of susceptibility did exist particularly in "Attraction" and "Hilde" types.

Sources of tolerance have been mainly sought in butterhead lettuces. After two repeated inoculations at 4-6 leaf stage, plants were planted in repeated blocks of 10 under a screenhouse and assessed for number of yellowing leaves at two different times after inoculation and at maturity; yield loss before trimming (growth reduction) and after trimming were calculated."Mantilia" was always the most susceptible - 15 to 20 leaves affected, 30 to 50 % yield loss -, "Divina", "Audran" and "Verian" were tolerant - 7 to 10 leaves affected, 15 to 25 % yield loss-.

A breeding program was conducted to get summer type varieties (bolting resistant) with BWYV tolerance ("Mantilia" x"Verian" and "Audran" x "Verian"). Two lines 4607 and 5614, obtained from these original crosses, are fixed for these characters and shows transgression for this tolerance. Table 1 shows that line 5614, selected also because a high weight head, have a low yield loss when compared with "Mantilia"; benefit after trimming is still more significant.

Virus concentration estimated by ELISA after different incubation periods does not show any difference between susceptible and tolerant parents or lines; the virus can be detected in the youngest leaves 10 to 12 days after inoculation in all material revealing no difference in virus migration rate. Involved mechanism is a true tolerance but the inheritance of that resistance is not known.

The tolerance was as effective when tested with four different lettuce isolates. In replicated field tests, at two locations, and in natural contamination conditions, the comparison of plants proportion showing symptoms in parent cultivars and breeding lines is very favourable to 4607 and 5614 lines. Fig.1 shows the results of such an assay where 9, 25 and 65 % of the plants are judged unmarketable because of BWYV yellowing symptoms for 4607, 5614, and "Mantilia" respectively.

RESISTANCE IN *LACTUCA VIROSA*

In preliminary assays, several accessions of *Lactuca saligna, L. serriola, and L.virosa* were tested in a replicated field test submitted to natural infection. All *L. saligna* and *L. serriola* were susceptible harbouring interveinal yellowing. Among the *L. virosa* accessions, one, IVT 280, did not show any symptom, another one, IVT 1398 was susceptible with typical yellowing. IVT 280 - obtained from EENINK, Wageningen, NL - is known to be almost completely resistant to *Nasonovia ribis-nigri* (one dominant gene) and partially resistant to *Myzus persicae* (several genes).

Artificial inoculations where BWYV-FL1 infected *M. persicae* were forced to feed on young plantlets (two to three repeated inoculations) confirmed the resistance of IVT 280 and the susceptibility of others accessions. ELISA tests revealed that infection does not occur in IVT 280 in either inoculated or non inoculated leaves and that the virus concentration is high in IVT 1398 and others accessions. Similar assays performed with five BWYV lettuce isolates showed that IVT 280 may multiply the virus at a very low rate in the inoculated leaves but there is no migration of the virus and the plants remains always symptomless.

The inheritance of the resistance was studied between *L. virosa* resistant and susceptible accessions since the interspecific crosses between *L. virosa* and *L. sativa* are very difficult. Crosses between IVT 280 and IVT 1398 - which possesses an anthocyanin marker gene - gave results compatible with the hypothesis of a single dominant gene as shown in Table 2 : both reciprocal F1 were resistant ; the F2 population segregated 3 resistant to 1 susceptible. On eight F3 lines issued from resistant F2 plants (20 to 40 plants per line), 3 were homogeneous resistant, 5 were segregating confirming the monogenic dominant hypothesis. A breeding program to introduce IVT 280 resistance in *L. sativa* is in progress. Interspecific crosses were successful using *in vitro* culture of immature embryos. F 4 of BC1, (IVT 280 x *L. sativa*) x *L. sativa*, are studied for symptom expression and virus multiplication: some lines seems interesting.The resistance can be inherited but, as it is sometimes the case after interspecific crosses, the disjonction does not fit with the former hypothesis.

For the control of BWYV, which has a large host range and several vectors as *M. persicae* and *M. euphorbiae*, on a crop, the lettuce, generally grown in small or medium size plots at least in Europe, a genetic resistance is probably the best answer. Tolerance will provide a short-term way and could be associated with pyrethroid sprays which are efficient to reduce secondary contaminations (unpublished results). Several lines of tolerant butterhead experimented in France, Spain and Italy gave satisfactory results: they will be submitted for Catalogue inscription. However, more complete resistance is needed. Transfer of quasi-immunity from *L. virosa* will be continued but it is a longer term solution.

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Table 1. Comparative effect of BWYV-FL1 on a susceptible cultivar and a tolerant line.

Cultivar	Mean weight of head (g) [a]		Yield reduction (%) before trimming	Yield reduction (%) after trimming [b]	
	Healthy	Infected		Healthy	Infected
Exp.1					
"MANTILIA"	487	4 25	16	15	34
" 5614 "	647	632	3.4	13	18
Exp.2					
"MANTILIA"	385	316	18	11	33
" 5614 "	480	430	10	12	13

a Mean of 20 plants

b $\frac{\text{Mean weight-Mean weight after trimming}}{\text{Mean weight}}$

Table 2 . Inheritance of the resistance to BWYV in L.virosa IVT 280 .

Line	Number of plants Resistant	Susceptible	Theor.segregation (1 dominant gene)	Chi-square Value
IVT 280	30	0		
IVT 1398	0	26		
F1(IVT280 x IVT1398)	15	0		
F1(IVT1398 x IVT280)	5	0		
F2(IVT280 x IVT1398)	67	26	3 R : 1 S	0.43 NS
BC1 (F1 x IVT280)	7	0	1 R : 0 S	0.0
BC1 (F1 x IVT 1398)	10	9	1 R : 1S	0.05 NS

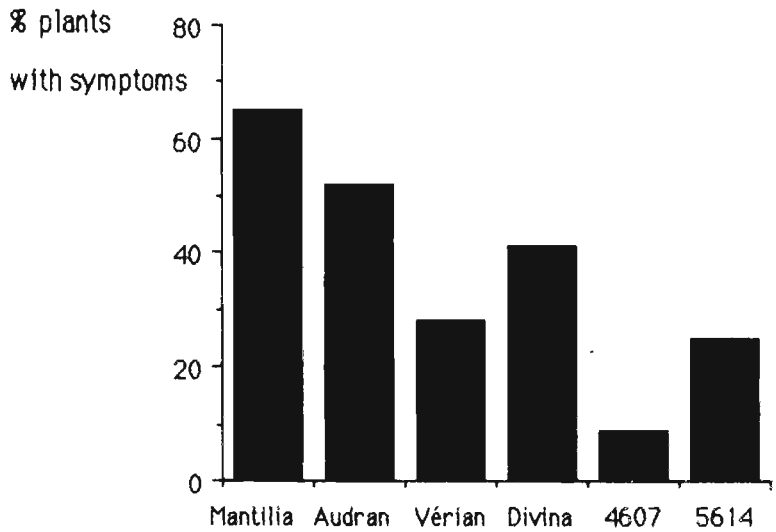

Fig 1. Comparative proportion of unmarketable heads in susceptible and tolerant cultivars or lines after natural BWYV contamination.

DIVERSITY OF PAPAYA RINGSPOT VIRUS (PRSV). IMPORTANCE FOR BREEDING FOR RESISTANCE.

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Occurence of strains with differential properties has been observed for many plant viruses. Breeding for resistance to viruses must take account of this virus diversity, specially at the level of biological properties. In the case of papaya ringspot virus (PRSV), 2 pathotypes were already identified: type P able to infect papaya and type W unable to infect papaya. these two pathotypes were antigenically indistinguishable (Purcifull *et al.*, 1984). A serological variant has also been described (Quiot-Douine *et al.*, 1986).

We studied serological and several biological properties of 20 strains and isolates related to PRSV and coming from different parts of the world.

Using immunodiffusion tests, we have distinguished nine groups

using 7 antisera prepared against the virus particles of 6 of these strains or isolates. The common serotype already described is present in different parts of the world, but a serological heterogeneity has been observed among isolates from Africa or nearby and among those from the Carribean.

Antisera prepared against amorphous and cylindrical inclusion proteins have shown also heterogeneities among the 20 strains or isolates.

Four pathotypes have been defined using 8 lines of muskmelon mechanically inoculated ("Ouzbèque", "Védrantais", "Charentais T", "Yoatango", "PI 161375", "72025", "PI 414723", and "WMR 29"). Two different pathotypes have been found among strains and isolates having the common serotype, and only one pathotype grouped most of the isolates from Africa.

The aphid transmission frequency was measured by transmission from squash to squash by *Aphis gossypii* Glov.. With percentages of aphid transmission ranging from 22 to 86% PRSV isolates have a moderate to good aphid transmissibility, except strains which have been maintained during a long time by mechanical transmissions which can lead to a loss of aphid transmissibility.

Diversity between strains or isolates has been observed by comparing the quantity of protein detected by ELISA tests with antisera against virus particules in infected squashes incubated at different temperatures: 15°C, 20°C, 35°C, or 40°C. Most of PRSV strains or isolates produced more antigens at 35°C than at 15°C. A few isolates from Africa or nearby produced more antigens at 15°C than at 35°C.

The diversity observed in PRSV shows the difficulty of breeding plants for virus resistance. Moreover, there is no accurate correlation:

1) between serological and biological properties so that biological types cannot be recognized only by immunological tests.

2) between biological properties, specially pathotypes, and geographic origin. So, breeding of plants for resistance to a virus must take account of all virus types already present or capable of settling in the biotope where these plants will be used.

3) between several biological properties, for example, pathotype and optimum temperature. So, breeder has to take account of the different pathotypes of the virus and also of their behaviour according to environmental conditions.

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ANALYSIS OF POLYGENICALLY CONTROLLED RESISTANCE IN POTATO TO POTATO LEAFROLL VIRUS

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INTRODUCTION

In most potato breeding programmes an important objective is the incorporation of resistance to the very damaging disease caused by potato leafroll virus (PLRV). The sources of resistance to PLRV which are usually used in resistance breeding programmes are polygenically controlled (Ross, 1958). They are expressed as a quantitative resistance to infection which is difficult to measure, although resistance ratings of cultivars can be obtained from the results of field trials. Selection for a polygenically controlled character in a breeding programme is difficult and the pattern of inheritance of all characters in Solanum tuberosum is further complicated because it is a tetraploid species. Therefore, improved techniques to assess and screen for resistance to PLRV and to study the inheritance of resistance are highly desirable.

In recent work, a more precise analysis of resistance to PLRV was attempted and three components of resistance, which can be found in some breeders clones and cultivars, were identified (Barker & Harrison, 1985; Barker & Harrison, 1986; Barker, 1987). Of these components a hitherto unrecognised form of resistance to PLRV, that is expressed as a severe restriction on the amount of virus which accumulates in infected plants (Barker & Harrison, 1985), is probably the most important. When potato clones with this type of resistance are grown, few aphids feeding on infected plants are able to acquire enough virus to transmit it to other plants (Barker & Harrison, 1986). Among about a dozen examples, this type of resistance occurred most commonly in the potato cultivars and clones that had been found to be most resistant to infection with PLRV in field exposure trials (Barker & Harrison, 1985), suggesting that there might be a link between the two kinds of resistance (Barker, 1897). The work to be reported has: (1) examined in more detail the association between resistance to PLRV multiplication and field resistance to infection in a large range of clones; (2) made a preliminary assessment on the nature of inheritance of ability to restrict PLRV multiplication among individual seedlings (genotypes) in progenies obtained by crossing a resistant and a susceptible parent.

MATERIALS AND METHODS

Test material was of two types (1) A range of about 50 potato clones, nearly all bred at SCRI, and most tested previously for resistance to infection with PLRV in field exposure trials. (2) 40 genotypes from two seedling progenies produced by reciprocal crosses between Maris Piper and a breeding clone G7445(1), in which PLRV attains higher and lower concentrations respectively.

Virus-free plants of the above material were graft inoculated with scions from PLRV-infected plants. The daughter tubers were collected and plants with secondary infection grown from them in a glasshouse. PLRV concentration in leaf extracts was estimated by an accurate and sensitive quantitative ELISA technique in which reactions of extracts of leaf were compared with those of a series of known concentrations of purified PLRV particles.

RESULTS

ASSOCIATION BETWEEN VIRUS CONTENT AND FIELD RESISTANCE

Young and old leaves of the range of 50 clones were tested by ELISA on a number of occasions during the growing season. The mean virus concentration in leaves of a selection of clones from this range is shown in Table 1.

Table 1 Tests for association between PLRV concentration in leaf tissue and resistance to infection in the field.

Clone	Field rating*	Mean virus titre (ng/g leaf) Young leaf	Old leaf
G8107(1)	R	34	104
G7445(1)	R	30	149
G6642(2)	S	161	383
G8213(3)	R	201	228
G6582(3)	S	287	1328
G8105(1)	R	618	880
G6867(2)	R	897	3974
G6907(1)	R	961	2446
Maris Piper	S	1636	1528
G7714(1)	S	1583	2374

* R = Resistant to infection, in most instances more so than cv. Pentland Crown.
S = Susceptible to infection.

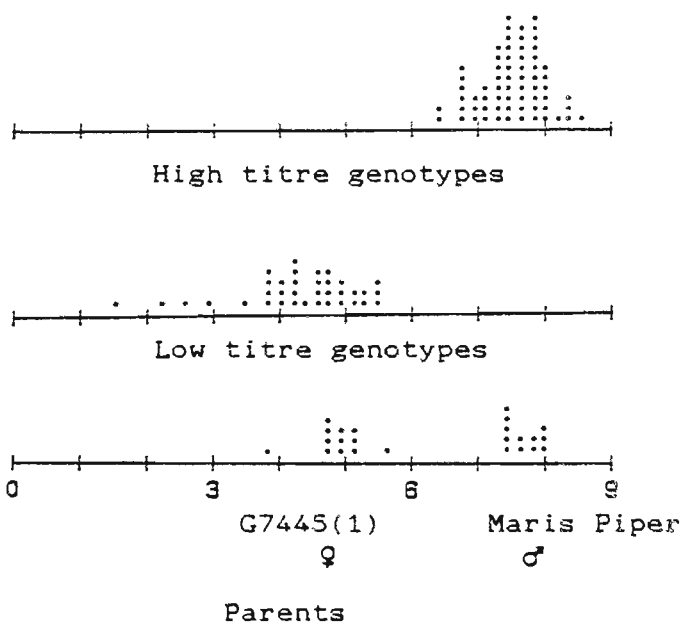
Tests on the same clones on different occasions gave relatively consistent estimates of virus concentration although virus titre tended to be greater in all clones when the plants were young. In young leaves, the virus concentrations in different clones range from 34-1635 ng/g leaf and in old leaves from 104-3974 ng/g leaf. Despite the indications from previous work (Barker & Harrison, 1985), the more extensive tests reported here provide no evidence for an association between restriction of virus accumulation in a clone and its resistance to infection with PLRV (Table 1).

INHERITANCE OF ABILITY TO RESTRICT PLRV MULTIPLICATION

Virus concentration in genotypes of the progenies from crosses between Maris Piper and G7445(1) was tested using similar techniques on a number of occasions and the data analysed after a natural log transformation. When data was plotted as a histogram it was apparent that the observed variation of virus concentration between the genotypes was of a discontinous rather than continous

nature (Fig. 1). Indeed, when the mean virus concentration in each genotype was analysed it was found that the concentrations could be divided into two classes, high and low, the values being approximately the same as those in plants of their two parent clones. The ratio of numbers of high and low virus titre genotypes differed in the progenies of the reciprocal crosses but more extensive tests will be required to determine whether this effect is reproducible (Table 2). When the results from both crosses were combined there were 19 genotypes with high virus concentration and 21 with low virus concentration, which is close to a ratio of 1:1 (Table 2).

Fig. 1 Histogram of Log e of virus titre measurements* from 17 genotypes from the progeny of the cross Maris Piper (male) X G7445(1) (female).



* Virus titre measured on 6 occasions

Table 2 Partitioning the genotypes of progenies from crosses between Maris Piper and G7445(1) into high and low virus titre classes.

Cross		Virus concentration of genotypes	
Female parent	Male parent	High Titre	Low Titre
Maris Piper	x G7445(1)	8	15
G7445(1)	x Maris Piper	11	6
Total		19	21

DISCUSSION

The results suggest that PLRV content of leaves is not associated with field resistance ratings and therefore cannot alone provide the basis of a rapid method for breeders to screen for resistant material. Instead it seems that there are at least two important features which should be incorporated into PLRV resistant breeding material, ie. resistance to infection (such as is measured by field exposure trials) and ability to restrict virus accumulation in plants with secondary infection. Of these two features, low virus concentration may be the more important because of the dramatic effect it has on the potency of plants as sources of virus for aphids. However, in the future it should be possible to select potato clones with both types of resistance by measurements of virus content in the foliage of resistant clones identified by field exposure trials.

The preliminary results of tests with progenies from crosses between parents in which PLRV reaches high and low concentrations, respectively, indicate that the genotypes could be assigned in equal numbers to two phenotypic classes based on virus concentration. Virus concentration therefore is likely to be controlled by a major gene or genes. But although a major gene may be operating, this may not account for all the observed variation and it is possible that minor genes can affect virus accumulation. There are two main alternative explanations of the 1:1 segregation ratio. Either the resistant parent, G7445(1), has a dominant resistance gene in a simplex state and the susceptible parent, Maris Piper, is homozygous recessive; or Maris Piper has a dominant major gene for susceptibility in a simplex state and G7445(1) is homozygous recessive. Further tests will be required to determine which of these possibilities is correct and to find whether this type of resistance is inherited similarly in other clones.

Thus, although one component of resistance to PLRV may be under major gene control there are other components of virus resistance (Barker, 1987) whose inheritance has yet to be determined. The complete pattern of inheritance of the several components of resistance to PLRV, and of resistance to vector aphids, is likely to be complex. Therefore, in the future it may be profitable for breeders to concentrate their effort on those components of resistance which are controlled in a simple manner.

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Screening cereals for resistance to barley yellow dwarf in Mexico.

P.A. Burnett and M. Mezzalama

Screening cereal lines for barley yellow dwarf resistance is conducted by the CIMMYT Wheat Program in Mexico. Currently the program screens cereal lines in small, space-planted plots that are exposed to natural epidemics of barley yellow dwarf virus. Lines selected for apparent resistance can be tested in experiment infested with greenhouse-reared viruliferous aphids. Lines showing visual resistance in Mexico have been distributed worldwide. There is a large of among-site variation in resistance but some lines of bread wheat, durum wheat, barley and triticale are visually resistant at most sites.

NEW APPROACH TO BREEDING FOR BEAN GOLDEN MOSAIC RESISTANCE

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Immunity to bean golden mosaic virus (BGMV) has not yet been detected in thousands of common bean (Phaseolus vulgaris L.) genotypes evaluated to date. So far, only a few black-seeded bean cultivars possessing BGMV tolerance have been used in breeding for bean golden mosaic resistance projects. Unfortunately, these black-seeded tolerant materials often introduce undesirable phenotypic seed characteristics in commercial genotypes, most of which have seed colors other than black. Consequently, a different germplasm evaluation and selection approach, based on the identification of previously unexploited BGMV-resistance traits, was followed in this study. The main resistance mechanisms detected in non-black-seeded bean genotypes were: disease escape, delayed symptom expression, tolerance, and resistance to flower and pod distortion. Since these resistance mechanisms have been observed in genotypes belonging to different gene pools of Middle-American and South American origin, it would be advisable to study their general combining ability prior to their use as parental materials. Fortunately, the main sources of BGMV resistance (Great Northern, Pinto, Red Mexican) identified in this investigation, belong to gene pool 5 which has a high positive combining ability (Singh, S.P., Econ. Bot. 43:39-57, 1989).

RESISTANCE TO MAIZE STREAK VIRUS IN AFRICA

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Maize streak virus (MSV) is an indigenous African geminivirus which evolved with various native grasses. It is misnamed since it has no evolutionary connection with maize, a new-encounter host following its introduction into Africa from the Americas post 1600's. There are periodic local and regional MSV epidemics in Africa causing major yield losses of maize. The virus, vectored by *Cicadulina* spp. leafhoppers invades maize fields seasonally across tropical and subtropical Africa. It also occurs in some neighbouring and Indian Ocean Islands.

The sources for the seasonal re-occurrence of MSV in maize following winter or dry season periods are inadequately known. In West Africa, the disease is sporadic in early plant stages and the epidemic develops subsequently mostly from within-field spread. At the end of the maize season in West Africa it is not clear if the virus is taken from maize back to the grasses, or if it is a "dead-end" disease in maize. If it is, then, the subsequent maize crop is exposed to the virus diversity in native grasses, which will remain fairly constant from year to year, and only those strains capable of infecting maize will create a new epidemic on the crop. In contrast, if grasses become infected from maize fields, enabling cyclic virus transmission: maize-grasses-maize, the potential exists for virus strain selection with the deployment of resistant maize.

Resistance in maize was discovered by Dr. Storey in East Africa in the 1930's but was never effectively utilized in a breeding programme, thus, it never reached farmer's fields. Resistance was rediscovered at IITA, in Nigeria in 1975. Strategies and methods were developed which enabled incorporation of MSV resistance into a breeding program targeted at developing high yielding, disease resistant varieties adapted to various African ecosystems. Many MSV-resistant varieties have been developed and deployed across Africa.

STABILITY AND DURABILITY OF RESISTANCE

All evidence so far indicates that resistance operates effectively across Africa from Angola to Zimbabwe and from Senegal to Ethiopia. Given the apparent

great genetic variation of the virus population on native grasses, this result is amazing. Possibly this is due to the original strategy of avoiding challenge of maize by a specific virus strain only. Instead, the diversity of vectors and virus strains in grasses in Nigeria were used in the original attempts to search for resistance in maize. In addition the strategy involved selecting for tolerance only, i.e. plants with mild symptoms which were hardly affected by the virus (Soto et al., 1982). Thus, escapes and potentially immune plants (virus strain-specific immunity) were avoided. Selection for increased levels of tolerance in every generation automatically lead to the accumulation of minor genes affecting the host-virus relationship in favor of the host. Recent genetic analysis indicates quantitative inheritance of resistance (Kim et al., 1989). It is highly likely that this approach of continuously selecting for tolerance, which results in selection of any genes influencing the virus negatively will result in resistance that is durable.

The development of rapid and effective screening procedures was critical to the incorporation of resistance as a breeding target in a maize breeding program with many other objectives. Mass rearing of leafhopper vectors, means for virus acquisition and field infestation techniques, were all developed on a massive scale. Approximately one million young maize plants are challenged in three cycles of breeding per year.

Originally, resistance was found at low frequency in TZ yellow, a maize population composited from many sources and thus the actual origin of the resistance is unknown. In addition resistance was developed by IRAT in Reunion Island and utilized in developing the variety "La Revolution". The origin of this separate source of resistance is also unknown. Both sources have been used in developing MSV tolerant varieties at IITA.

The overall resistance conferred by having bred for tolerance results in a population of maize in which the incidence of streak in an epidemic is much lower than in a susceptible (intolerant) variety. In one study, virus incidence in the tolerant variety reached 10%, in contrast to 63% in the intolerant variety (Soto et al., 1982). This characteristic of a variety - developing low incidence of a systemic disease with apparent equal opportunity for epidemic development - has been called tolremicity (Buddenhagen, 1983). In this case, by selecting for tolerance in individual plants and recombining these, it was possible to develop varieties in which individual plants, if infected, suffer only minor yield loss. Recent work at IITA, wherein plants were artificially infested two weeks after planting showed that where an intolerant variety suffered a 64% yield loss, a highly tolerant variety and hybrid were reduced in yield by 12% and a hybrid classed as moderately tolerant was reduced by 17%. In addition it resulted indirectly in varieties which are tolremic i.e. virus incidence remains

low. The combined effects of tolerance and tolremicity compose resistance. The low virus incidence in tolremic varieties during a potentially serious epidemic could be due to one or more of many factors involving vector behavior, acquisition/transmission , virus replication and virus systemicity.

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REYNAUD B
CIRAD/IRAT - LA REUNION

LA RESISTANCE AU MAIZE STRIPE VIRUS (MStpV) ET A SON VECTEUR *PEREGRINUS MAIDIS* (AHSMEAD, 1890) (HOMOPTERA DELPHACIDAE) CHEZ LE MAIS (*ZEA MAYS*) IRAT 297, VARIETE COMPOSITE DES MASCAREIGNES (OCEAN INDIEN)

Le maize Stripe Virus (MStpV), tenuivirus, transmis par le delphacide *Peregrinus maidis* (ASHMEAD, 1890) provoque d'importants dégâts dans les régions maïsicoles de la zone tropicale. Récemment, l'incidence de cette maladie s'est accrue, particulièrement sur le continent américain et dans l'Océan Indien alors qu'aucune source de résistance n'avait encore été découverte.

A l'île de La Réunion, les observations faites de 1985 à 1988 sur des semis mensuels de l'hybride INRA 508 et du composite IRAT 297 montrent qu'en moyenne seulement 3 % des plants d'IRAT 297 présentent des symptômes alors que 16 % de ceux d'INRA 508 sont atteints. Même au cours des fortes épidémies, aucune parcelle de composite ne présente plus de 25 % de plants virosés.

25 jours après semis, on dénombre en moyenne 1,36 *P. maidis* adultes par plant dans les parcelles d'INRA 508 pour seulement 0,87 dans celles d'IRAT 297.

En unité insect-proof, le comportement de *P. maidis*, sous bonnettes ou non, confirme la résistance au vecteur chez le composite. Après 24 h de dépôt, la fuite des insectes adultes est quantitativement plus élevée que la mortalité. Les mécanismes de résistance s'apparentent donc bien à des phénomènes d'antibiosis mais surtout d'antixenosis.

Dans ce même essai, INRA 508 présente 98 % de plants avec symptômes pour seulement 20 % chez IRAT 297 et 50 % chez l'hybride IRAT 143 (Révolution x INRA 508). De telles différences de proportions de plants atteints et le fait que, quelque soit la variété, les plants virosés évoluent toujours vers une grande sensibilité, font suspecter l'existence d'une résistance complète au virus.

Les infestations artificielles faites sur S1 de plants résistants d'IRAT 297 donnent des pourcentages de plants virosés allant de 8 à 48%. Chez quelques plants, l'apparition de symptômes atténués montre également l'existence d'une certaine résistance partielle.

La résistance au MStpV pourrait être contrôlée par une combinaison d'un ou 2 gènes majeurs dominants et de gènes mineurs récessifs modificateurs.

Breeding for resistance to tomato yellow leaf curl virus

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Tomato yellow leaf curl virus (TYLCV), which is transmitted by the tobacco whitefly, **Bemisia tabaci**, is the major disease affecting tomatoes in the summer and autumn in Israel. The disease causes severe damage to fresh market tomatoes grown for local consumption and export.

F_1 hybrid TY-20 is the first tomato cultivar released for commercial cultivation with tolerance to TYLCV. The tolerance trait, derived from **Lycopersicon peruvianum**, is recessive and controlled polygenically. Plants of TY-20 when inoculated with TYLCV show mild disease symptoms, but recover from the disease and give an acceptable marketable yield.

TY-20 is a fresh market tomato for the local market. Plants have a determinate vine habit. Fruits are flat-round in shape, with green shoulders, and have an average fruit weight of 110 g. Fruit firmness is fair.

In laboratory tests, transmission rates of TYLCV by the vector whitefly from plants of TY-20 and a susceptible cultivar were found to be similar.

RESISTANCE OF GROUNDNUT TO COMPONENTS OF ROSETTE DISEASE

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Groundnut rosette is the most important virus disease of groundnut (Arachis hypogaea) in Africa but is not reported from other parts of the world. Resistance to rosette was found in groundnut germplasm collected from Cote d'Ivoire (Sauger & Catharinet, 1954a, b; De Berchoux, 1958). The resistance is governed by two independent recessive genes (De Berchoux, 1960; Bock & Nigam, 1988; Nigam & Bock, 1988 and unpublished data).

Rosetted groundnut plants contain groundnut rosette virus (GRV) and groundnut rosette assistor virus (GRAV). GRV depends on GRAV for transmission by Aphis craccivora (Hull & Adams, 1968). GRAV, a luteovirus (Casper et al., 1983; Reddy et al., 1985a), is not manually transmissible but it has been purified and a polyclonal antiserum is available (Rajeshwari & Murant, 1988). GRV is transmissible manually but no virus-like particles have been observed; infectivity is associated with single-stranded RNA of c. 1.5×10^6 molecular weight.

Groundnut plants infected by GRAV alone are symptomless. Symptoms of rosette in groundnut are associated with infection by GRV, but recent studies (Murant et al., 1988; I.K.Kumar & A.F.Murant, unpublished data) have shown that the symptoms are caused not by GRV itself but by a satellite RNA that depends on GRV for its replication; moreover, some forms of the satellite infect groundnut without causing symptoms. Thus plants that show no symptoms following exposure to viruliferous aphids may nevertheless be infected by one or more components of the virus complex.

The resistant cultivars RG1, RMP40, RMP91, RMP93, RRI/6 and RRI/24 were compared with the susceptible cultivar Spancross for their resistance to the components of rosette disease. Seedlings were raised in an aphid-proof greenhouse at Chitedze and were inoculated either by grafting or by A. craccivora. The virus content of groundnut test seedlings was determined in Dundee. GRAV was detected by ELISA with the GRAV polyclonal antiserum or with monoclonal antibody SCR6 raised to potato leafroll virus (PLRV) (Rajeshwari et al., 1987). GRV was detected by manual transmission to Chenopodium amaranticolor, Nicotiana benthamiana and N. clevelandii (Reddy et al., 1985b). Representative GRV isolates were examined for the presence of satellite RNA by electrophoretic analysis of dsRNA extracted from infected N. benthamiana (Murant et al., 1988).

All inoculated plants of the susceptible cultivar Spancross developed obvious rosette symptoms within 1-4 weeks after inoculation by either method. Most

plants of the resistant cultivars showed no symptoms after 6 months but some developed moderate to severe rosette after several weeks. The proportion of plants of the resistant cultivars that showed rosette symptoms seemed to be increased in plants grown at high temperatures (i.e. in glasshouse conditions in summer in Malawi). GRAV was detected in most plants of all cultivars, whether or not they showed rosette symptoms. In contrast, GRV was detected only in rosetted Spancross plants and in those plants of resistant cultivars that had rosette symptoms or eventually showed them. DsRNA analysis of representative GRV isolates recovered from resistant and susceptible cultivars showed that all contained the satellite RNA.

The results show that rosette-resistance in groundnut is directed against GRV and that the resistant plants are fully susceptible to GRAV. Resistance to GRV brings with it resistance to the GRV satellite RNA which is the component of the complex responsible for rosette symptoms in groundnut but can multiply only in the presence of GRV. The resistance to GRV does not amount to immunity; it seems to be overcome to some extent in unfavourable environmental conditions, possibly high temperature.

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Aspects of virus control in ornamental bulbous crops in The Netherlands.

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Ornamental bulbs suffer from stylet-borne viruses. This may be either by the overall infection by one virus at least, like in the trade material of narcissus, bulbous iris and gladiolus, or by the additional infection of one or more viruses in crops like tulip, hyacinth, crocus, and virus-tested material of lily and bulbous iris. The seasonal period of virus spread is different in the various crops (Diagram 1).

Diagram 1: Virus spread above soil level in ornamental bulbous crops in The Netherlands.

Month / Crop	April	May	June	July	August	September
Tulip	xxxxxxxxxxxxxxxxxxxxxxxxxx					
		oooooooooooo				
Hyacinth	xxxxxxxxxxxxxxxxxxxxxxxxxxxx					
		ooooooooooooooooo				
Narcissus	xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx					
		oooooooooooooooooooooo				
Bulbous iris - trade material	xxxxxxxxxxxxxxxxxxxxxxxxxxxx					
		ooooooooooooooooo				
Bulbous iris - virus-tested material	xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx					
		oooooooooooooooooooooooooo				
Crocus	xxxxxxxxxxxxxxxxxxxxx					
		oooooooooooo				
Lily	xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx					
		oooooooooooooooooooooooooooooooooooo				
Gladiolus	xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx					
		oooooooooooooooooooooooooo				
Dahlia	xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx					
		oooooooooooooooooooooooooooo				

xxxxxxx = field growth above soil level
ooooooo = flying aphids active in virus spread

The primarily vegetative propagation of ornamental bulbs implies that viruses spread to plants mostly persist in the offspring. Generally the elimination of virus-diseased plants is a laborious task. Therefore, the control of the virus spread is necessary to be developed. The increase in different rates of virus infection endangers the efficacy of virus control. The increase may even be enhanced if the more profitable yields of bulbs are likely to be obtained. A few examples in tulip will be dealt with in the following.

Tulip

In tulips the spread of tulip breaking virus (TBV) is affected by ecologically determined factors, e.g., temperature regimes to store the bulbs from the lifting (July) until planting (October-November), the sprinkling of water to enhance the bulb growth during the field growth above soil level.
The indirect effect on the spread of TBV due to temperature regimes during storage are shown in table 1.

Table 1: Spread of tulip breaking virus in tulip cv. Halcro affected by different temperature/regimes during storage.

Temperature treatment	Planting date	Bulb weight ratio	% virus
1. 20°C till September 1 and 17°C afterwards	October 15	109	24.6
2. 20°C continuously	October 15	100	21.0
3. 17°C continuously	October 15	90	19.5

The first treatment is best in yields of bulbs but the virus spread is likely to be more troublesome to reduce.

In 1981 plots of tulip cv. Halcro were abundantly sprinkled with water or not during the virus-spread season from the beginning of May onwards. The infection rates obtained were 50.7% TBV for the unsprinkled plots and 67.7% TBV in the plots which were sprinkled.

Virus control by mineral-oil and synthetic pyrethroid insecticides

In experiments in which plots with healthy tulips cv. Halcro were planted adjacent to infected tulips cv. Texas Flame the weekly spraying was applied from the first week of May until the third week of June. The results are shown in table 2.

Table 2: Effect of sprays of mineral oil and synthetic pyrethroid insecticide on the field spread of tulip breaking virus in tulip cv. Halcro.

Treatment	Active amount per ha	% virus		% reduction		Weight ratio	
		1980	1981	1980	1981	1980	1981
Untreated		27	56	0	0	100	100
Luxan oil H	15 l	--	16	--	72	--	92
Permethrin	100 g	18	39	34	31	102	98

The effect of mineral oil was more pronounced than that of the insecticide. A substantial reduction of the bulb yield was obtained by the mineral oil sprays.

Lily

The length of the growing period implies that substantial virus spread above soil level may occur from May till October. In 1988 the source plants of the non-persistently transmitted tulip breaking virus, and lily symptomless virus and the persistently transmissible lily virus X were planted in the plots. The results on the control of virus spread are shown in table 3.

Table 3: Effect of mineral-oil and synthetic pyrethroid insecticide sprays in different dosages on the spread of tulip breaking virus, lily symptomless svirus, and lily virus X altogether in the lily cv. Enchantment in 1988.

Treatment (mineral oil + insecticide)	Dosage interval		% virus 7 days	% virus 14 days	% reduction 7 days	% reduction 14 days	Weight ratio 7 days	Weight ratio 14 days
Untreated	0		42.5	42.5	0	0	100	100
Luxan oil H[1]	¼[3]	+ 0	11.4	15.4	73	64	96	99
Luxan oil H	½	+ 0	4.7	14.5	89	66	95	101
Luxan oil H + PP[2]	¼	+ 0	5.2	8.1	88	81	99	104
Luxan oil H + PP	¼	+ ½	8.2	9.4	81	78	99	102
Luxan oil H + PP	½	+ 1	2.0	5.4	95	87	94	97
Luxan oil H + PP	½	+ ½	2.7	2.7	94	94	94	97
PP	0	+ 1	12.4	15.1	71	65	99	101

1) 12.5 l/ha
2) PP = cyhalothrin; 15 g/ha
3) 1 = full, ½ = half, ¼ = quarter dosage

The mineral-oil sprays were more effective at a half dosage than at a quarter. The effect was improved by the addition of insecticide, which was slightly better at the full than half dosage. The insecticide spraying was substantially effective. The reduction of the virus spread was better in the weekly than fortnightly aplications.

Growers, advice to limit virus spread

The best control may be obtained by the use of mineral oil at half dosage plus pyrethroid inecticide at full dosage to reduce the spread of the viruses above soil level. This mixture earlier proved to be as effective as the mineral oil used at full dosage to limit the spread of the non-persistent transmitted viruses. The half dosage of mineral oil is favourable on to cultivars which may suffer from a reduction of the bulb yield at full dosage or even so at lower dosages of oil. The surplus of insecticide may be reduced by the use of half dosages. Factors additionally affecting the spread of viruses in lily and consequently the efficacy of the control are listed in table 4.

Table 4: Factors which affect the control of the virus spread above soil level in lily by minreal oils and synthetic pyrethroid insecticides from the first week of May till October. The treatment to be applied: + = yes, - = no, and ± = doubtful, in different dosages: 1 = full, ½ = half and ¼ = a quarter.

Factor	Dosage of mineral oil + pyrethroid insecticide ½ + 1	½ + ½	¼ + 1	¼ + ½
1. Adjacent virus sources				
- normal trade material	+	+	-	-
- certifiable 'virus-tested' material	+	+	±	-
Internal virus sources				
- less than 5%	+	+	±	-
- more than 5%	+	+	-	-
2. Susceptibility of cultivars				
- substantial	+	+	-	-
- little	+	+	+	±
3. Seasonal susceptibility				
- 'early' (May, June, July)	+	+	±	-
- 'late' (August, September)	+	+	+	±
4. Planting density				
- low	+	+	-	-
- high	+	+	+	±
5. Lily plus weeds				
- many	+	-	-	-
- little	+	±	-	-
6. Frequency of sprays				
- weekly	+	+	+	+
- fortnightly	±	±	±	-
7. Efficacy of oil brand				
- Luxan oil H	+	+	+	+
- 11E, Albolineum AK	+	+	±	-
- Duphar-7E oil	±	±	-	-
8. Daily weather conditions				
- strongly drying	±	±	±	-
- clouds/later during daylight	+	+	+	±
9. Seasonal weather conditions				
- strongly drying/nice	±	±	±	-
- clouds/fairly bad	+	+	+	±

Factor	Dosage of mineral oil + pyrethroid insecticide ½ + 1	½ + ½	¼ + 1	¼ + ½
10. Susceptibility of cultivars to reduction of bulb yields				
- little	+	+	+	+
- substantial	±	±	±	±

Note: Only the liquid formulations of the synthetic pyrethroid insecticides ought to be used.

Conclusions

The virus spread may be enhanced by factors which stimulate the yield of bulbs, e.g., in tulip by the optimal temperature regime during storage, or by the water sprinkling in the field. In bulbous iris the period of susceptibility to virus infection is considerably increased by the culture of virus-tested material. The virus-spread may be limited by synthetic pyrethroid insecticides in crops in which fairly short virus-spread seasons occur, and/or if they they suffer from substantial reduction in bulb yields by the use of mineral oil, e.g., in tulip. The insecticides may be used in different mixtures with mineral oil in dependance on several conditional factors which affect virus spread, e.g., in lily.

Resistance to a whitefly transmitted virus in muskmelon and wild relatives species

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Since at least 1982 the plastic-house muskmelon cultivation on the south east coast of Spain has been seriously affected by a yellowing disease (Esteva et al. 1988). The greenhouse whitefly, Trialeurodes vaporariorum Westwood, acts as the vector of the causal agent of this muskmelon yellowing disease (Soria and Gómez-Guillamón, 1988).

Only two greenhouse whitefly-transmitted diseases of cucurbits have been reported in the world, which are beet pseudo-yellows (BPYV) and cucumber yellows virus (CuYv). Other diseases reported in Netherlands (Dorst et al. 1980; Dorts et al. 1983), in France (Lot et al. 1980), in Bulgaria (Hristova and Natskova, 1986) and Australia (Duffus and Johnstone, 1981) have been tentatively identified as the BPYV. CuYV was described in Japan (Yamashita et al. 1979). In France was reported a disease which seems to be very close , if not identical,to CuYV.BPYV and CuYV could be caused by the same causal agent,although there are differences with regard to their host ranges. BPYV was described for the first time in California (Duffus, 1965).

The causal agent of Spanish muskmelon yellowing disease seems to be a virus whose morphology is like that of CuYV (Alfaro and Jordá, personal communication) and could be serologically related to BPYV (Duffus, personal communication). All the muskmelon hybrids and cultivars grown on the south east coast of Spain show high levels of susceptibility to muskmelon yellowing disease. Therefore in 1985 we initiated a programme to search sources of resistance.

During 1985, 1986, 1987, and 1988, 187 accessions of Spanish muskmelon landraces, one accession of Cucumis melo var. agrestis and the muskmelon lines 'Kafer Hakin', 'Nagata Kim Makuwa', Ginsen Makuwa','Muchianskaja','Freeman's cucuber', Miel Blanc', PI 161375, PI 157080 and PI 157084 were evaluated under natural infection conditions. Only one Spanish landrace, which belongs to 'Tendral'type and the C. melo var. agrestis accession did not show yellowing disease symptoms, but these two accessions showed unequivocal signs of infection after artificial inoculation by T. vaporariorum. The lines'Nagata Kim Makuwa', PI 161375 and PI 157084 displayed a notable reduction of the severity of symptoms. The 186 remaining landraces accessions and the other evaluated lines were highly susceptible to yellowing disease.

During 1989, 38 muskmelon accessions from Afghanistan, Crete, China, India, Hungary, South of Balkans, Turkestan, USA and Yugoslavia were evaluated under artificial inoculation conditions. The artificial inoculation was achieved by infecting muskmelon seedlings with 40 viruliferous for 72 hr. All these 38 accessions presented the characteristc symptoms of yellowing disease.

The species Cucumis anguria, C. meeusii, C. dipsaceus, C. figarei, C. africanus, Momordica involucrata, Lagenaria siceraria and Citrullus colocynthis, which were tested under natural infection conditions, behaved like resistent to yellowing disease. Cucumis ficifolius, C. zeyheri, C. metuliferus and Cucurbita martinezii showed yellowing disease symptoms under the same conditions. One accession of Cucumis myriocarpus behaved like resistant and another one was susceptible. The resistant accession of Cucumis myriocarpus and C. africanus have been afterwards tested under artificial inoculation conditions. The observed resistance under natural conditions was confirmed under artificial conditions.

Acknowledgments

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DEPLOYMENT OF RESISTANCE TO THE BARLEY YELLOW MOSAIC VIRUSES IN THE UK

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INTRODUCTION

Yellow mosaic symptoms on winter barley in the UK are caused by two distinct viruses, barley yellow mosaic virus (BaYMV) and barley mild mosaic virus (BaMMV). These were previously regarded as strains of BaYMV but they are now known to differ in their serological properties and in their nucleic acids. Both have filamentous particles of two lengths and both are transmitted by the fungus, *Polymyxa graminis*. Some cultivars remain symptomless when grown on fields infested with one or both of the viruses and cultivar resistance is the only practicable means of control.

BASIS OF RESISTANCE

All cultivars tested were equally susceptible to the vector (Adams, Swaby & Macfarlane, 1986) but neither virus can usually be detected in resistant cultivars growing on infested fields or inoculated in the laboratory. There is, therefore, total resistance (immunity) to the viruses in some cultivars. Immunity in UK cultivars appears to operate against both viruses but susceptible cultivars differ in their relative responses to the two viruses: for example, Maris Otter is more susceptible than Igri to BaMMV but Igri is the more susceptible to BaYMV. When a viruliferous isolate of *Polymyxa graminis* is grown in sand culture on roots of an immune cultivar, BaMMV is not transmitted by the zoospores or resting spores of the vector that are produced (Adams, Jones & Swaby, 1987).

FIELD EXPERIMENT

A field experiment was designed in collaboration with the Agricultural Development and Advisory Service of the Ministry of Agriculture to test whether growing an immune cultivar might decrease the concentration of virus carried by the fungal vector in the field. The four-year experiment began in autumn 1987 and has plots growing 1, 2 or 3 years of immune barley (cv. Torrent) or 1, 2 or 3 years of winter wheat before returning to susceptible cultivars. Control plots with continuous susceptible cultivars are also included. The experiment is being done at two sites, in Gloucestershire and Cambridgeshire, both infested with BaMMV. After harvest in 1988, soil samples were taken from the plots and seedlings of a BaMMV-susceptible cultivar were grown in a dilution series of soil in sterile sand. Roots were subsequently examined microscopically for the presence of *Polymyxa graminis* and by ELISA for BaMMV. A maximum likelihood computer program was then used to assess the most probable numbers of

infective propagules in the soil. At the end of this first crop season, there were no significant differences between the crop treatments; samples will be taken after further seasons to determine whether a change in inoculum levels of either virus or vector occurs. The combined results suggested that 20-30% of the *Polymyxa graminis* propagules were carrying BaMMV.

BREAKDOWN OF RESISTANCE

In winter 1988, disease symptoms were noticed for the first time on an immune cultivar (Torrent). Serological tests using polyclonal antisera could not distinguish the virus from the normal BaYMV. Several outbreaks occurred at well separated sites in 1988 and were confirmed in 1989. Attempts are being made to develop monoclonal antibodies to what is presumed to be a distinct strain of BaYMV. The virus has not been transmitted mechanically and experiments to obtain a viruliferous isolate of the vector in sand culture are in progress.

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VARIATION OF BARLEY YELLOW DWARF VIRUS CONTENT OVER TIME IN SPRING AND WINTER BARLEYS, WHETHER OR NOT CARRYING THE RESISTANCE Yd_2 GENE

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INTRODUCTION

In barley, the Yd_2 resistance gene has been the most useful gene in improving resistance of commercial cultivars to BYDV infection. However, when cultivars carrying Yd_2 have been sown in different places contrasting results have been obtained. Recently, investigations have demonstrated that the Yd_2 gene, whether in winter or spring barleys, protects the plants when they are infected with isolates belonging to group I or BYDV-MAV, but this protection is much less when infected with isolates belonging to group II or BYDV-RPV (HERRERA & PLUMB, 1988). Although, the mechanisms involved in the resistance are unknown, it has been suggested that the resistance shown in barleys containing the Yd_2 gene is associated with a decreased virus titre. This paper describes experiments that compared variation of virus content over time in shoots and roots of two pairs of barleys, with and without the Yd_2 gene.

MATERIALS AND METHODS

In the first experiment virus content was monitored over time in two pairs of barleys. The winter barleys Vixen (Yd_2+) and Igri (Yd_2-), and the spring near isogenic lines Atlas 68 (Yd_2+) and Atlas 57 (Yd_2-). British BYDV isolates PAV-like (G), MAV-like (F) and RPV-like (R-568) were inoculated simultaneously to all the cultivars 8 days after germination using five aphids per plant. Ten plants were inoculated for each host/isolate combination. On each sampling date the plants were harvested, separated into shoots and roots, and stored at -28°C, until extracted. Individual shoots or roots were pulverized in a mortar in liquid nitrogen, then ground in 1:10 (w/v) phosphate-buffered saline 0.5M, pH 7.4 containing 0.05% Tween-20 and 0.5% PVP. The extracts were centrifuged for 3 min. in an Eppendorf bench centrifuge before testing. The virus content was measured by indirect ELISA using monclonal antibodies MAC91, MAC92 and MAFF 2 for PAV, MAV and RPV-like isolates respectively as the second antibody.

In a second experiment the distribution of PAV and RPV-like isolates was studied in different plant sections over time, in a pair of winter barleys Vixen and Igri. Five plants at each host/isolate combinations were harvested 2, 4, 6, 8, 10 and 12 days after the inoculation. At each sampling day, the plants were separated into roots, stem (leaf sheaths), 1st leaf, 2nd and 3rd leaves and their virus content measured by indirect ELISA.

TABLE 1. Relative virus content (R.V.C.) of resistant cultivars with respect to susceptibles, based on ELISA values.

DAYS AFTER INOCULATION	BYDV-ISOLATES PAV SHOOTS	PAV ROOTS	MAV SHOOTS	MAV ROOTS	RPV SHOOTS	RPV ROOTS
VIXEN RELATIVE TO IGRI (Igri = 100)						
6	76.4	62.1	39.3*	22.5*	106.0	94.7
9	108.0	60.5*	33.5*	8.4*	100.0	92.7
13	131.0	73.6*	32.9*	8.6*	52.4*	75.7*
16	74.8	69.0*	31.7*	11.6*	83.9	89.9
19	[1] 45.1*	71.3	78.6	27.0*	61.7*	113.0*
23	[2] 35.3*	47.9*	127.0*	29.0*	65.1*	109.0
28	65.9*	48.1*	86.8	100.0	56.8*	92.4
ATLAS 68 RELATIVE TO ATLAS 57 (Atlas 57=100)						
6	23.6*	8.8	6.5*	3.0*	84.2	89.1
9	14.6*	7.1*	22.6*	10.6*	67.3*	82.7
13	30.9*	12.2*	34.3*	9.5*	77.6	118.0*
16	21.6*	50.2*	28.4*	48.6*	58.6*	18.2*
19	8.6*	14.6*	70.6	94.6	164.0*	88.0
23	16.7*	13.3*	60.0	19.1*	120.0	112.1*
28	15.5*	50.9*	42.0*	100.0	102.0	111.0*

[1] *, Significative difference ($P<0.05$) between pairs of cultivars at each sampling date.

[2] Underlining means date in which the susceptible pair had the largest virus concentration.

RESULTS AND DISCUSSION

When the variation of virus content was monitored over a period of 4 weeks after inoculation, the results showed that, in shoots and roots of cultivars carrying Yd_2 gene Vixen (Yd_2+) and Atlas 68 (Yd_2+) infected with either PAV or MAV had less virus than the paired cultivar without the gene Igri (Yd_2-) and Atlas 57 (Yd_2-) (TABLE 1). By contrast when the plants were inefcted with this RPV-like there were fewer statistical significant differences in virus content and sometimes the resistant cultivar had more virus than the susceptible.

Differences in virus content between resistant and susceptible cultivars were also noted when the distribution of virus content in different plant parts were compared (Fig. 1). Although, in cultivars with and without Yd_2 the virus content varied among different sections, the proportion of virus in roots was much less in resistant barleys 2 and 4 days after the inoculation than in the susceptible. For example, Vixen had 1% and 7% of the total virus content in root tissues 2 and 4 days after the inoculation, while Igri had 64% and 66% respectively. However, when the cultivars were infected with the isolate (RPV-like) virulent against Yd_2 gene,there were no differences in the distribution of virus content between resistant and susceptible cultivars.

Previous suggestions have indicated that, in field infection the isolates PAV and MAV are avirulent against Yd_2 gene, while the isolate RPV is virulent against the same gene (HERRERA & PLUMB, 1988). CARRIGAN et al., (1983) found no evidence that differences in virus translocation are involved in resistance. However, this study has shown that difference in virus multiplication between resistant and susceptible cultivars may explain differences in virulence among the isolates, and such difference appeared to be related to differences in roots rather than shoots.

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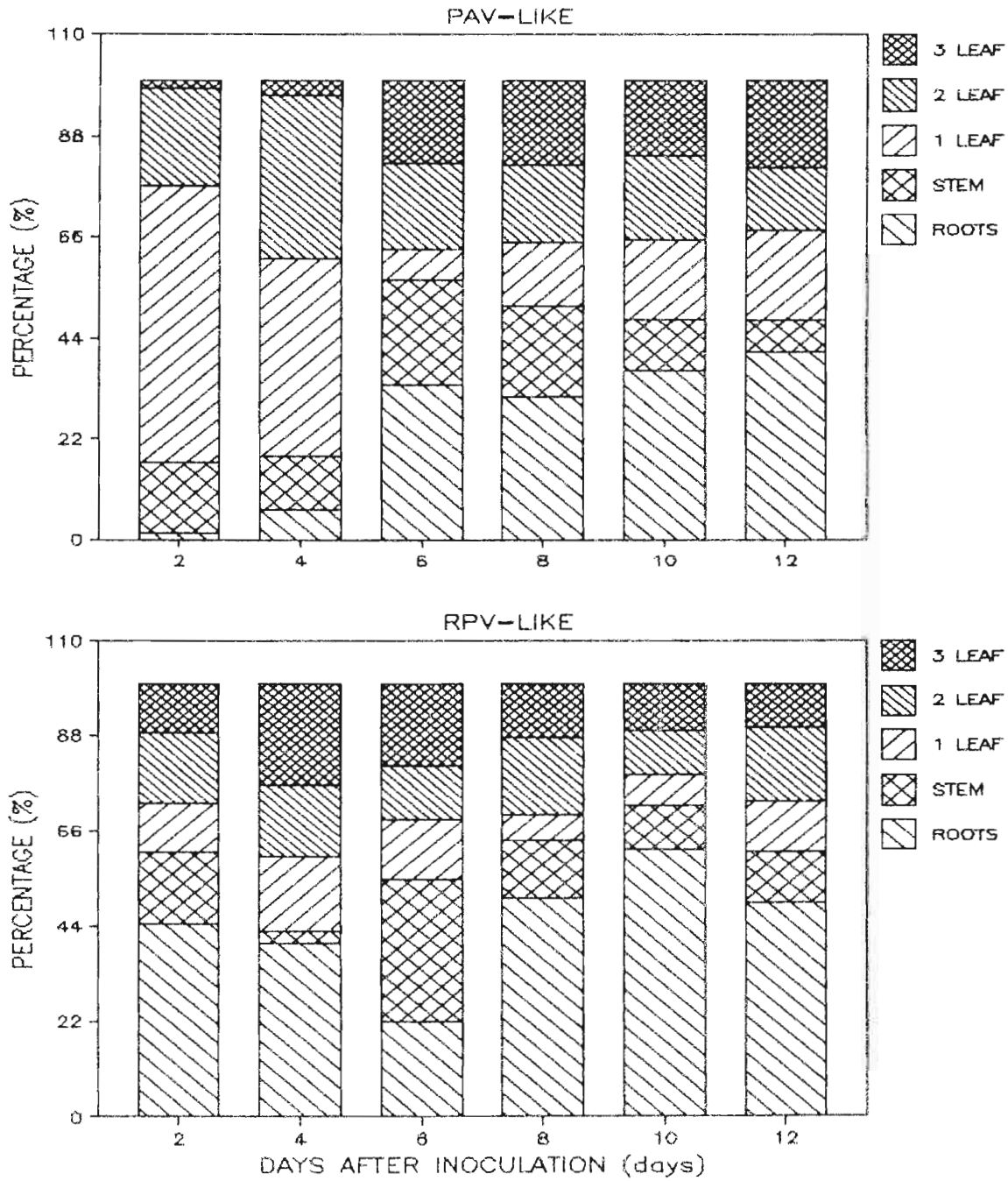
FIGURE 1. Variation in virus content as percent of total virus content in the plant among individual parts of Vixen (Yd_2^+) plants infected with either PAV or RPV-like isolates.



ISOLATION AND CHARACTERIZATION OF TOBAMOVIRUS INFECTING PEPPER PLANTS IN THE SOUTHEAST REGION OF SPAIN. THE INTERACTION AMONG PEPPER PATHOTYPES AND THE RESISTANCE GENES IN CAPSICUM SPP.

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In a survey of virus causing diseases in commercial pepper plants grown under plastic in the Southeast region of Spain, it was found that member of the tobamovirus group were the main disease-causing agents (Alonso et al. 1989). By studying the responses of Capsicum spp. which carry the L^0, L^1, L^2 and L^3 resistance genes to tobamoviruses (Boukema 1984), it has been determined that the majority of the tobamoviruses isolated corresponds to the $P_{1,2}$ pathotype, and some of them to the $P_{1,2,3}$ pathotype.

By immunodiffusion tests and radioimmunoassay, it was determined that both pathotypes are closely related. Further analysis of the nucleotide sequence of the RNA region which codifies for their coat proteins has allow us to corroborate the previous findings.

To better understand the interaction among the different pathotypes and the resistance genes in Capsicum spp, it has been analyzed the influence of different factors in the hypersensitive reaction in pepper plants. In addition, we have determined the ability of the P_0 and P_1 pathotypes (Boukema 1984) to induce acquired resistance against challenging inoculations with the $P_{1,2}$ and $P_{1,2,3}$ pathotypes. It was found that the P_0 pathotype is more effective in protecting pepper plants than the P_1 pathotype. The inoculation with P_0 protects completely the Capsicum spp. against a challenging inoculation with the $P_{1,2}$ pathotype. However, it does only partially protect the inoculated pepper plants against a challenging inoculation with the $P_{1,2,3}$ pathotype.

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SESSION 5

RESISTANCE AUX VECTEURS/ VECTOR RESISTANCE

GROUNDNUT RESISTANCE TO APHIS *CRACCIVORA* (KOCH)

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INTRODUCTION

Aphis *craccivora* (Koch) (groundnut aphid, cowpea aphid) is a vector of at least seven viruses which attack groundnut (*Arachis hypogaea L.*) including the non persistent peanut stripe virus (PStV) and the persistent Groundnut rosette virus complex (GRV) (Feakin 1973).

In Africa, GRV is a serious viral disease and potential control methods include host plant resistance to the aphid vector. It has been demonstrated that use of aphid resistant genotypes lowers the aphid population and slows down the spread of the virus thereby reducing GRV incidence in the crop (Evans 1954, ICRISAT 1988). Screening trials of germplasm by the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) have identified several aphid resistant genotypes. An understanding of the resistance mechanisms would enable plant breeders to develop new genotypes which would possess aphid resistance as well as desirable agronomic features. A collaborative project between the Overseas Development Natural Resources Institute (ODNRI) and ICRISAT has been established to investigate these mechanisms and the techniques used and results are summarised here.

METHODS

The following techniques have been used with *A. craccivora* on the susceptible and resistant genotypes:

1. Behavioural observations in field and screenhouse conditions.
2. Measurements of growth and development.
3. Electronic monitoring of stylet penetration behaviour.
4. Stylectomy by VHF microcautery.
5. Phloem sap collection.
6. Ultrastructure of phloem sieve elements.

RESULTS AND DISCUSSION

In the field no significant differences were found between the rate of arrival of alates (winged aphids) onto the susceptible and resistant genotypes but population development was slower on EC 36892 and after heavy rainfall, population decline was faster on the resistant genotype EC 36892 than on susceptible TMV 2. Behavioural studies in the greenhouse also showed that both alates and apterae were not inhibited from alighting or walking onto EC 36892, but in choice tests a significant redistribution of aphids in favour of the susceptible genotype TMV 2 occurred over the following 10 h period.

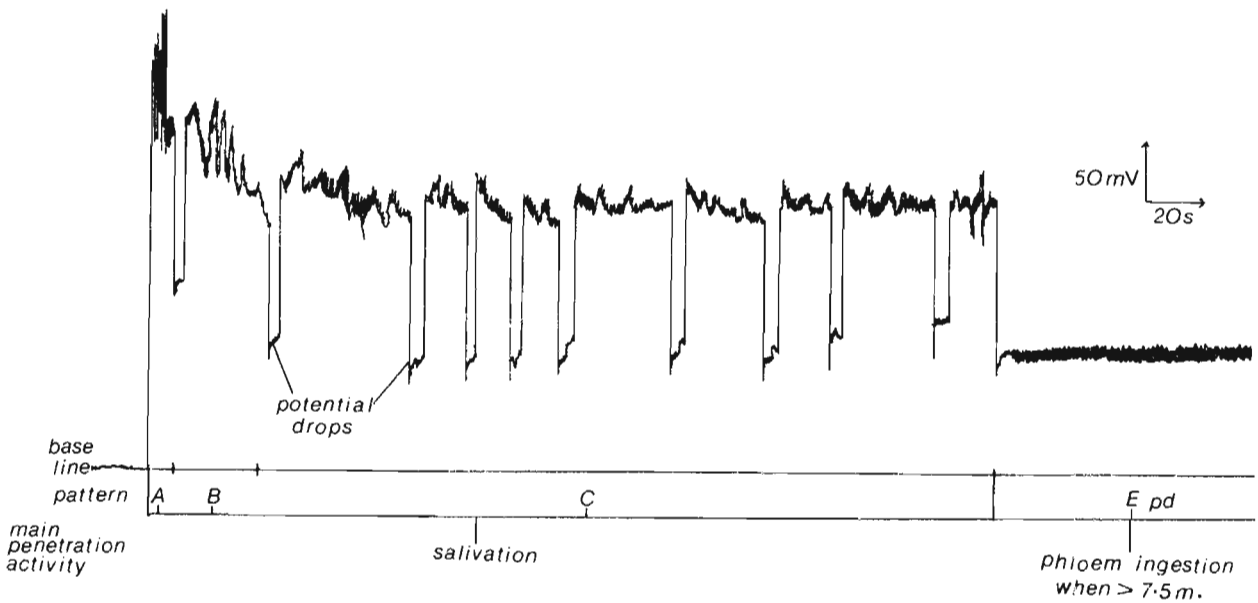
Aphid development from 1st instar nymph to maturity took approximately 1 day longer on EC 36892 and nymphal production over the first five days of reproduction was 70% lower compared to TMV 2.

The behavioural and developmental studies indicated that the aphids were not deterred from alighting on or exploring plants of EC 36892 and that aphid resistance is reflected by longer development time and poorer reproduction. These effects could be due to one or more of the following: an inability to locate the phloem sieve element (the preferred feeding tissue of most aphid species), interference of sustained phloem feeding and or ingesting phloem sap which is nutritionally inadequate.

To investigate these factors a DC electronic system which monitors stylet penetration activities of homopterans has been used (Tjallingii 1988). The waveforms or Electrical penetration graphs (EPGs) (FIG 1) were recorded for 8h and the results indicated that the time taken to initiate phloem ingestion (as measured by time taken to reach Pattern E(pd) from the start of recording) was similar on both TMV 2 (120min) and EC 36892 (134min) but that the mean total duration of phloem ingestion (duration of Pattern E (pd)) was significantly shorter (a reduction of 40%) on the resistant genotype. Assuming that the rates of ingestion are similar on both genotypes, the EPG data suggests that chemical and/or physical factors in the sieve elements of the resistant genotype inhibit phloem sap ingestion by the aphid. Reduced feeding from the phloem would explain the poorer development of the aphid and lower incidence of GRV on EC 36892.

Chemical analysis of phloem sap from the two genotypes is underway using sap exudates from severed aphid stylets. Physical comparisons of the sieve elements from the two genotypes will also be made using the high resolution of transmission electron microscopy.

Figure 1. EPG aphid patterns and the penetration activities.



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Rice resistance to *Nilaparvata lugens* (Stal)

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INTRODUCTION

The brown planthopper, *Nilaparvata lugens* is a serious pest of rice, causing feeding damage by sap removal and transmission of the propagative ragged and grassy stunt viruses (Sogawa 1982). Plant breeders at the International Rice Research Institute (IRRI), Philippines have produced rice varieties which offer resistance to *N. lugens* (Khush 1979) but their effectiveness can be reduced by the rapid development of populations which overcome this resistance (Claridge and den Hollander 1980). An understanding of the resistance mechanisms in a range of rice varieties would assist in the development of others with more durable field resistance and in planning the sequential release of varieties possessing different mechanisms. In 1984 a collaborative project between the Overseas Development Natural Resources Institute, U.K. and IRRI was established to investigate the bases of rice resistance mechanisms to *N. lugens* in cultivated and wild rices. This summary gives a brief description of the methods and results.

METHODS

A number of techniques were developed to study the specialised feeding behaviour of *N. lugens* on a range of rice varieties. These included:

1) a high resolution video system to study the specialised feeding behaviour and activity of the insect (Cook et al., 1987).

2) an electronic system to monitor stylet penetration activities within plant tissues (Kimmins 1989).

3) honeydew clocks to monitor feeding activity (Padgham and Woodhead 1988, 1989).

4) Bioassays of water soluble and surface extracts from the tested varieties/species (Woodhead and Padgham 1988).

5) laser stylectomy to obtain samples of the insects' principal feeding substrate, phloem sap (Spiller and Padgham in prep.).

The rice varieties/species used:

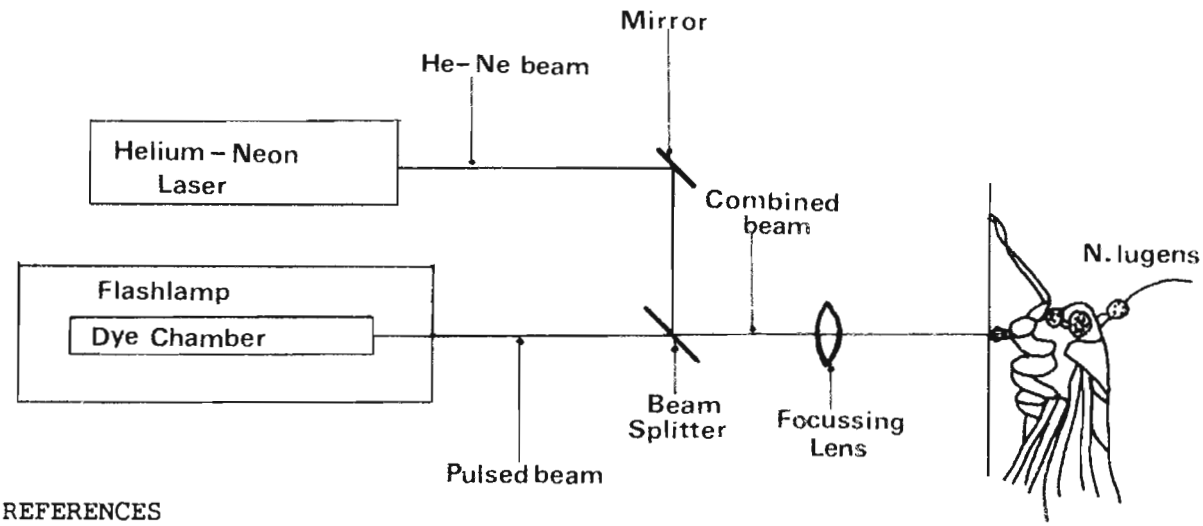
	variety/species	resistance gene
Oryza sativa	TN 1, IR22	none
	IR26, IR46	Bph 1
	IR36, ASD7	bph 2
	Rathu Heenati/IR62	Bph 3
	Babawee	bph 4
	Oryza perennis	?
	O. officinalis	?
	O.punctata	?
	Leersia hexandra	?

RESULTS AND DISCUSSION

In the initial phase the activity and feeding behaviour of *N. lugens* were studied in detail on three varieties: IR 22 (susceptible), IR 46 (moderately resistant) and IR 62 (highly resistant). Observations of feeding behaviour over four hours showed more frequent, shorter probes on IR 46 and IR 62 and less honeydew resulted from feeding on IR 46 and IR 62 over the four hour period (Cook et al., 1986, Kimmins et al., 1987). Over a 24 hour period, insect weight gain was lowest on IR 62 though they were similar on IR 22 and IR 46. These results suggested that different mechanisms were operating in the two resistant varieties. Histological observations of saliva sheaths left in the rice tissues and the electronic monitoring system indicated that *N. lugens* had no difficulties in locating their principle feeding site, the phloem, in IR 46 and IR 62, but that ingestion from this tissue could not be sustained in the 4h period. Bioassays using surface chemicals showed that the increased movement and probing activity which was observed on IR 46 was due to the chemical composition of the surface wax, while the poorer development and growth rates of *N. lugens* on IR 62 appeared to be caused by water soluble chemicals which inhibit feeding. The latter effect could be due to either a lack of ingestion stimulants and/or the presence of ingestion deterrents. Presumably their absence or presence is detected in the phloem. At present attempts to collect samples of phloem sap from IR 62 are underway using laser stylectomy (Fig 1).

The study was extended to compare the feeding responses of *N. lugens* on eight cultivated varieties, three wild rice species and the weed *Leersia hexandra* (Padgham and Woodhead 1989). Strong surface effects were observed in IR 36 and *O. punctata* while phloem constituents were implicated as the main resistance factors in the other varieties and species.

Figure 1. Diagram of laser-stylet cutting system



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HOST PLANT RESISTANCE TO APHID VECTOR AND THE SPREAD OF COWPEA APHID-BORNE MOSAIC VIRUS IN COWPEA LINES

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The settling behaviour of Aphis craccivora Koch, the vector of cowpea aphid-borne mosaic virus (CAMV) in cowpea, Vigna unguiculata (L.) Walp., on aphid-resistant, aphid-tolerant and aphid - susceptible cowpea lines was investigated. Although apterae counts on the aphid-susceptible TVu3629 and aphid-tolerant TVu 1190 lines were higher than on aphid-resistant TVu 408-2 and TVu 3273, apterae were more widely dispersed among the latter. This indicated that aphid-resistance could induce aphids to be more restless and mobile. The incidence of CAMV was not necessarily positively correlated with alatae numbers in the aphid-resistant lines.

Thus aphid activity, such as rapid dispersal and short, frequent probes on aphid-resistant lines, may be behaviourally more suited to the spread of nonpersistent CAMV in aphid-resistant cowpea lines. Furthermore, unless resistance to all the Known vectors of CAMV could be bred into a given cultivar, aphid resistance may not prevent the spread of CAMV by non-colonizing, transitory vectors.

We conclude that attention should be directed towards the development of cultivars with direct resistance to CAMV, since several cowpea lines are known not only to be resistant to CAMV, but also to the other known viruses of cowpea.

IMPROVEMENT OF PEACH RESISTANCE TO GREEN PEACH APHID : MAIN FACTURES OF THE RESISTANCE USED

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The green peach aphid (GPA) is the most serious aphid pest of peach in French orchards (ACTA, 1974). Studies of resistance to GPA in peach, *Prunus persica* L. Batsch, began at Bordeaux in 1976.

Preliminary work showed different patterns of resistance to GPA in peach cultivars that have not been improved for fruit quality (Massonié 1979 ; Massonié *et al* 1982). The S.2678 peach cultivar was selected for a breeding program assigned to introduce the GPA resistance into cultivated varieties.

In the orchard only the sexual forms but not the fundatrices of the GPA colonize the cultivar S.2678. *Fundatrigeniae apterae* artificially placed on orchard or nursery trees or seedlings in laboratory leave these plants one day to one week later. The few nymphs deposited by the adults leave or die. The cultivar is also resistant to *Myzus varians* Davids. But it is susceptible to *Brachycaudus persicae* Pass. *Brachycaudus prunicola* Kltb. and *Hyalopterus amygdali* Blanch. (Massonié & Maison, 1979). The resistance to *M. persicae* and *M. varians* is linked with a necrosis of plant tissues at the feeding site. The resistance is controlled by a single dominant gene (Massonie & *al*, 1984). No resistant breaking biotypes have been observed in experimental orchards located in the south west and the south east of the French country. Plum pox virus transmission by *M. persicae* and *M. varians* is less efficient on necrotic resistant peach seedlings (Massonié & Maison, 1986).

Susceptible and necrotic resistant varieties of hybrids, young and old leaves, differ in their qualitative composition in phenolic compounds. Necrotic reaction induces changes, essentially in phenolic acids. Studies about the direct effect of phenolics on GPA, by incorporating them in a synthetic media, have given conflicting results. First studies showed that phenolic extracts were deleterious and resistant hybrid extracts more deleterious than susceptible hybrid extracts (Bastide & *al*, 1988) ; control tests don't corroborate these results (Rahbe & *al*, 1988).

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Characterisation of resistance in raspberry to the virus vector aphid, Amphorophora idaei

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Amphorophora idaei is the main, possibly the only, natural vector of importance for four viruses commonly found infecting raspberry in Europe (Jones, 1986). Several genes for resistance to **A. idaei** have been identified in **Rubus**, some differing in their effectiveness against four described biotypes of **A. idaei** in England (Briggs, 1965; Keep & Knight, 1967; Keep, Knight & Parker, 1970). When tested under field conditions, resistance was shown to be very effective not only in controlling **A. idaei** numbers but also in preventing infection with the viruses it transmits (Jones 1976, 1979). Currently, more than 80% of the UK raspberry hectarage is planted with cultivars containing resistance genes to **A. idaei** (Jones, 1988). In some localities an increase has been found in the occurrence of a biotype of **A. idaei** able to colonise raspberry cultivars containing the **A. idaei** resistance gene, A_1. Although other resistance genes are known that are effective against this biotype, the occurrence of this and other biotypes emphasises the need for a more fundamental understanding of the precise mechanism(s) of resistance so as to plan a sound strategy for the continued effectiveness in crops of this biological form of aphid and virus control.

Recent experiments have determined the components and the relative effectiveness of resistance to **A. idaei** in raspberry cultivars containing major genes A_1 and A_{10} and those containing only minor genes (Birch & Jones, 1988). Both antixenosis and antibiosis were identified in each resistance source, with the strongest resistance being expressed in cultivars containing gene A_{10}, and the weakest in cultivars containing only minor gene resistance (Table 1). More recent studies have indicated that the expression of resistance may be influenced by environmental conditions.

The level of resistance of raspberry to **A. idaei** was decreased by wiping leaves with some organic solvents. Principal component analysis of data, obtained by gas chromatography (GC) of dichloro-methane extracts from a range of **A. idaei**-resistant and -susceptible cultivars, identified two or three principal components associated with resistance. The first of these components was associated with a compound which mass spectroscopy (MS) showed to be a pentacyclic triterpenoid of the amyrin series. Leaf volatiles emitted from **A. idaei**-susceptible and -resistant cvs trapped onto Tenax TA and analysed by thermal desorption in a Perkin Elmer ATD_{50} apparatus coupled to a GC-MS, indicated a five-fold greater concentration of several volatile compounds in resistant cultivars than in susceptible ones.

Table 1. Responses of **Amphorophora idaei**, biotype 1 to raspberry cultivars differing in resistance to this aphid

Cultivar and resistance source	No. alatae settling 40 h after placing in an infestation house	No. nymphs produced during 5 days after removal from infestation house	% apterae remaining on excised leaves floating on water after 48 h
Malling Jewel (not resistant)	337	492	100
Norfolk Giant (minor genes)	239	298	90
Malling Landmark (gene A_1)	104	40	20
Glen Prosen (gene A_1)	135	181	50
Joy (gene A_{10})	98	6	20

Further work in progress is aimed at identifying the specific chemicals involved in resistance using principal component analysis of GC and GC-MS data from progeny seedlings segregating for resistance genes A_1 and A_{10} together with analysis of volatiles using a GC linked to an electro-antennogram. Analysis of electrical penetration graph (EPG) data and olfactometer observations will seek to determine the effect of resistance on aphid probing and feeding behaviour and the likely consequences for virus acquisition and transmission.

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RESISTANCE TO NEMATODE VECTORS TO REDUCE GRAPE FANLEAF VIRUS SPREAD IN VINEYARDS

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At present, control of grape fanleaf virus (GFV) in vineyards is currently based on production of virus-free plants by clonal selection and soil fumigation to kill the dagger nematode, *Xiphinema index*, vector of the disease. But probably the use of fumigants will be prohibited shortly in many vineyards, due to the action of environmentalists. Thus, the only available method to prevent spread of the virus in vineyards will be to leave the contaminated soils without grape cultivation during 6 to 8 years or more, which is obviously unrealistic, especially in vineyards producing high-quality wines. So, it is important to develop quickly new techniques for breeding rootstocks resistant to the virus and its vector would they be induced by conventional or biotechnological approaches.

Since ten years, the muscadine grape (*Muscadinia rotundifolia*), native from USA, is known to be highly resistant to *Xiphinema index* and GFV-transmission. But this species is not suitable as rootstock because its redhibitory defects (poor rooting ability and graft-incompatibility with *Vitis vinifera*).

From 1985 to 1988, 60 intergeneric hybrids of *V. vinifera* x *M. rotundifolia* were screened in pots for resistance to the nematode. Resistance ratings were based on density levels in nematode populations found on the roots of tested plants over an inoculation period of 12 months or more. In the majority of cases, each test was replicated on 12 plants, propagated by *in vitro* culture.

For all the hybrids tested, the populations of nematodes decreased significantly : On average, they were one-hundred times lower on hybrids than on classic rootstocks used as checks. Virus infection of the hybrids was tested by ELISA after planting in highly contamined soil. Some of the most nematode-resistant hybrids show a high but not complete level of field resistance to GFV-transmission.

Based on the results of this study, the use of intergeneric hybrids of *Vitis* x *Muscadinia* as rootstock cultivars or as parents in rootstock breeding programs is discussed, taking into account the cultural and biological characteristics of these plants.

	Number of varieties tested	Number of inoculated plants	Initial inoculum (nemas/Kg soil)	Inoculation period (months)	Final population estimate (nemas/Kg soil)	Rate of multiplication
M. rotundifolia	3	29	400	15	32* (1 - 270)**	0.08
Rootstock cultivar (Check)	1	10	400	15	3700 (900 - 8500)	9.3
F 1 hybrids	11	159	25	6	3.2 (0 - 94)	0.13
Rootstock cultivars (Check)	4	25	25	6	184 (6 - 670)	7.4
F 1 hybrids	18	200	50	18	10.6 (0 - 172)	0.21
Rootstock cultivars (Check)	4	44	50	18	735 (28 - 5242)	14.7
F 1 hybrids	23	254	50	12	7.6 (0 - 142)	0.15
BC1 hybrids	8	84	50	12	112 (4 - 994)	2.2
Rootstock cultivar (Check)	1	12	50	12	407 (58 - 596)	8.1

( )* Mean ( )** Range

RESISTANCE TO XIPHINEMA INDEX OF MUSCADINIA ROTUNDIFOLIA AND ITS HYBRIDS WITH VITIS VINIFERA

(Tests made from 1985 to 1988)

IDENTIFICATION		GFV CONTAMINATION : ELISA TESTS *				NEMATODE RESISTANCE
F 1	N° hybrid	Parent M. rotundifolia	Number of plants	Positive reaction	Doubtful reaction	Negative reaction	X. index rate of multiplication **
VRH	8624	cv. "Trayshed"	11	10	0	1	0.57
VRH	8731	cv. "Noble"	19	2	0	17	0.02
VRH	8736	cv. "Noble"	13	12	0	1	0.69
VRH	8771	cv. "NC 184-4"	16	8	0	8	0.03
VRH	8712	cv. "Carlos"	16	5	1	10	0.00
VRH	83/9/79	cv. "Carlos"	16	1	2	13	0.05
VRH	87/16/79	cv. "Carlos"	15	3	1	11	0.03
VRH	87/20/79	cv. "Carlos"	10	0	2	8	0.03
VRH	97/71/79	cv. "Carlos"	15	5	2	8	0.04
VRH	99/80/79	cv. "Carlos"	16	5	3	8	0.06
VRH	99/91/79	cv. "Carlos"	13	5	3	5	0.06
CHECK	Rootstock cultivar "Fercal"		37	35	0	2	7.7

* Three years after inoculation

** After an inoculation period of six months (25 nemas/Kg soil)

- TABLE 2 -

RESISTANCE TO GRAPE FANLEAF VIRUS CONTAMINATION IN SOME F 1 HYBRIDS RESISTANT TO XIPHINEMA INDEX

SESSION NOCTURNE/
EVENING SESSION

SOME EXAMPLES OF USING REGIONALIZED VARIABLES THEORY IN EPIDEMIOLOGY STUDIES.

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The interest in regionalized variable theory and its application for sampling estimates of disease rate through the kriging technics was described previously in a communication and a publication.

This notion makes it possible to follow the *a posteriori* epidemiological dynamics of a disease and so build hypotheses on possible vectors by studying the figures of semi-variograms.

Last, it is possible to extend these applications, through the cokriging methods, by proving the relationships between the spread dynamics of a supposed vector and the disease dynamics. It could then limit the transmission trials to the few species whose spread dynamics seem to fit with the disease one.

CROP PROTECTION AND PLANT ARCHITECTURE.

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The meristem control process of plants during growth allows measurement of the interaction of plant architecture with immediate environment.

The modelling and simulation of the growth of crop plants rest on the qualitative knowledge obtained by the Laboratory of Botany of Francis HALLE with the concept of architectural model and reiteration and also on the mathematical theoretical model founded by the Modelling Laboratory of CIRAD based on the stochastic controls of the lengthening of the internodes of young vegetative axes.

This model allows estimates of good accuracy of the damage intensity on crops and permits them to be visualized through computer graphic methods.

Potential of remote sensing for detection monitoring and forecasting virus infections

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Virus infection modifies leaf pigment content, plant architecture and transpiration rate. Thus the reflectance and radiative temperature of a plant canopy can be affected and remote sensing techniques enable these changes to be detected.

Earth resource satellite data can be used if a 20 x 20 m ground resolution is adequate. If more detailed information is needed it is necessary to use airborne or ground based radiometrie measurements.

Meteorological satellite data (ground resolution 1 x 1 or 5 x 5 km) enable the ground surface temperature and the global status of the vegetation to be determined sequentially but without detailed information on the different fields. Such data can be used to forecast a virus infection. It depends on the growth of the host plants and crops and also on the growth of the population of vectors (aphids etc.) and of the possibility of flight. So infection models based on these data can be developed.

RESISTANCE INDUITE/
INDUCED RESISTANCE

SESSION 6

PROTECTION CROISEE/
CROSS PROTECTION

CONTROL OF ZUCCHINI YELLOW MOSAIC VIRUS IN SQUASH BY CROSS PROTECTION.

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Zucchini Yellow Mosaic Virus (ZYMV) is an aphid borne potyvirus causing very severe diseases of Cucurbits in most regions in the world. In Zucchini Squash (**Cucurbita pepo** L.) symptoms include mosaic and distortion of leaves and fruits. Generally one or two weeks after inoculation infected plants do not produce anymore marketable fruits. ZYMV presents a high variability, and variants differing in symptomatology, host range, virulence towards resistance genes, and aphid transmissibility have been reported (Lisa and Lecoq, 1984).

No control measure has been found to be both economical and efficient in the epidemiological situation of Southern France, and resistance genes to ZYMV have not been identified so far in **C.pepo** germplasm collections. The only available resistance to ZYMV was reported in the related species **C. moschata** Duch. (Provvidenti et al., 1984). Breeding for resistance to ZYMV in squash will require interspecific crosses, and will probably be a long process. In this context, cross protection appeared to be an interesting alternative approach to control ZYMV.

A variant (ZYMV WK) which induces very mild mottle symptoms in melon and squash leaves and no symptoms on fruits, was selected from a poorly aphid transmissible isolate (E15 PAT) deficient in the helper component function (Lecoq, 1986). E15 PAT produces severe symptoms and was itself derived from the highly aphid transmissible and severe isolate E15 AT. ZYMV WK was obtained from a melon plant infected by E15 PAT which developped axillary branches with very attenuated symptoms. Extracts from these branches were mechanically inoculated to melon which subsequently exhibited mild symptoms. This subculture was then passed through single local lesion transfers on **Chenopodium amaranticolor** Coste et Reyn. and **C. quinoa** Willd. before being increased in Cucurbits. ZYMV WK is poorly aphid transmitted, to the same extent as E15 PAT.

ZYMV WK could not be differentiated from E15 AT or E15 PAT either serologically in SDS immunodiffusion or slide precipitin tests, or by its host range. However ZYMV WK produced milder symptoms both in Cucurbits (melon, cucumber or squash) and non Cucurbits (**C. amaranticolor**).

In preliminary tests carried on in the greenhouse, a first inoculation by ZYMV WK conferred a protection against a challenge inoculation by severe isolates from various geographical origins (France, Algeria, Spain, Jordan, and the USA). However when a serological variant from the Réunion island was used the protection was only partial, while no protection at all was observed against Watermelon Mosaic Virus 2 (WMV2).

Taking advantage of a severe natural outbreak of ZYMV in summer 1988 an experiment was designed to evaluate the field efficiency of the protection conferred by ZYMV WK. 2 blocks of 40 zucchini squash F1 hybrid "Diamant" were planted 2 weeks following mechanical inoculation with ZYMV WK, next to 2 blocks of non inoculated plants. Fruits were collected daily or every 2 days, estimated for commercial value, and weighted. Four weeks after planting 98% of the uninoculated plants presented severe ZYMV symptoms, while at the end of the experiment none of the inoculated plants showed severe ZYMV symptoms. Results are presented in Fig. 1 and 2.

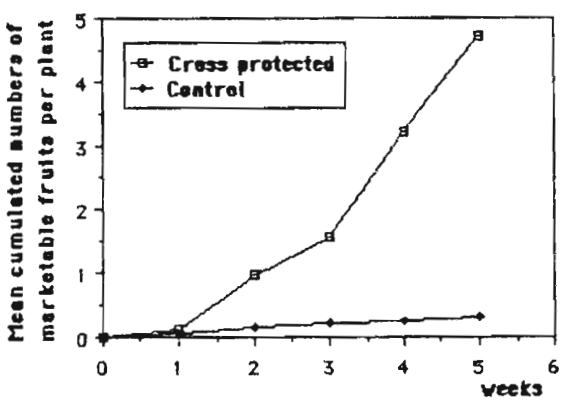

Figure 1: Production in marketable fruits of Zucchini squash plants inoculated or not with ZYMV WK, under severe natural ZYMV epidemic conditions, field test, summer 1988.

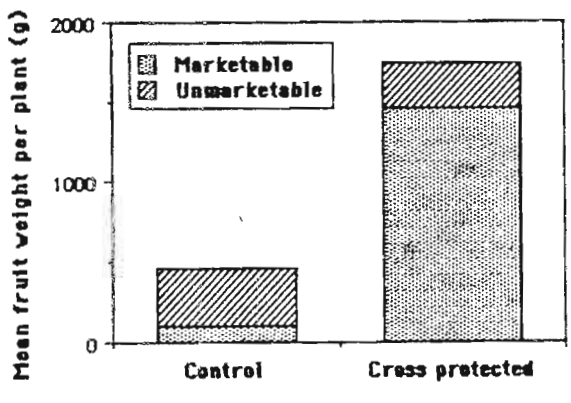

Figure 2: Mean fruit weights produced by Zucchini squash plants inoculated or not with ZYMV WK under severe natural ZYMV epidemic conditions, field test, summer 1988.

It is worthwhile noting that most of the unmarketable fruits collected in the cross protected plots were produced by plants coinfected with Cucumber Mosaic virus (CMV) which also alter fruit quality. Preinoculation with ZYMV WK did not apparently affect the spread of CMV or WMV2.

To further investigate the potential interest of ZYMV WK, an experiment was done to compare the production of plants inoculated by ZYMV WK to that of non inoculated plants. To avoid contamination by other viruses, 2 blocks of 16 plants for each treatment were planted in early spring under a plastic tunnel. Fruits were collected and evaluated as previously. No infection by CMV, WMV2 or severe ZYMV was detected during the experiment. Results are presented in Fig. 3 and 4.

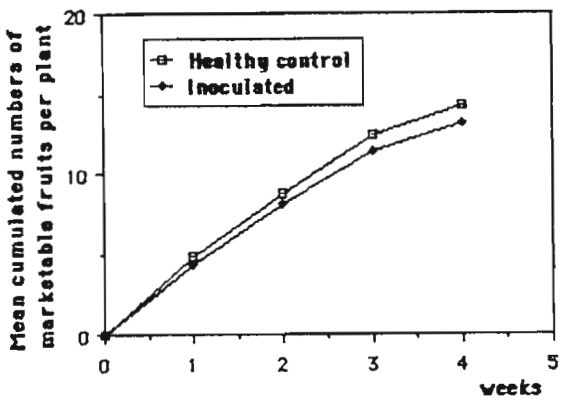

Figure 3: Production in marketable fruits of Zucchini squash plants inoculated or not with ZYMV WK, under protected conditions, tunnel test, spring 1989.

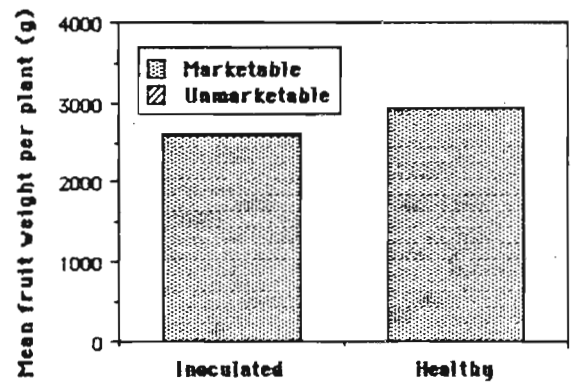

Figure 4: Mean fruit weights produced by Zucchini squash plants inoculated or not with ZYMV WK, under protected conditions, tunnel test, spring 1989.

There is a slightly lower marketable fruit production on inoculated plants. The difference observed in the number of marketable fruits (7%) is not significant at the 5% level, while that observed on the marketable fruit weight per plant (11%) is significant at the 5% level. The percentage of unmarketable fruits was very low in both treatments (less than 1%).

ZYMV WK is efficient in protecting Zucchini squash against severe ZYMV infections under field conditions, without affecting greatly the potential production of the crop. Further investigations are needed to demonstrate the feasability of using ZYMV WK to control ZYMV in the field on a large scale basis. However, its poor aphid transmissibility makes it very attractive for this purpose.

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ANALYSIS OF THE CROSS PROTECTION BETWEEN TOMATO BLACK RING NEPOVIRUS AND HUNGARIAN GRAPEVINE CHROME MOSAIC NEPOVIRUS AND COMPARISON OF THEIR NUCLEOTIDE SEQUENCE.

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Tomato black ring virus (TBRV) and Grapevine chrome mosaic virus (GCMV) are members of the Nepovirus group of plant viruses. They have a divided genome composed of two separately encapsidated, single stranded, messenger sense RNA molecules of molecular weight approximately $1.6x10^6$ kDa (RNA2) and $2.6x10^6$ kDa (RNA1). They are distantly related as shown by the ability to construct viable pseudorecombinants by exchanging genomic segments between the two viruses (Doz *et al.*, 1980) and by slight cross-reactivity levels both in nucleic acids hybridization (Dodd & Robinson, 1984) and in serology (Kerlan & Dunez, 1983).

On the experimental herbaceous host *Chenopodium quinoa* (Wild), these two viruses induce very different symptoms : TBRV causes a severe systemic necrosis often leading to plant death while GCMV induces only a mild chlorotic mottle with slight growth retardation. Previous inoculation of *C. quinoa* plants with GCMV protects them from the expression of the severe symptoms normally caused by TBRV infection (Doz *et al.*, 1982).

We have analyzed the cross-protection phenomenon between GCMV and TBRV by quantitating both the viral capsids (by ELISA, using specific antisera) and the viral RNAs (by molecular hybridization, using specific cRNA probes) in singly and doubly infected plants. A space/time study of the virus repartition in the plant has been achieved by quantitating the coat protein and viral RNAs at several levels of the infected plants and at several times after inoculation. The behaviour (spreading, accumulation) of each virus in doubly infected plants is only marginally affected by the presence of the other one, suggesting that they replicate independently even in protected plants. These results suggest that mechanisms different from the mere inhibition of infection/spreading of the challenging strain are at work in this interaction : the expression of the viral symptomatology seems to be inhibited.

In an attempt to understand the molecular bases of this particular type of protection, we have cloned (in a cDNA form) and completely sequenced the genome of GCMV and compared the data with the sequence of TBRV RNA1 (Greif *et al.*, 1988) and RNA2 (Meyer *et al.*, 1986). Whereas cross protection is usually observed only between closely related strains of a virus, our results show that the two viruses share only about 60 % homology (both at the nucleotide and at the amino acid level) and that they should thus be regarded as distinct viruses. Attempts have also been made to get transgenic plants expressing either of the full-length genomic RNAs or the isolated coat protein gene. The results of these experiments will be discussed.

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Isolation and Study of Mild Strains of Cocoa Swollen Shoot Virus for Possible Cross Protection

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INTRODUCTION

Swollen shoot disease is caused by a mealybug-transmitted virus that occurs in virtually all the cocoa-growing areas in West Africa. A national "cutting-out" programme has been in operation since 1946 in attempts to control the disease. To date, over 190 million trees have been removed yet infection is now more prevalent than ever, especially in the worst-affected 'Area of Mass Infection' in Eastern Region. Recent reports of the exploitation of mild virus strain protection in disease control in other crops such as citrus (Grant & Costa, 1951; Muller & Costa, 1977) and papaya (Yeh et al., 1988) have rekindled interest in earlier studies of this phenomenon in cocoa (Crowdy & Posnette, 1947; Posnette & Todd, 1955) and their possible application in areas where cutting out has been least effective.

This paper reports on the initial results of cross-protection studies conducted in the gauzehouse.

MATERIALS AND METHODS

Isolates of mild strains of CSSV (SS70, SS90, SS167E and SS365B) have been maintained in cocoa for several years. Some of the plants infected with these mild strains produced symptoms of both mild and severe strains which were separated by serial mealybug transfers to Amelonado cocoa beans. Uncontaminated cultures of SS70 and SS90 were then used to inoculate 60 seedlings of each of the following Amazon hybrids: T85/799 x T17/524, T63/971 x T17/524, T85/799 x IMC76, T85/799 x Amelonado by patch grafting. Seedlings of the sensitive Amelonado cultivar were used as standards.

In a second test both hybrids and Amelonado seedlings were inoculated using mealybugs and patch-grafting to find the best inoculation technique. Seeds of T85/799 x Amelonado and Amelonado were inoculated using only mealybugs.

In another test, seedlings of Amelonado and hybrids which had been infected with SS70, SS167E and SS365B for 18 months were challenged with severe New Juaben strain (IA).

RESULTS

ISOLATION OF PURE CULTURES

The four mild strains produced similar leaf symptoms. After several transfers

to Amelonado seeds the usual symptoms produced were red vein banding which disappeared within a few days. Fine vein flecking, vein banding, yellow chlorosis and stem swellings were also produced but all the leaf symptoms were transient. Two of the original source plants infected with strain SS90 as well as one infected with strain SS70 induced symptoms of both mild and severe strains in the test seedlings. This indicated that these sources were contaminated with virulent strains. From the serial transfers, pure cultures of the four mild strains were isolated and maintained in Amelonado seedlings. The transmission rates of SS167E and SS365B by mealybugs were very low.

REACTION OF INTER-AMAZON HYBRIDS TO MILD STRAINS

The symptoms produced in the hybrids were similar to those produced in Amelonado but were less prominent. Moreover, the leaf symptoms usually disappeared within three days in the hybrids compared with about five days in Amelonado seedlings. There was a higher transmission rate of the mild strains to Amelonado than to the hybrids. Percentage infection ranged from 23-40 in Amelonado compared to 5-15 in the hybrids.

COMPARISON OF INOCULATION BY GRAFTING AND MEALYBUG TRANSMISSION

The results of inoculation by patch-grafting and mealybugs (Table 1) showed that more plants were infected by patch-grafting than by mealybug transmission. Neither method resulted in 100% infection. The percentage infection obtained using patch-grafting ranged from 21.7 to 53.3 compared with 10.0 to 11.7 using mealybugs. However, more infections were obtained by mealybug transmission to seeds. Percentage infection of 68.3 to 70.0 was then obtained.

The incubation period from patch grafts to symptoms ranged from 91 to 154 days, while that using mealybugs was from 86 to 149 days.

CHALLENGE OF MILD STRAIN-INFECTED SEEDLINGS WITH SEVERE NEW JUABEN STRAIN

The number of seedlings previously infected with mild CSSV strains which produced persistent mild symptoms after the challenge are detailed in Table 2. Symptoms of the severe strain first appeared three to five months after the challenge inoculation. In some of the seedlings, symptoms of the severe strain persisted for some weeks. Thereafter, only symptoms of mild strains were consistently produced. However, in seedlings pre-inoculated with SS167E and SS365B, most of them produced permanent leaf symptoms and swellings.

DISCUSSION

Because swollen shoot is one of the major limiting factors for growing cocoa in Ghana, the selection of mild strains to protect cocoa trees from the severe effects of virulent strains is of great importance. The results indicate that the effectiveness of cross-protection was influenced by the mild strain used. For example SS167E and SS365B strains do not appear to offer complete protection when challenged with severe 1A. The challenged seedlings developed permanent

leaf symptoms with swellings. Similar results were reported by Yeh et al., (1988) in cross protection studies of papaya ringspot virus.

The reaction of the inter-Amazon hybrids to CSSV mild strains was similar to that of Amelonado and therefore these hybrids of the type now being issued to farmers could be used in cross-protection work. However, because of the early disappearance of the leaf symptoms, the hybrids need much more attention when recording symptoms after inoculation.

Patch-grafting appeared to be the most efficient method for inoculating seedlings with mild strains. Although higher rates of infection were obtained when seeds were inoculated with mild strains using mealybugs the practical use of this method in the field will pose many problems as seeds would have to be inoculated before distribution to farmers.

Posnette and Todd (1955) used only Amelonado trees and reported that only 35 of 416 plants protected with mild strain showed severe symptoms compared with 273 of 387 unprotected trees. In the present study using Inter-Amazon hybrids and the SS70 strain, successful cross-protection ranged from 50 to 100%. These promising results are being investigated further in the field.

ACKNOWLEDGEMENTS

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Table 1. Comparison of patch-grafting and mealybug transmission of mild strains of CSSV to cocoa seeds and seedlings

Progeny	Patch-grafting		Mealybug transmission	
	No. tested	% infection	No. tested	% infection
Seedlings				
T85/799 x Amelonado	60	53.3	60	11.7
T63/971 x T17/524	60	21.7	60	10.0
Amelonado	60	50.0	60	11.7
Seeds				
T85/799 x Amel	--	--	60	68.3
Amel	--	--	60	70.0

Table 2. Seedlings pre-inoculated with different mild strains of CSSV and later challenged with severe strain

Progeny	No. challenged	No. showing only mild symptoms	No. showing persistent severe symptoms
SS70 Strain			
T85/799 x Amelonado	4	2	2
T85/799 x T17/524	5	4	1
T85/799 x IMC76	2	2	0
T63/971 x T17/524	5	3	2
Amelonado	5	4	1
SS167E Strain			
T85/799 x Amwl	4	1	3
T63/971 x T17/524	5	2	3
Amelonado	4	2	2
SS365B Strain			
T85/799 x Amelonado	3	1	2
T63/971 x T17/524	4	1	3
Amelonado	3	1	2

THE QUESTION OF THE CROSS PROTECTION PHENOMENON IN VIROID INFECTION BETWEEN TWO STRAINS OF CITRUS EXOCORTIS VIROID

(1) (2)
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INTRODUCTION

The first evidence of cross protection with viroid systems was reported by Fernow (1967) as a delay in symptom expression when tomato seedlings which had been inoculated with a nearly symptomless strain of potato spindle tuber viroid (PSTV) were chanllenge inoculated severe strain of PSTV. Recently, Khoury et al., (1988) showed that the severe strain of PSTV replicated at detectable levels in potato plants previously inoculated with a mild strain. Studies by Niblett et al., (1978) indicated that a similar "protection" response could be observed with the sequential inoculation of even four different viroids but simply the delay in symptom expression depended on the viroids and even the host used in each assay.

In contrast with these results, no evidence for cross protection has been found between mild and severe isolates of the exocortis disease agent (Kapur et al., 1974; Garnsey et al., 1989). With the present understanding of the "exocortis disease" as a complex of different viroids (Duran-Vila et al., 1988), caution must be exercised in characterizing field isolates based solely on the symptom reaction on the bioassay host, citron (Citrus medica L.). Therefore, in the absence of a clear identification of the exocortis isolates used in previous cross protection assays, the interpretation of such tests is questionable.

The citrus exocortis viroid (CEV) is a well characterized 371 nucleotive viroid and has been found associated with the severe forms of the exocortis disease. It induces severe stunting, epinasty, leaf rugosity and necrosis on inoculated Gynura aurantiaca DC plants. In this report we present data on the characterization of a new strain of CEV that causes mild symptoms on gynura as well as evidence for temporary interference with a standard severe CEV strain that might be considered as a "cross protection" response.

METHODS

Infectivity assays. The field sources used were selected from

the citrus collection maintained at IVIA (Moncada, Valencia). Inoculated citron (Citrus medica L.) seedling clone Arizona 861-1, was used as source of tissue for preparation of inocula. Preparations of nucleic acids from inoculated citrons were assayed for infectivity by slash inoculation of Gynura plants. The biological activity was determined by the appearance of symptoms on gynura over a 40 days period. Relative infectivity was estimated as the total number of infected plant-days on 3 inoculated gynura per treatment.

In cross protection assays, gynura plants were slash inoculated with nucleic acid extracts from gynura infected with an exocortis strain (CEV-129) which induced mild symptoms. Lots of 6 plants were challenge inoculated with the severe strain of CEV at weekly intervals beginning at the second week after inoculation with the mild CEV. In every inoculation with the challenge strain, 3 non-protected gynura were also inoculated as controls.

Viroid detection. Tip tissue consisting of young leaves and stems (5-6 g) were homogenized in a extraction medium containing water-saturated phenol and partitioned in 2M LiCL (Semancik et al., 1975). These nucleic acid preparations were used for infectivity assays and/or sequential polyacrylamide gel electrophoresis (s PAGE) (Semancik and Harper, 1984).

RESULTS AND DISCUSSION

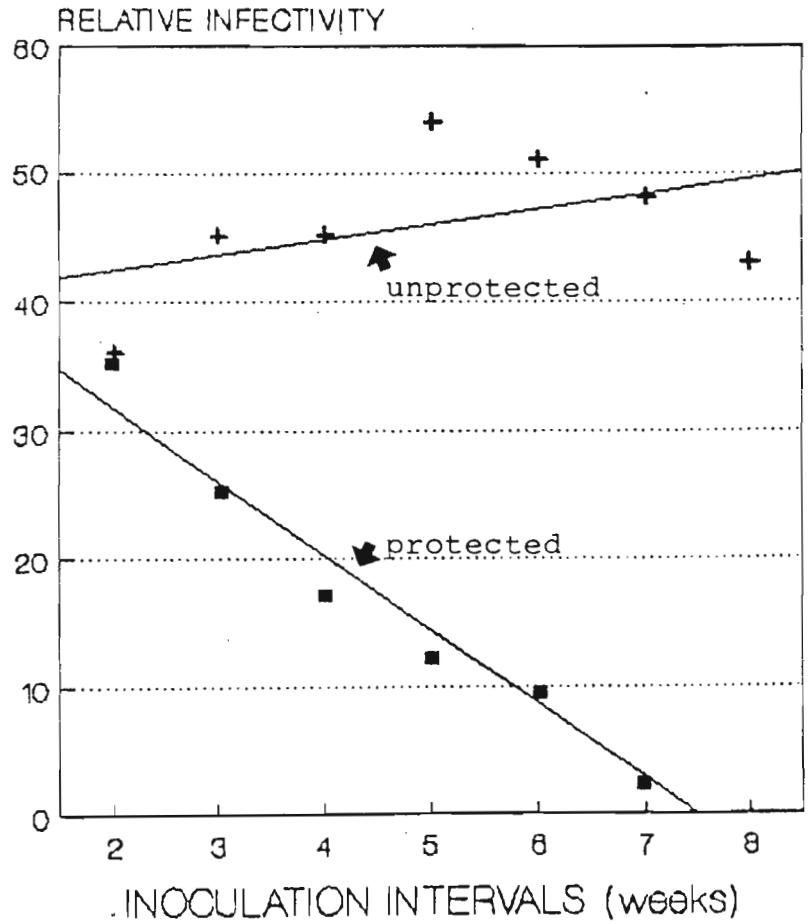
Detection of a CEV Strain Inducing Mild Symptoms on Gynura.

Nucleic acid extracts from citrons induced symptoms on inoculated Gynura 4 weeks after inoculation. Nine out ten isolates induced severe stunting, epinasty and leaf rugosity symptoms identical to the standard CEV isolated from California. A single source induced wilting of the top leaves followed by scorching and necrosis of the tip and edge of the leave blade. Nucleic acid extracts from inoculated Gynura were analyzed by sPAGE, and only a single viroid, CEV-129. However the titer of CEV-129 was markedly lower than in the nucleic acid a extracts from plants inoculated with standard CEV. The relative infectivity of CEV-129 was also 6 to 9 fold lower than the standard CEV estimated between 36-54. Further characterization showed that CEV-129 was a true strain of CEV probably containing minor changes in the basic CEV sequence.

Cross-protection Between Mild and Severe CEV

Cross protection tests demonstrated that inoculation of gynura with mild CEV resulted in a progression of effects in symptom expression ranging from no difference over that observed in non-protected controls to apparent complete "protection". The development of the severe symptoms induced by the severe CEV depended on the period of time elapsed between protecting and challenging inoculations. When plants were challenged 2-3 weeks after inoculation with the mild strain, the rapid development of

the severe symptoms prevented the development and/or observation of the wilting and necrosis of the tip and edge of the leaf blade, characteristic of the mild syndrome. When the plants were challenge inoculated after 6-8 weeks, only mild symptoms developed. The progressive decrease in the specific infectivity of the severe CEV in plants preinoculated with mild CEV as compared with the non-protected controls, is represented below.



After the plants were cut back, the second flush of tissue displayed the characteristic symptoms of the severe strain. When these plants were analyzed by s-PAGE, no differences in viroid titers were detected. Bioassay of the same extracts on gynura induced a common severe reaction indicating the complete absence of any protecting effect with prolonged time of mixed infection with two closely-related strains of CEV.

These cross protection tests demonstrated that the mild strain of CEV can induce a range of responses dependent upon the temporal relationships between the "protecting" and the "challenging" inoculations. The results using a true CEV strain as the protective and challenge inoculations showed a delay in symptom expression, as previously reported between mild and severe strains of PSTV on inoculated potato (Fernow, 1967; Khoury et al., 1988). The delay in symptom expression is further documented by the decrease in relative infectivity as the interval between protecting and

challenging inoculation is increased. The absence of plants showing severe symptoms when 8 weeks elapsed between the two inoculations could be interpreted as a true "protection" as already described by Niblett et al., (1978). However, the challenge strain did increase to subtantial levels. Therefore, an accurate description of the effect produced would indicate that any "protection" has been only from the symptoms induced by the severe strain. More importantly, this response is only temporary since the regrowth from plants receiving both protecting and challenging inoculations was also severe and in all cases, the severe strain prevailed.

In summary, the progressive decrease in the relative infectivity of the severe CEV with increased time between protecting and challenging inoculations indicates the importance of temporal considerations in the observation and interpretation of the "cross-protection" phenomenon. From this data, it is possible to record a series of responses with a single viroid-host combination which can include no protection, a delay in symptom expression, and "complete protection".

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SESSION 7

BIOLOGIE MOLECULAIRE - BIOTECHNOLOGIE - PLANTES TRANSGENIQUES / MOLECULAR BIOLOGY - BIOTECHNOLOGY -TRANSGENIC PLANTS

Nucleotide sequence of the coat protein gene of Lettuce Mosaic Virus.

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Lettuce Mosaic Virus (LMV) is a seed and aphid transmitted potyvirus. It occurs commonly on lettuce and may cause severe economic losses. Two genes of tolerance to LMV are known i.e. "g" and "mo"; they delay and attenuate symptom expression and prevent seed transmission. Since 1981, new pathotypes (LMV1, LMV9 and LMVE) have been identified in the mediterranean area which induce severe diseases on tolerant plants. However, these pathotypes could not be differentiated by serology.

The RNA of LMV was purified and cDNA (obtained by the method of Gubler and Hoffman, 1983) was cloned . An oligonucleotide probe derived from a conserved region of potyvirus coat protein was used to screen clones covering coat protein gene . Twenty five clones were obtained and sequenced. The N-terminal region of the coat protein has been microsequenced and the first amino acids determined. The sequence encodes for a 277 amino acids protein. The protein has a molecular weight of 34000 Da as estimated by SDS-PAGE . It is very homologous to other known potyvirus coat proteins (55 % of homology with that of PVY) but the N-terminal region is quite different. The cleavage site between the coat protein and the polymerase, is unusual. The 3' non coding was also determined and shows no homology with other potyvirus.

The strategy of introducing the coat protein of LMV into lettuce plants has been adopted to protect them against new pathotypes . Different constructions derived from coat protein gene of LMV are being undertaken which would serve to transform lettuce protoplasts by electroporation .

Transgenic Plants as a Tool for Studying Cucumber Mosaic Virus Satellite RNA Replication

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Certain satellite RNAs, such as STobRV, replicate via a rolling circle mechanism, whereas the mechanism for CMV satellite RNA replication has never been clearly determined. We have observed in transgenic plants that precursor transcripts bearing a single copy of CMV satellite RNA can serve as template for efficient satellite RNA replication, which suggests that circular intermediates are not involved. We are currently testing the hypothesis that satellite RNA is replicated by internal initiation on the precursor transcript, upon CMV infection.

Molecular Basis for the Necrogenic Potential of Cucumber Mosaic Virus Satellite RNA

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Comparison of the published sequences of CMV satellite RNAs does not make it possible to distinguish between the two major classes of CMV satellite RNA, which differ in their ability to either reduce or aggravate symptom expression on tomato. Using as starting material cDNAs corresponding to the two satellite RNAs of the two groups (necrogenic and non-necrogenic) which have the fewest sequence differences, we have prepared recombinant molecules in order to localize the region for the necrogenic response of tomato.

Cloning of the Coat Protein Gene of Beet Necrotic Yellow Vein Virus and its Expression in Sugarbeet "Hairy Roots"

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Beet necrotic yellow vein virus (BNYVV) which causes the rhizomania disease of sugarbeet possesses a segmented genome of 4 RNA species (Putz 1977). The viral coat protein (CP) cistron is located near the 5'-extremity of the second-largest RNA (RNA-2) (Bouzoubaa et al. 1986). The synthesis, amplification and cloning of a cDNA comprising the CP gene with or without its leader sequence, and the stable integration and expression of the CP gene in transformed sugarbeet roots is shown on this poster.

Viral RNA was extracted from purified BNYVV, isolate Yu2. cDNA for the CP gene and its leader sequence was synthesized using two synthetic oligodeoxynucleotide primers. cDNA first strand synthesis was carried out using unfractionated BNYVV-RNA and AMV reverse transcriptase, second strand synthesis and amplification was done by polymerase chain reaction (PCR). The cDNA was cloned into a standard in vitro transcription vector pGEM3zf (+) (Promega). A fragment from the above construct containing the coat protein gene and the 5'-leader sequence was then cloned into the plant expression vector pRT103 (Töpfer et al. 1987). In this construct, the CP gene is under the control of the constitutive 35S-promoter of cauliflower mosaic virus (CaMV). A Hind III-fragment containing the 35S-promoter, the CP gene and the polyadenylation signal of CaMV was cloned into the binary vector pLX222 for plant transformation (Landsmann et al. 1988). This vector provides the selectable marker gene neomycin phosphotransferase under the control of the nos-promoter.

The binary vector construct was transferred to Agrobacterium strain C58C1(pRiA4) via triparental mating to test the expression of the CP gene in transformed sugarbeet tissue. Explants from sterile sugarbeet seedlings were inoculated with the Agrobacteria, and the induced "hairy roots" were cultivated in liquid medium with or without selection for kanamycin resistance. Southern blot analysis of "hairy root" DNA revealed the presence of one to several copies of the transferred CP gene in different "hairy root" clones. In these clones, production of BNYVV coat protein could be detected by ELISA and Western blotting using polyclonal antiserum against complete virus particles. Experiments are under way to infect transgenic "hairy roots" with BNYVV to see if CP synthesis inhibits virus multiplication.

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INTERFERENCE WITH TURNIP YELLOW MOSAIC VIRUS REPLICATION *IN VITRO* BY MEANS OF ENGINEERED DEFECTIVE INTERFERING RNAs.

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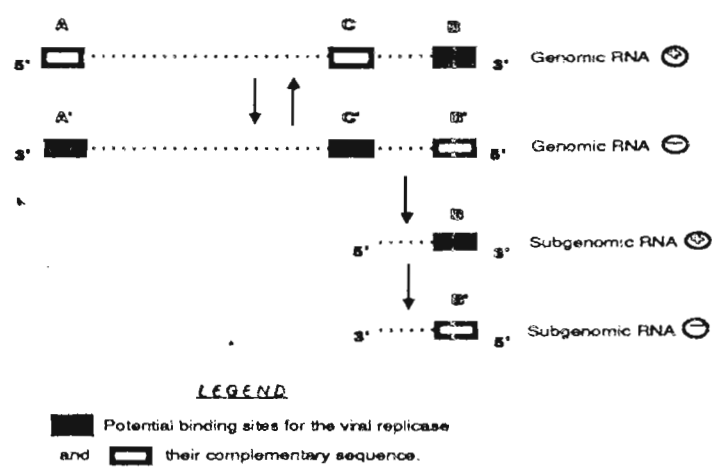
The monopartite genome of Turnip Yellow Mosaic Virus (TYMV), the type member of the Tymoviruses, has been recently sequenced (1). It is a 6318 nucleotide-long positive stranded RNA from which the non structural viral proteins are expressed, whereas the coat protein is translated from a subgenomic RNA that results from internal initiation of replication on the (-) RNA of genomic length (2).
The multiplication and the genetic expression of TYMV require the use of three initiation sites for the viral replicase during the life cycle of the virus. These are the 3' end of the (+) RNA, the 3' end of the (-) RNA and an internal initiation site on the (-) RNA for the synthesis of the subgenomic RNA.They are respectively named B, A', and C' on figure 1.

Fig.1: Schematic representation of TYMV replication.



Since these cis-active sequences are involved in a fundamental process in infectivity, different antiviral strategies based on molecular interference with viral replication have been envisaged. Among them, the "sense RNA approach" seems promising. TYMV is one of the few plant viruses from which the viral replicase has been sufficiently purified to allow a reliable *in vitro* replication assay. Therefore it has been possible to test for the efficiency of this sense approach *in vitro* : it was shown in our laboratory that TYMV RNA replication can be inhibited by 90% when transcripts from cDNA clones corresponding to the 3' end of the genomic RNA are introduced in an *in vitro* replication assay (3). This inhibition is attributed to a competition phenomenon between the 3' end "genome-like fragments" and the viral RNA for binding to the replicase.Transgenic plants expressing these "genome like fragments" have been obtained and we are currently testing them for their resistance against TYMV infection.

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Following this approach, we have constructed Defective Interfering (DI) RNAs encompassing different combinations of the binding sites for the TYMV replicase (ACB, AB, CB, A'C'B', A'B', C'B', BB'...). When present in DI genomes, the A and A' sites in our constructs have no extraviral nucleotides at their extremities. We are currently analyzing the behaviour of the engineered DI genomes as simple templates for the replicase on one hand, and as inhibitory competitors for the binding of this enzyme in the presence of the genomic RNA on the other hand. Together with a better understanding of the interactions between the viral genome and the replicase, we think that these *in vitro* studies might provide precious information about the sense RNA approach before being transposed and tested *in vivo* .

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STRATEGY TO OBTAIN SYNTHESIS OF RANDOM PVY PEPTIDES IN TRANSGENIC PLANTS : A TOOL TO STUDY VIRAL FUNCTIONS AND TO CREATE NOVEL TOLERANCE GENES.

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Potato Virus Y (PVY) is able to infect various *Solanaceae*; it is the type member of the Potyvirus family which form the largest and economically the most important group of plant viruses. The potyvirus genome consists of a single-stranded positive-sense RNA molecule with a poly-A tail at the 3' end and a small viral protein (VPg) covalently linked to the 5' end.

After shotgun cloning of cDNAs corresponding to the entire genome of PVYn in the vector Bluescript (Stratagene), the entire nucleotide sequence of PVYn (9704 nts) was determined by the dideoxy method (Robaglia *et al.* 1989). A polyprotein of 3063 amino acids is encoded by the single long open reading frame of the 9704 nucleotide genomic RNA.

We decided to get insight in potential protection against a challenge infecting virus and in the viral products responsible for symptoms, by scanning expression of sub-regions of the viral genome in transgenic plants.

A way to obtain tolerance or resistance of plants to viruses is to specifically disrupt the pathogen's life cycle with a pathogen gene that is expressed at the wrong time, in the wrong amount, and/or in a counterfunctional form (Sanford and Johnston 1985).

This has been illustrated by the creation of transgenic plants expressing anti-sense RNA corresponding to parts of a pathogen genome or the capsid protein of the challenge virus. The first partial protection of transgenic plants expressing a viral capsid protein was reported for the Tobacco Mosaic Virus (Powell Abel *et al.* 1986). Since then, similar results have been reported for an increasing number of plant viruses.

Our goal is to create a large number of plants expressing random parts of the PVY polyprotein, in order to screen the ability of each peptide to either protect these plants against a PVY infection or produce(s) symptoms in infected plants. We are also expecting some information about the function of the polypeptides encoded by the NH2 terminal part of the genome.

Although this has not been demonstrated so far, it is likely that expression of fragments of viral proteins other than the capsid might also protect the plant by :

- inducing the production of a host substance toxic to the challenge virus,
- sequestering a compound necessary for the life cycle of the virus,

- competing for a binding site of the virus,
- creating a mutated viral protein.

Concerning proteins responsible for symptoms of potyvirus infected plants, nothing is known up to now. In the case of Cauliflower Mosaic virus, it was shown that that gene VI expression in transgenic tobacco plants produces a symptomatic phenotype (Baughman *et al.* 1988).

For that purpose, we have have designed the following strategy. A cDNA library of PVY will be established in a plant expression vector. The cDNA will be ligated to BamH1 adaptor oligonucleotides containing an ATG codon within a consensus sequence for translation initiation in plants. As a result in the transgenic plants, fragments of the viral polyprotein will be translated, fused to the functional part of a protein conferring kanamycin resistance (NPTII). Transformed tobacco plants obtained by electroporation will be selected for their resistance to kanamycin and then tested for their tolerance to the pathogen as well as examined for their phenotype compared with healthy and infected control plants.

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ABSTRACT

THE EXPRESSION AND FUNCTIONS OF POTYVIRAL GENE PRODUCTS IN TRANSGENIC AND NON-TRANSGENIC PLANTS

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cDNAs corresponding to various parts of the genome of the potyvirus, tobacco vein mottling virus (TVMV), have been introduced into tobacco plants via Agrobacterium vectors. These include the cylindrical inclusion protein (CI) gene, the coat protein (CP) gene, the small nuclear inclusion protein (NIa) gene, a cassette containing the CI-NIa-large nuclear inclusion protein (NIb)-CP genes, and the same cassette with a large deletion in the CP gene. The CI- and CP-transgenic plants have been shown to express the appropriate TVMV proteins; analysis of the other plants for expression of viral RNA and proteins is underway. The transgenic plants are also being tested for their susceptibility to infection by TVMV and other potyviruses.

Antibodies to the putative 34 kDa product of the cistron located at the 5' end of TVMV RNA have been obtained from rabbits injected with a polypeptide synthesized in a bacterial expression vector. The antibodies have been used to detect a 34 kDa protein in TVMV-infected plants. This protein is one of the two gene products which had not previously been detected in potyvirus-infected plants.

Clones containing full-length TVMV cDNA have been produced and introduced into vectors containing bacteriophage T3 or T7 RNA polymerase promoters. In vitro transcription of linearized plasmids yields RNA which is infectious in tobacco plants and protoplasts. Typical

vein mottling symptoms and progeny TVMV RNA and proteins are produced in transcript-inoculated plants. A series of mutants are being produced by in vitro, site-specific alterations in nucleotide sequence of the cDNA and used to examine the roles of the various potyviral gene products in replication, transport, aphid transmissibility and symptom development. Mutant genes will also be used in the production of transgenic plants in order to more closely define those parts of potyviral genes which have potential for the expression of resistance phenotypes.

CHARACTERISATION OF THE GENOME OF PLUM POX VIRUS, STRAIN D, AN APHID-TRANSMISSIBLE VIRUS.

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Plum pox virus (PPV), strain D, was isolated from apricot orchard of the South eastern of France. This virus is vectored by aphids (Myzus persicae, Aphis citricola...). To control the spread of this virus, we have projected molecular approaches to analyze the expression of PPV genome and to produce tools for viral detection.
Genomic RNA, 3.5×10^6 d, was copied into cDNA and cloned in plasmid Bluescribe M13+. Five inserts with size of 1.6 - 1.8 - 3.4 - 5.5 and 1.5 Kbp overlapped and covered the 99.6 % of the genomic RNA. These cloned cDNA was basically used to generate deleted cDNA subclones from DNaseI treatment. These deleted subclones were used as template for the double-stranded DNA sequencing.
The PPV, strain D, genome encodes a long open reading frame of 3141 amino acids. As in another potyvirus (TVMV, tobacco vein mottling virus) the Helper Component of PPV shared significant sequence homology to the aphid transmission factor (gene II product) of CaMV (Cauliflower mosaic virus). Comparison of the PPV, strain D, polyprotein sequence with that of PPV-NAT (strain not-aphid transmissible) has shown considerable homology. Variabilities were observed in the Helper Component (HC) and the Coat protein (CP) genes. Deletions were obseved into HC and CP genes of PPV-NAT.
We have proposed from these genetic variabilities, the possible explanation to support the difference in biological properties of these strains.

OBTENTION OF A FULL - LENGTH COPY DNA OF THE GENOMIC RNA OF POTATO VIRUS Y .

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We are interested in understanding the molecular basis of development and pathogenicity of Potato Virus Y (PVY), a potyvirus responsible for important crop loss in potato as well as tobacco.

Robaglia *et al.* (1989) have sequenced the entire genomic RNA of a N isolate of PVY. This RNA consists of 9704 nucleotides, and codes for a 3063 amino acid polyprotein. Analysis of the sequence of this polyprotein, together with either N-terminal sequence determination of certain mature viral proteins, or comparison with other potyvirus proteins, allowed us to propose that a protease activity (amino acids 1843 to 2275) is auto-cleaved and ensures the cleavage of the COOH part of the polyprotein giving the following proteins: capsid, replicase, cytoplasmic inclusion and probably the VPg. Cleavage sites near the NH2 terminus of the polyprotein as well as the functions of proteins in this region present more ambiguities. This region includes three proteins, among which the helper component necessary for the transmission of the virus by aphids, and a protein which might be a cell to cell movement protein. For another potyvirus, the Tobacco Etch Virus (TEV), a proteolytic activity responsible for the maturation of polypeptides in the NH2 region of the polyprotein was recently shown to be associated with the helper component (Carrington *et al.* 1989).

As for most RNA viruses, the lack of well-defined mutants of PVY has impeded studies on the molecular biology of this pathogen. However, the recent development of recombinant DNA technology makes it possible to generate such mutants indirectly by *in vitro* transcription of DNA copies modified by site-directed mutagenesis. Such RNA molecules, if expressed *in vivo*, i.e. replicated upon inoculation of host cells, are very useful tool for the genetic study of RNA viruses. Such full-length cDNA clones from which infectious transcripts could be synthesized have been reported for brome mosaic virus (Ahlquist *et al.* 1984), human rhinovirus type 14 (Mizutani and Colonno, 1985), black beetle virus (Dasmahaparta *et al.* 1986), tobacco mosaic virus (Dawson *et al.* 1986; Meshi *et al.* 1986), poliovirus (Van der Werf *et al.*1986), cowpea mosaic virus (Vos *et al.* 1988) and tobacco rattle virus (Hamilton and Baulcombe, 1989).

Knowledge of the nucleotide sequence of the genomic PVY RNA has allowed us to attempt to synthesize and clone in *E. coli* the full-length cDNA of the PVY(N) RNA downstream of a procaryotic promoter (T7).

At present, we have two separate clones. One contains a 5.5 Kb insert representing the 3' end of the genomic RNA, containing the polyA stretch. The other represents the first 500 nucleotides of the viral RNA immediatly 3' of the promoter for the T7 RNA polymerase. We are now trying to obtain clones representing the remaining parts of the viral RNA. These separate clones will then be joined by use of unique restriction sites. The full-length cDNA clone will then be transcribed *in vitro* and the RNA tested for infectivity.

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cDNA Cloning of Two Viruses of Barley: Barley Yellow Mosaic Virus and Barley Mild Mosaic Virus.

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In the last ten years yield reduction of winter barley (*Hordeum vulgare*) cultivars due to the disease caused by Barley Yellow Mosaic Virus (BaYMV) has become a serious problem to growers of this crop in Western Europe. BaYMV is soil-borne being transmitted by the fungus *Polymyxa graminis* and thus control measures are extremely difficult. At present the only means to stop the spread of the virus is by cultivation of resistant cultivars which are agronomically inferior to currently cultivated susceptible cultivars and have a very limited resistance gene pool. In Europe, distinct serotypes of BaYMV have been identified. These serotypes may occur simultaneously in infected plants but differ in mechanical transmissability and particle stability. However there is increasing evidence that these serotypes are in fact distinct viruses and they have now been termed BaYMV (includes So and NM isolates) and Barley Mild Mosaic Virus (BaMMV, formerly BaYMV-M type). Both of these viruses contain a bipartite ssRNA genome with an approximate size of 8kb (RNA1) and 4kb (RNA2).

cDNA clones corresponding to the RNA genomes of these two viruses were prepared using oligo(dT) to prime first strand synthesis. Using restriction enzyme and hybridisation analysis it was possible to identify 4 distinct classes of cDNA clones and the largest of each was taken for further analysis. In Northern blot hybridisation experiments each was found to correspond to a single RNA species from one of the viruses. Under stringent washing conditions there was no cross hybridisation between each cDNA clone and other viral RNAs suggesting that they contain large regions of substantially different base composition. This was further demonstrated when the clones were compared using restriction enzyme analysis as no similarities between the different cDNA clones were detected. The cDNA clones corresponding to RNA1 were 6.4 and 6.3 kb in size and those to RNA2 were 3.7 and 3.5 kb for BaYMV and BaMMV respectively.

The protein coding capacity of the cDNA clones was investigated using *in vitro* transcription and translation systems. Only one strand of the cDNA clones was found to code for proteins *in vitro*. Comparison of these products with the *in vitro* translation products of the natural viral RNAs demonstrated that the cDNA transcripts code for the major translation products coded for by

the viral RNAs. It was also possible to distinguish between products coded for by RNAs 1 and 2 in total viral *in vitro* translation products.
Antibodies specific for either BaYMV or BaMMV were used in immunoprecipitation studies with the *in vitro* translation products to determine if the cDNA clones code for antigenic proteins such as the viral coat proteins. In the case of BaYMV and the corresponding cDNA clones there was one major immunoprecipitation product which was coded for by the cDNA representing RNA2. However for BaMMV several less abundant products were immunoprecipitated corresponding to products encoded by the cDNA prepared to RNA1. Deletion experiments revealed that the major immunoprecipitation product of BaYMV, thought to be the coat protein is encoded by a region at the 5' of RNA-2.

Work is now concentrated on characterizing those regions of the cDNAs coding for the coat proteins as well as producing full length clones which may be used in infection studies.

The Nucleotide Sequence of the Coat Protein Gene of a Non Transmissible Isolate of Zuccini Yellow Mosaic Virus.

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Complementary DNA of a non transmissible isolate of ZYMV was cloned into a bluescript plasmid in E. coli.

The nucleotide sequence of the 3'-end region of ZYMV was determined. The sequence contains one long open reading frame (ORF) terminated by a TAA stop codon followed by a non-coding region of 134 nucleotides.

A poly(A) tail of about 48 residues was found at the 3'-end. It has been previously reported for several potyviruses that the last translated region within the virus genome codes for the CP polypeptide. The predicted amino acid sequence of that region was derived from the cloned cDNA sequence of ZYMV (888) nucleotides from the 3'-end). The putative unique cleavage site (ala-gln) within the predicted polyprotein was thus located. From this data it was concluded that the CP molecule contains 296 amino acids.

Computer analysis of the amino acid sequence of the putative ZYMV capsid protein revealed about 65% homology with other potyviruses. A highly variable region was located close to the N-terminal of the CP.

Sequences homologous to other potyviruses were found throughout the putative CP molecule correlating with the highly conserved region within the CP of various potyviruses as described by Shukla et al. (1988).

RNA2 of Grapevine Fanleaf Virus (strain F13) cloning, sequence analysis and coat protein location

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Grapevine Fanleaf Virus strain F13 (GFLV-F13) is a member of the nepovirus group and is responsible for the "court-noué", an economically significant disease in vineyards in France and in many other countries. The genome is made of two single-stranded positive-sense polyadenylated RNA which carry a genome linked protein (VPg) at their 5' end. This genome is encapsidated in isometric particles and the capsid is composed of a single protein species.

The nucleotide sequence of the genomic RNA2 (3774 nt) of GFLV-F13 was determined from overlapping cDNA clones and its genetic organization deduced. The complete sequence contained only one long open reading frame of 3555 nt (1184 residues) borded by a leader of 7 nt and a 3' non-coding region of 212 nt. RNA2 sequence analysis and comparison with RNA2 of Tomato Black Ring Virus (TBRV) and M RNA of Cowpea Mosaic Virus (CPMV) showed a highly conserved 3' non-coding region but a less extended consensus sequence at the 5' end than that reported for other nepovirus RNAs.

Analysis of the N-terminal sequence of purified coat protein (CP) and identification of the C-terminal residue has allowed the precise position of the CP cistron within the 131 KD polyprotein of RNA2 to be deduced. A proteolytic cleavage at an Arg/Gly site at residues 680-681 produces the CP. This represents the first cleavage site for a nepovirus coat protein obtained by direct by N-terminal amino acid sequence analysis and is the first Arg/Gly cleavage site reported for plant viruses expressing their genomic RNAs by processing of a polyprotein. The coat protein of 504 amino acids (M_r 56 KD) has very hydrophobic properties, similar to those of the TBRV coat protein.

SESSION 8

STRATEGIES DE SELECTION/ BREEDING STRATEGIES

BREEDING FOR VIRUS RESISTANCE : PLANT BREEDERS POINT OF VIEW.

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Unfortunately all virus resistances are not monogenic, dominant and effective against all the strains of the virus. In such a simple case a backcross method can be used to introduce the resistance into cultivars having good agronomic characteristics.

Several problems can arise :

1- **Defective characters** can be linked with the resistance. Genotypes occur where the linkage has been broken (*Tm-2* gene for Tobacco Mosaic Virus resistance in Tomato linked with *nv*) or genetic combinations where the defective character is not expressed (TMV resistant Tomato lines have a low pollen fertility; in F_1 hybrids the fertility is restored).

2- **Only low level of resistance** is found. Transgressive phenotypes can be created by recombination of partial resistances occuring at different steps of the virus infection (resistance to the transmission by vectors, to virus multiplication, to virus migration in the plant...). Such characters may be inherited independently and higher levels of resistance than in the parents can be obtained through recurrent selection. Such is the case with Cucumber Mosaic Virus resistance in Pepper (*Capsicum*).

3- How to manage **polygenic resistances** (created by the plant breeders as in the previous paragraph or found in natural plant populations)? Which breeding methods will permit accumulation of all the genes controlling resistance with good agronomic characteristics (including resistances to other diseases)? Backcross methods can be used with intercrossing resistant plants for one or two generations or recurrent selection.

4- **Genetic analysis** of complex resistances may be easier by using haploidy. Dihaploid lines obtained in an F_1 hybrid between a resistant plant and a susceptible one can be inoculated with different viruses or strains, using various methods (mechanical inoculation, aphids...), incubated in various conditions (normal or high temperature...), etc. Inheritance of the mechanisms and above all the correlations between the reactions studied.

5- **Different tests** must be used according to the degree and the mechanism of resistance : vectors, strains, inoculum concentration, incubation conditions... Particularly when the level of resistance is increasing (as in paragraph 2) the tests must be changed and become more severe. In multi-disease resistance breeding, tests for several diseases must be done on the same plant with the problems of induced resistance or induced susceptibility. The inoculation method must be reliable and if possible there should be no escapes : this can be difficult to obtain with viruses transmitted by aphids in a persistent manner or when inoculation is made by grafting. Another problem can be the localization of the virus in the plant : for instance with fruit trees which part of the tree must be tested with ELISA or other methods to verify the resistance and the absence of virus multiplication after inoculation?

6- **Screening for resistance**/susceptibility is done as early as possible. This supposes a good correlation between the reaction of young and adult plants. There may be some problems for instance when testing for tolerance to symptoms on the fruits.

7- One must take great care not to **introduce susceptibility** to other diseases or other undesirable characters when breeding for resistance to a particular virus. Indeed, the sources of resistance are often 'wild' or 'exotic' and may be highly susceptible to pests or diseases not present in their country of origin but common where the future variety will be grown.

8- Particular problems can be found with **perennial plants** and moreover with shoot/rootstock interactions as in fruit trees. For instance susceptibility may be expressed as grafting-incompatibility.

ROLE ET UTILISATION DES VARIETES RESISTANTES AUX VIRUS ET A LEURS VECTEURS EN LUTTE INTEGREE

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Les objectifs de la lutte contre l'infection des plantes par les virus sont en général de limiter au maximum les diminutions de rendement qualitatif et quantitatif des cultures et d'assurer à l'agriculteur la rentabilité financière de la récolte.Ils se situent à deux niveaux d'intervention:

+ à l'échelle de la plante et de la culture,il s'agit selon les cas:

- d'empêcher que le virus soit inoculé à la plante par son ou ses vecteurs puis qu'il s'y multiplie suffisamment pour affecter le rendement et/ou qu'il puisse être véhiculé par translocation vers la descendance de la plante(plant de pomme de terre,par exemple),
- de retarder suffisamment longtemps l'infection des plantes pour que leur rendement ne soit pas significativement affecté,
- d'empêcher que les plantes ne servent à leur tour de sources de virus pouvant être à l'origine d'infections secondaires et de la généralisation de l'infection à l'ensemble de la culture.

+ à l'échelle d'une région de production et dans un contexte de rotations culturales,il s'agit:

- d'abaisser ou de contenir les populations de vecteurs et de virus dans des limites telles qu'elles permettent de maintenir la pression d'infestation et d'infection dans le temps et dans l'espace à un niveau minimum,
- d'interrompre le cycle épidémiologique d'un virus susceptible de trouver toute l'année des cultures à infecter(par exemple,cas du virus de la jaunisse nanisante de l'orge sur céréales à paille et sur maïs).

Trois grandes possibilités sont offertes pour réaliser ces objectifs:

+ implanter la culture à une période de l'année qui lui permette d'échapper au maximum à l'infestation par les vecteurs et dans certains cas particuliers(plant de pomme de terre),éliminer soigneusement et précocément les sources de virus internes à la culture et détruire celle ci avant que les virus transmis au feuillage n'aient

le temps d'atteindre la descendance(**méthodes culturales**),
+ supprimer les vecteurs avant qu'ils aient le temps et la possibilité de transmettre le virus(infections primaires)ou de se multiplier sur les plantes et assurer les infections secondaires(**lutte chimique**),
+ utiliser directement des plantes qui échappent à l'infestation par les vecteurs(**résistance par antixenosis**)ou qui ne les multiplient pas(**résistance par antibiosis**)ou encore qui peuvent échapper partiellement ou totalement à l'infection par le/les virus.

La mise en oeuvre des différentes méthodes de lutte contre la dissémination des virus aux plantes a subi des fluctuations au cours du temps mais elle n'a jamais été vraiment optimisée.
Des succès considérables ont été remportés par la sélection de cultivars à haut rendement alliée à la mise au point de nouvelles méthodes culturales intensives(blé,riz,pomme de terre..).Mais ces cultivars"améliorés"se sont souvent révélés plus sensibles aux attaques des déprédateurs et des maladies.Dans le domaine des maladies à virus,l'opinion encore très répandue dans les milieux agricoles que la lutte chimique dirigée contre les seuls vecteurs pourrait à elle seule réglér le problème s'est traduite à partir des années 60 par une augmentation extraordinaire du marché des pesticides.Mais très tôt,l'abus et les conditions anarchiques de leur emploi ont entraîné l'apparition des phénomènes de résistance des insectes aux insecticides aboutissant à la pullulation provoquée des ravageurs.Un autre effet,indirect mais semblable dans ses conséquences peut aussi résulter de l'élimination des ennemis naturels des déprédateurs par les insecticides.Ensuite,l'usage répété de ces derniers et le non respect des doses prescrites,entraînant souvent la présence de résidus dans les chaînes trophiques,ont posé avec beaucoup d'acuité les problèmes de la protection de l'Environnement en général.Enfin,l'efficacité de la lutte *per se* s'est souvent révélée inférieure aux espérances car le choix des matières actives et les modalités d'application des produits n'ont pas toujours été évalués correctement:on connaît par exemple la relative inefficacité des aphicides pour empêcher l'infection des cultures par les virus non persistants.
Devant les imperfections de la lutte chimique lorsqu'elle est utilisée comme seule méthode de lutte,il fallait donc raisonner en termes différents les moyens d'assurer une productivité optimale des cultures en identifiant toutes les techniques et méthodes adéquates,compatibles entre elles et pouvant être utilisées de manière harmonieuse pour contenir les populations virales et leurs vecteurs en dessous d'un seuil économique de dommages.C'est dans ce **contexte de lutte intégrée** que se place l'utilisation de variétés résistantes,non pas comme une alternative à la lutte chimique mais

comme une des composantes de la lutte intégrée permettant de répondre aux objectifs définis plus haut.

Parmi les avantages offerts par l'utilisation de telles variétés, les plus importants sont évidemment ceux qui permettent de pallier les insuffisances et les inconvénients de la lutte chimique et de réduire les contraintes culturales.Il est ainsi possible:
+ d'assurer à l'agriculteur des rendements et une rentabilité financière au moins égaux ou supérieurs à ceux des variétés sensibles nécessitant des interventions phytosanitaires.Ceci est particulièrement vrai des pays où les agriculteurs ne peuvent ou ne savent pas correctement utiliser les pesticides:Heinrichs *et al.*(1986)ont bien montré que l'utilisation de la variété de riz IR28,résistant à la cicadelle verte *Nephottetix virescens* agent vecteur du virus Tungro ,peut procurer de bons profits car elle ne nécessite pas d'applications d'insecticides,
+ de préserver au maximum le potentiel de lutte biologique naturelle exercée par les ennemis des vecteurs de virus en réduisant le nombre des interventions chimiques ou en les programmant à des dates où ils ne seront pas touchés,
+ de réduire les contraintes culturales:un des facteurs d'augmentation des risques d'infection virale est certainement la possibilité de faire successivement plusieurs cultures par an dans un même environnement,ce qui assure la permanence dans le temps et dans l'espace de sources de vecteurs et de virus et favorise l'augmentation de la pression d'infection;des variétés résistantes peuvent libérer l'agriculteur des contraintes du choix de dates optimales de plantation ou de semis destinées à éviter les infestations par les vecteurs et les infections par les virus et maintenir dans des limites tolérables les niveaux des populations virales et de vecteurs présentes localement.

Mais le recours aux variétés résistantes peut aussi présenter à terme certaines limites.
Pour pouvoir assurer la rentabilité de la culture tous les ans,il faut que la résistance soit durable dans le temps.Or le contournement de la résistance au vecteur et/ou au virus est possible;il peut avoir les mêmes conséquences que l'apparition des souches résistantes du vecteur aux insecticides:la cicadelle brune du riz, *Nilaparvata lugens*,agent vecteur du grassy stunt a ainsi développé des biotypes plus agressifs en réaction à la culture intensive de variétés de riz à résistance monogénique(Heinrichs,1979).
Il n'est en général pas possible de sélectionner une variété résistant à tous les vecteurs et à tous les virus à la fois;la résistance à un vecteur peut alors entraîner une sensibilité accrue à un autre vecteur et il est souvent observé qu'une plante résistant à

un vecteur par antixenosis est susceptible d'être plus fortement infectée par un virus non persistant du fait d'une fréquence plus grande de visites des plantes par les vecteurs.
La plupart des variétés manifestant un niveau de résistance horizontale variable,le succès de leur utilisation dépendra essentiellement de la pression d'infection observée localement(Gabriel *et al.*,1987).

Enfin l'utilisation nécessaire des variétés résistantes en lutte intégrée implique en particulier certaines contraintes supplémentaires:
+ la nature de la résistance ne doit pas interférer avec l'efficacité de la lutte chimique(Raman,1988),
+ la lutte biologique et la résistance des plantes doivent être compatibles et complémentaires(Obrycki *et al.*,1983).

A l'heure actuelle il faut réaffirmer que ces principes ne sont pas encore appliqués de manière cohérente et raisonnée.C'est pourtant la seule possibilité raisonnable qui reste offerte pour éviter tous les problèmes rencontrés lors de l'utilisation séparée de chacune des méthodes.

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Field Evaluation of Genetically Engineered Virus Resistant Crops

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Potato and tomato plants resistant to some viruses were developed through genetic engineering and tested under field conditions to evaluate their level of efficacy. These tests were conducted following review and approval by the U.S. Department of Agriculture.

Transgenic potato plants were obtained by *Agrobacterium*-mediated transformation of Russet Burbank potatoes with a gene construct coding for the expression of the coat proteins of both PVX and PVY viruses. Transgenic plants expressing the coat proteins to a sufficient level were tested in the field at a Monsanto farm at Jerseyville, Illinois. Plants in the field were either inoculated with a mixture of PVX and PVY viruses, or left uninoculated. Dramatic differences could be detected at an early stage between transgenic and nontransgenic plants. The unprotected plants showed severe stunting and foliar deformation, while the best transgenic plants did not show any trace of symptoms. They grew as actively as uninoculated controls. Among the uninoculated plants, no obvious difference in growth could be detected between transgenic and nontransgenic plants, showing that the inserted gene did not negatively affect the overall development of the plant.

Transgenic tomato plants were obtained by *Agrobacterium*-mediated transformation of UC82B processing tomatoes with a gene construct coding for the coat protein of a tobacco mosaic virus strain. Here again, very good protection was obtained when the plants were infected by tobacco mosaic virus. Severe chlorosis and up to 70% yield reduction were observed on nontransgenic plants infected with the TMV strain PV230, while the transgenic plants did not show any symptoms and yielded as well as the uninfected controls.

DEPLOYMENT OF RESISTANCE GENES: IMPLICATIONS FROM STUDIES ON RESISTANCE-BREAKING ISOLATES OF TOBACCO MOSAIC VIRUS

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INTRODUCTION

Plant resistance genes have been used as the major strategy for combatting virus diseases in numerous crop species (reviewed in Fraser, 1986). Many examples deployed by the breeder have involved single gene resistance, but in some crops, several genes with activity against a particular virus are available, and cultivars with oligogenic resistance have been constructed. In other crops, resistance is on a very narrow genetic base, or is not available for a particular virus.

Breeding for resistance may be negated if the virus can evolve virulent forms, capable of overcoming the resistance genes deployed against it. However, virulence in viral pathogens has arguably been studied less than the corresponding phenomena in fungal and bacterial pathogens (Flor, 1956; Staskawicz et al., 1984). In a previous review (Fraser, 1986) it was noted that virulence had been reported against just over one half of a random sample from the literature of 54 resistance genes. Gene-for-gene systems of interaction between host resistance and viral virulence have been proposed for a small number of plant/virus combinations, such as tomato/tobacco mosaic virus (TMV) (Pelham, 1972); Phaseolus bean/bean common mosaic virus (BCMV) (Drijfhout, 1978) and Capsicum/TMV (Tobias et al., 1989).

About one tenth of the genes in the sample had been outstandingly durable, in that no virulent isolates had been reported after many years of deployment in crops, and after challenge by numerous isolates of virus under agricultural or experimental conditions. For the remaining genes in the sample, the literature was unclear, and there is uncertainty about whether the genes had shown durability under realistic test, or whether the question had not been investigated.

On the face of it, the existence of virulent isolates against more than half of the sample of resistance genes would tend to suggest that resistance-breaking behaviour is common in viruses, and that this may create problems for resistance breeding. As noted earlier, however, it is likely that this is an oversimplification, and an unduly pessimistic one at that (Fraser and Gerwitz, 1987). For some of the 'defeated' resistance genes in the survey, geographical isolation of genes from some strains of virus could provide a measure of protection. Furthermore, at least some virulent isolates of any particular

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virus may be definable under experimental conditions, but may be impaired in the pathogenic fitness which is required for spread under agricultural conditions. Our objective in this paper is to review some aspects of virulence, and discuss consequences for resistance gene deployment, using resistance to tobacco mosaic virus (TMV) in various crop species as the principal model.

GENETIC MAPPING OF VIRULENCE DETERMINANTS

Until recently, virulence determinants could only be mapped on viral genomes using indirect methods, such as by construction of pseudorecombinants between the genomic components of viruses with multipartite genomes. Recently, it became possible to perform genetic mapping on a much finer scale. cDNA copies of virulent and avirulent viral RNAs can be cut at specific restriction enzyme sites, then re-ligated to form artificial recombinants. In combination with sequence analysis of the region of the genome involved, this has allowed virulence/avirulence determinants to be pinpointed as particular base changes.

For TMV, the determinants of virulence have now been mapped against three resistance genes, and interestingly, they are located in genes specifying three different viral functions. Virulence against the N' gene in Nicotiana, which restricts some virus isolates to necrotic local lesions, is located in the TMV coat protein gene, although mutations at several places in this sequence can affect the virulence behaviour (Culver and Dawson, 1989). One possibility is that features of the secondary or tertiary structure of the coat protein may be recognized as the virulence/avirulence determinant. This is supported by indirect evidence that the interaction of the virus with the resistance mechanism specified by the N' gene is affected by the biochemical properties of different coat proteins (Fraser, 1983).

Virulence against the Tm-2 resistance gene in tomato, which prevents movement of the virus from the initially-infected cell, maps in the viral 30 kDa protein involved in cell-to-cell spread (Meshi et al., 1989). Virulence against the Tm-1 gene, which inhibits TMV multiplication but allow spread throughout the plant, maps to the 126/183 kDa proteins which are thought to be involved in the viral replicase (Meshi et al., 1988).

IS THERE A LIMIT TO THE NUMBER OF VIRULENCE 'GENES' A VIRUS ISOLATE CAN CONTAIN?

It is unlikely that viral genes could have the determination of virulence/avirulence as their sole or even primary function. As the examples above show, the virulence determinants are located within genes with other functions in viral replication or particle structure. However, it is clear that this primary function can be maintained while allowing mutation to the virulent form. It may be, however, that the frequency of such successful mutation to virulence will vary for different resistance genes, and will depend on the sensitivity to mutation of the viral function involved. It is reasonably well established that different resistance genes are overcome by virulent mutants which arise at different frequencies. For example, in tomato, the Tm-1 gene has been overcome frequently in horticulture by resistance-breaking isolates, while the Tm-2 gene seems to have been overcome less frequently. It may be that the replicase gene is more tolerant of mutations to virulence than the transport protein gene.

If virulence determinants against different resistance genes are located in different viral functions, it seems reasonable to expect that a virus isolate might be able to contain several virulence determinants. However, if virulence against two resistance genes maps in the same viral function, there may be a constraint on mutation to the doubly-virulent condition. Thus TMV isolates carrying virulence against both Tm-1 and Tm-2 are known (Pelham, 1972), but there do not appear to be any confirmed reports of isolates virulent against Tm-2 and the allelic $Tm\text{-}2^2$, which probably operates by a similar mechanism. The two virulences might be mutually exclusive. Alternatively, the comparative rarity of virulence against $Tm\text{-}2^2$ might simply mean that doubly-virulent mutants are statistically unlikely, but not biochemically impossible.

The opposite of mutually exclusive virulence determinants may also exist, in that mutation to virulence against one resistance gene may automatically confer virulence against another. In resistance to BCMV in Phaseolus bean, all known virus isolates which overcome the $bc\text{-}1^2$ gene also overcome the allelic bc-1 (Drijfhout, 1978), although the converse is not true.

IS VIRULENCE ACCOMPANIED BY LOSS OF PATHOGENIC FITNESS?

It is conceivable that the mutation which confers virulence might impair the major function of the viral gene, leading to a reduced ability to multiply or spread. This may vary also for different resistance genes. To test this, we mutagenized avirulent TMV with nitrous acid, and selected mutants able to overcome the Tm-1 resistance gene. The pathogenic fitness of these mutants was assessed by measuring their ability to multiply, and the severity of symptoms caused, in non-resistant tomato plants. All 19 mutants tested multiplied to lower concentrations, and caused less severe symptoms than the avirulent parent isolate (Fraser and Gerwitz, 1987). This suggests that initial mutation to virulence was coupled with a quantitative loss of pathogenic fitness, although possible effects of second mutations elsewhere in the genome cannot be entirely excluded. Interestingly, a Tm-1-breaking isolate of TMV which was obtained from a commercial tomato glasshouse multiplied as well as the avirulent parent. This suggests either that mutation to virulence is possible without loss of fitness, or that further mutation and selection of the virulent mutant can restore full fitness.

In related work with isolates overcoming the $Tm\text{-}2^2$ gene, we have found that four naturally-occurring virulent forms from different sources all share the same phenotype. All have lower specific infectivity than avirulent isolates, and all establish infection on $Tm\text{-}2^2$ resistant plants with difficulty. All multiply normally in susceptible plants, but to reduced levels in resistant plants - a 'ghost' effect of the defeated resistance gene. All cause extreme inhibition of growth of resistant plants, but not of susceptible plants. In this case, therefore, there does appear to be a persistant impairment of pathogenic fitness as an accompaniment of virulence. This may partly explain why isolates overcoming $Tm\text{-}2^2$ have been so rare in commercial horticulture.

CONCLUSIONS

Clearly, much remains to be discovered about the mechanisms of virulence, and the implications for resistance breeding. A fuller understanding should enable decisions to be made about resistance gene deployment in a manner which will ensure maximum durability.

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The virus variability: its implication in plant breeding for resistance

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For most of the agronomically important species of plants, there is a need to maintain a breeding work in order to produce yields of high level and high quality which remain stable from year to year. In several cases, the plant breeders must take account in their breeding programs of the main virus diseases which are able to induce losses of the quantity or quality of the crop. In many cases, virus strains have been found able to overcome the resistances introduced in commercial varieties.

When a breeding program for virus resistance is undertaken, it appears a need to have a knowledge of the virus variability in order to define a breeding strategy allowing to minimize the risk that a virulent strain become preponderent under natural conditions.

The traditional plant pathologists working with plant breeders used to distinguish and describe the virus strains by their biological properties using essentially a descriptive and populational approach. The molecular approach begins to offer a way to appreciate the level of analogy existing between the nucleic acid of two strains; it can allow to localize the modification which are responsibles for the changes in some biological properties as well as the cistron on which they occur. It opens the way to an explicative approach.

Until now, the virus variability concerns the classical plant breeder who try to obtain the best from its breeding material by sorting and combining the lines he has gathered. Their strategy is directly linked to the pathogenic properties of the virus variants that have been recognized. The transgenic technics offer the possibility to elaborate new modes of resistance against a given virus or a virus group whose the range of

variation is approximately known.

The selection under field conditions has been used during many years and allowed to produce several useful resistant varieties. The method allows to screen efficiently the breeding lines against the virus populations endemic in the areas where are the experimental fields. Difficulties occur when the climatic aleas modify the dissemination or the manifestation of the disease; interferences with other diseases or pests and the impossibility to test the material with foreign virus isolates can be also annoying.

Screening the lines by artificial inoculation under controlled environment give results easier to understand from a genetic point of view but it needs to have good technics of virus handling. This is easy with mechanically transmitted viruses and is feasible with some persistant ones.

The beginning of a breeding program needs the screening of numerous lines and varieties and a minimum knowledge of the virus variability is necessary to choose a panel of isolates for testing. The virus variants can be rare under natural conditions and several exemples show the advantage of diversifying the origins of the virus isolates (commercial fields, varietal collections, weeds known as virus reservoirs...).

Additional testing must be done on the lines selected after the first screening. This can be done by sending the breeding lines for testing in other countries or by inoculation with foreign virus strains under safe conditions.

When no true resistance can be found in the tested lines, attempts to identify and gather partial resistances need a more accurate testing for distinguishing between slighty differential responses of the lines and the random variations of the infecting strain. At this step, a larger panel of virus strains including anomalous strains could be useful.

The durability of a new resistant variety needs that the resistance incorporated will not be overcome ever by a newly produced or selected virulent strain nor by a strain introduced from other areas. Exemples of the comportment of resistant varieties under field conditions show that virulent strains can present various spatio temporal extensions according to their fitness in the agricultural ecosystem. The geographical diversity of a virus could contribute to the enlargement of the local virus variability; some virus seems able to take advantage of new opportunities for colonizing larger areas.

The genetic engineering offers a different and more rational approach to elaborate resistant varieties but careful experimentations could be necessary to evaluate the efficiency and durability of these resistances under natural conditions.

EPIDEMIOLOGIE GENERALE/ GENERAL EPIDEMIOLOGY

SESSION 9

VIRUS TRANSMIS PAR PUCERONS/ APHID TRANSMITTED VIRUSES

CHICKPEA VIRUS DISEASE EPIDEMIOLOGY IN CALIFORNIA

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In California, a shift of chickpea culture from summer to winter sowing to increase yields revealed their vulnerability to several devastating virus diseases. Previously, with summer-planted culture, viruses had not been important. Field trials were conducted in 1987-88 at Davis, Salinas and the central San Joaquin valley to monitor virus incidence and impact on yield. Aphid abundance and species composition, and effect of planting date on virus incidence on four varieties were studied in a separate trial at Davis. Viruses were identified by means of ELISA, immunodiffusion tests, host range studies and EM.

All viruses found were aphid-transmitted, the major ones being luteoviruses, with beet western yellows (BWYV) especially abundant, but also legume yellows (LYV) and subterranean clover red leaf (SCRLV) (Bosque-Perez et al., 1988). Lettuce mosaic (LMV) was found on chickpea for the first time and cucumber mosaic (CMV) also occurred, a first report on chickpea for the U.S.A. (Bosque-Perez and Buddenhagen, 1989; to be published). Alfalfa mosaic (AMV) and other mechanically transmissible viruses not fully characterized were also detected.

Aphid transmission studies were carried out in a greenhouse. The green peach aphid (GPA), *Myzus persicae* transmitted BWYV from chickpea to chickpea after 24 h acquisition access periods (AAP) and 48 h inoculation access periods (IAP). LYV and SCRLV were transmitted by the pea aphid (PA) *Acyrthosiphon pisum* in a similar fashion. AMV was transmitted by the PA and CMV by the cowpea aphid (CA), *Aphis craccivora* after 15 m AAP and IAP of up to 24 h. LMV was transmitted by the GPA after 15m AAP. Numerous attempts to transmit the luteoviruses and LMV with the CA were unsuccessful.

Monitoring of aphids at Davis by means of water pan traps and a suction trap showed strong seasonality in aphid occurrence. Aphid populations peaked in March-April coinciding with high levels of virus transmission

as detected in field trials and potted bait plants. The GPA was the most abundant aphid, followed by the PA and CA to a minor extent.

Fields of crops such as alfalfa, sugarbeets, safflower and various fruits, in which winter weeds were abundant were the most likely sources of viruses and vectors at Davis for no other chickpea fields were present in the area. Weeds included various Cruciferae, *Malva, Lactuca, Senecio* and *Chenopodium* some of which are known hosts of both the GPA and BWYV (Duffus, 1971).

Biology of aphids on chickpea was studied in a greenhouse. Aphid survival and fecundity were markedly reduced when fed on chickpea varieties with glandular hair exudates (mostly malic acid) as compared to a hairless variety. Mortality of GPA, PA and CA after a 48 h period ranged between 72 and 100% on hairy varieties Surutato and Chafa, compared to 13 to 28% on Chafa glabrous. However, vectors survived long enough to efficiently transmit the viruses under field conditions.

Symptoms of the different luteoviruses in chickpea differed little. Infected plants first became slightly yellow-green and remained stunted. In contrast, symptoms from mechanically transmissible viruses in chickpea consited of mottling of terminal leaves, followed by tip wilting and general yellowing. For most viruses phloem necrosis occurred resulting in stem darkening. If young, plants were often killed and external symptoms closely resembled those caused by the *Fusarium* wilt pathogen. Infection before flowering usually prevented seed set. Infection after flowering reduced yield and often caused seeds to shrivel.

Plot yields at Davis of the most susceptible variety were reduced by approximately 93% of yield potential; those of the most resistant, by about 53%. Paired plant (diseased/healthy) comparisons revealed that infection five months after planting reduced yield by about 95% at Davis but only by 60% in the central San Joaquin valley, presumably due to the higher level of BWYV at Davis. Infections six months after planting reduced yield by 70% at Davis but only by 15% in the central San Joaquin valley.

Virus incidence was monitored in plots of four varieties planted in Davis at monthly intervals, from November to February. Similar trends occurred with all dates of planting, but the December planting had higher virus incidence earlier in the season. At any given time variety

ILC 3375 had consistently higher incidence, in contrast to ILC 144 which had lower incidence, regardless of date of planting. These differences were reflected in a greater yield reduction for the variety having higher incidence. Individual plants of these two varieties had similar yield depression due to infection, but on a plot basis the effects differed significantly. Thus, they were equally intolerant, but differed in tolremicity (Buddenhagen, 1983).

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VIRUS TRANSMIS PAR PUCERONS/ APHID TRANSMITTED VIRUSES

VIRUS NON-PERSISTANTS/ NON-PERSISTENT VIRUSES

STUDY ON THE IMPORTANT VECTORS OF SOYBEAN MOSAIC VIRUS AND THEIR TRANSMISSION EFFICIENCIES IN CHINA

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Besides Aphis glycines and Acythosiphon solani can colonize on soybean plants,Aphis craccivora and other aphid species can also form small,temporary colonies on seedling soybeans in the fields in Harbin of China.The number and species of alate aphids in soybean fields varied with ecological enviroment changed,but the main flight alate aphid species were basically consistent during two years.The green-pantraps trapped alate aphids landing on soybean canopy and some of specimens were identified.The results indicated ,besides the three aphid species as mentioned above,Aphis gossypii ,Rhopalosiphom maidis,Rhopalosiphom padi,Myzus persicae accounted for the most of alate aphids alighting on soybean,but the number of A. glycines was absolutely the most abundent (see Table 1).

Tab.1 Species,number and ratio of alate aphids trapped by green-pantraps(Harbin)

Aphid species	in 1986					in 1987					Grand total	%
	Jun.	Jul.	Aug.	Total	%	Jun.	Jul.	Aug.	Total	%		
A. glycines	28	73	42	143	55.86	101	731	74	906	51.98	1049	52.48
A. gossypii	10	10	0	20	7.81	18	222	24	264	15.15	284	14.21
R. maidis	3	11	2	16	6.25	28	71	5	104	5.97	120	6.00
M. persicae	6	5	1	12	4.69	9	90	4	103	5.97	116	5.75
R. padi	3	24	0	27	10.55	27	22	2	51	2.93	78	3.90
A. craccivora	16	1	0	17	6.64	26	22	2	50	2.87	67	3.35
E. dilanuginosum	2	0	0	2	0.78	15	19	0	34	1.95	36	1.80
Ac. solani	1	3	0	4	1.56	1	19	1	21	1.21	25	1.25
Others	1	9	5	15	5.86	58	137	15	210	12.05	225	11.26
Grand total	70	136	50	256	100.0	283	1333	127	1743	100.0	1999	100.0

The number of alate aphids on soybean plant considerably correspond to that of alighting on a unit soybean canopy area calculated by the data of the green-pantraps.The frequency of alate aphids landing on soybean plant was greatly related to SMV epidemic rate (see Figure 1).

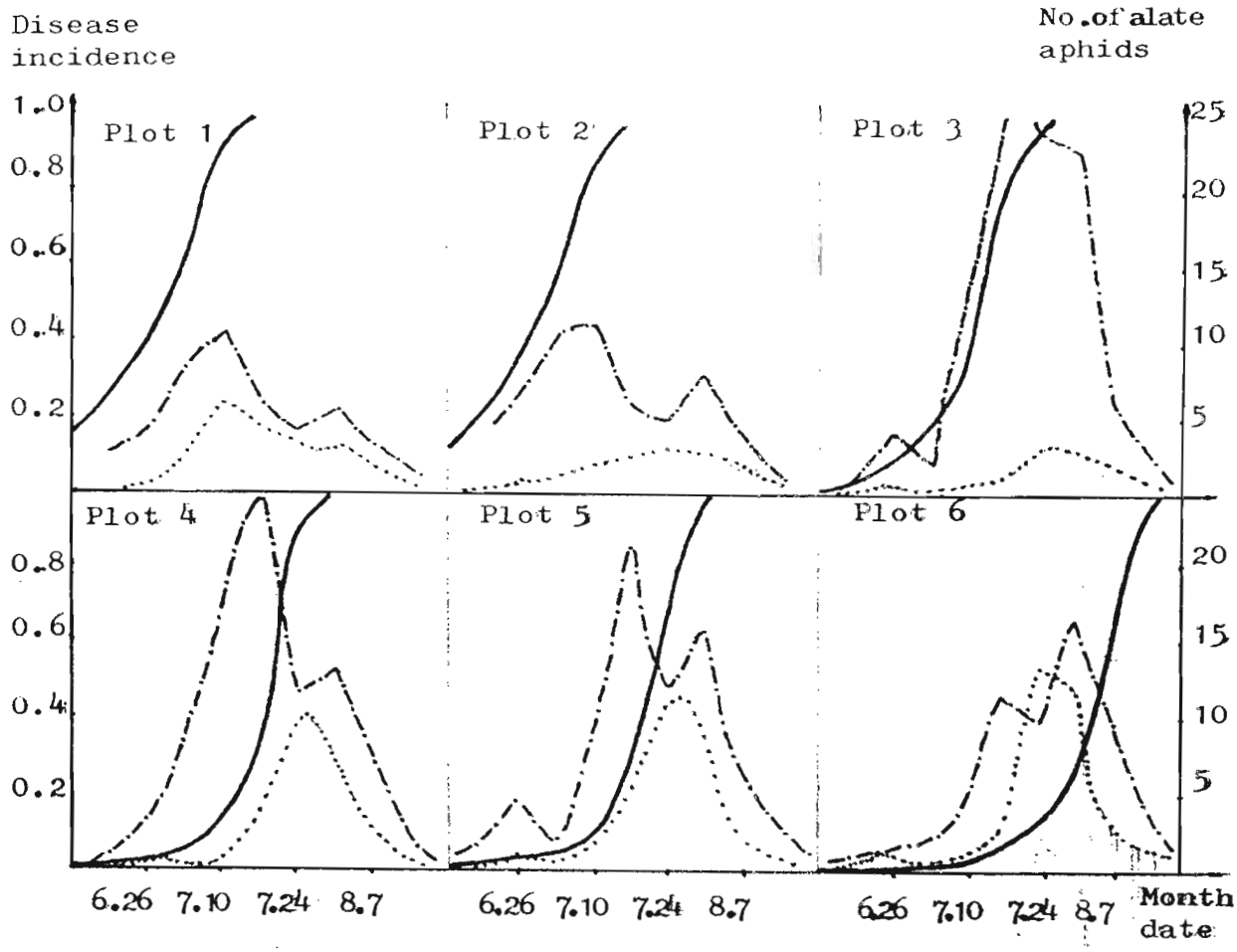

Fig.1 Relationships between the number of alate aphids per plant, alighting aphids per plant canopy area and SMV epidemics .

····· No.of alate aphids per plant ; ——— Disease incidence ;
—·—·—· No.of alate aphids alighting on per plant canopy area .

83-02 isolate of SMV transmitted by aphid vectors with acquisition probe of 30-60 sec. was optimal ; but the transmission efficiency was the highest with acquisition access of 40-50 sec.(see Tab. 2).

The most vector aphid species successfully transmitted the virus to the first plant after acquisition probe,but M. persicae could transmitted the second plant with much lower transmission probability (see Tab. 3).

Tab.2 Transmission rate of different acquisition times with A. glycines and A. craccivora(Harbin - 1986)*

Aphid species	Acquisition time(sec.) 10	20	30	40	50	60	ck
A. glycines	3.33	13.79	30.30	33.33	33.33	27.59	0.0
A. craccivora	10.00	22.45	28.21	53.33	50.00	20.00	0.0

*Average temperature about 22°C

Tab.3 Plants infected by continuous transfer after acquisition probe of vector aphids(Harbin-1986)*

Plant sequence of continuous inoculation	Vector aphid species A.glycines	M.persicae	Ac.solani	A.craccivora	ck
First plant	10.71	20.46	7.69	17.86	0.0
Second plant	0.0	2.94	0.0	0.0	0.0
Third plant	0.0	0.0	0.0	0.0	0.0

*Average temperature about 20°C

On the transmission of SMV isolat83-02 by single alate form of the vector aphid species with the optimal acquisition time, the results(see Tab.4)showed the transmission efficiency of A.craccivora

Tab.4 Transmission efficiencies of some major flight aphid species in soybean field (Harbin-1986,1987)

Items	Aphid species A. glycines		Ac. solani		A. craccivora		M. persicae		R. maidis		A. gossypii
	Alate	Apt.	Alate	Apt.	Alate	Apt.	Alate	Apt.	Alate	Apt.	Alate
No. of inoculated plants	60	60	59	60	60	60	59	58	62	40	70
No. of infected plants	20	18	14	14	35	32	28	27	8	5	3
Infection percent%	33.3	30.0	23.7	23.3	58.3	53.3	47.5	46.6	12.9	12.5	4.3
ck%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

was the highest : 58.33% , M. persicae : 47.66% , A. glycines :

33.33% , Ac. solani : 23.73% , R. maidis : 12.90% , and A. gossypii was the lowest : 4.29% among the tested aphid species . The most important vector species of SMV was Aphis glycines , and Aphis craccivora , Myzus persicae were also responsible for the spread of SMV in soybean fields .

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SIMULATION MODEL FOR EPIDEMICS AND DAMAGE OF SOYBEAN MOSAIC VIRUS

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The green-pantraps that accurately monitor the number and species of aphids alighting on soybean canopy were used to estimate the average frequency of aphids landing on the soybean canopy within a day.Measuring the daily infection rate with "trap plants" under certain disease incidence of soybean mosaic virus(SMV) and the number of flight aphids in the fields were used to calculate the transmission efficiencies of alate aphid population landing on and taking off the soybean canopy.According to the analysis of the infection probability and Poisson distribution,the model of daily infection rate of SMV was deduced.The model can be described as follow:

$$IRt1 = 1 - e^{e^{-0..0657C_{-1}}} \qquad 1 \leq t1 \leq 28$$

$$IRt2 = 1 - e^{e^{-0..0295C_{-1}}} \qquad t2 < 28$$

in which: $C = V \cdot Xt - Pt(Xt - Xt_{-1})$

The experimental results(Guo 1989)indicated that soybean plants infected by SMV can become the acquisition hosts for the aphid vectors only after systemic symptom of infected plants appeared. The rate of the systemic symptom appearance of soybean plants mechanically inoculated with the crude sap of 1 : 10 dilution was similar to that transmitted by aphid vectors.The soybean plants of V1 - R5 growing stages of five susceptible cultivars were inoculated with the crude sap and by aphid vectors and the inoculation experiments were repeated more than 30 times,the results suggested the rate of the systemic symptom appearance of infected plants was greatly correlated with temperature.The minimum average temperature for the symptom appearance was about 9C,the optimal average temperature was about 26 C(see Tab.1).

The effective cumulative temperature for the rate of symptom appearance of infected plants during developmental stages V1-R2 were very similar,but those during R3-R5 were a little higher.The figures of correlation points between the data of cumulative effective temperature and those of cumulative rate of systemic symptom appearance showed sigmoid curve.Among 8 formulas used to describe the data,the Gompertz was the best suitable for that during V1-R2, and the Weibull was the best during R3-R5(see Fig.1,2).

Tab.1 Relationship between the effective cumulative temperature and systemic symptom appearance of soybean plants infected by SMV under the different temperature

No. of sequence	1st			2nd			3rd			4th			5th	
DAT*	13.28C			15.19C			19.00C			25.02C			26.59C	
Items**	i	PP	TT	i	PP	TT	i	PP	TT	i	PP	TT	i	PP	TT
	24	30.0	78.9	12	14.3	78.5	8	15.3	82.7	5	23.4	77.2	4	36.7	72.2
	26	50.0	88.1	14	28.6	88.3	9	38.1	89.2	6	59.4	93.7	5	100	88.0
	28	70.0	103.6	16	71.4	99.6	10	64.2	101.8	7	89.1	110.0			
	30	80.0	120.2	21	97.8	131.7	12	91.5	124.6	8	98.4	126.9			
	34	100	145.5	23	100	142.4	14	100	140.1	9	100	144.2			

*Daily average temperature.

**i: Time since inoculating(days); PP: Cumulative percentage of symptom appearance; TT: Cumulative effective temperature since inoculating.

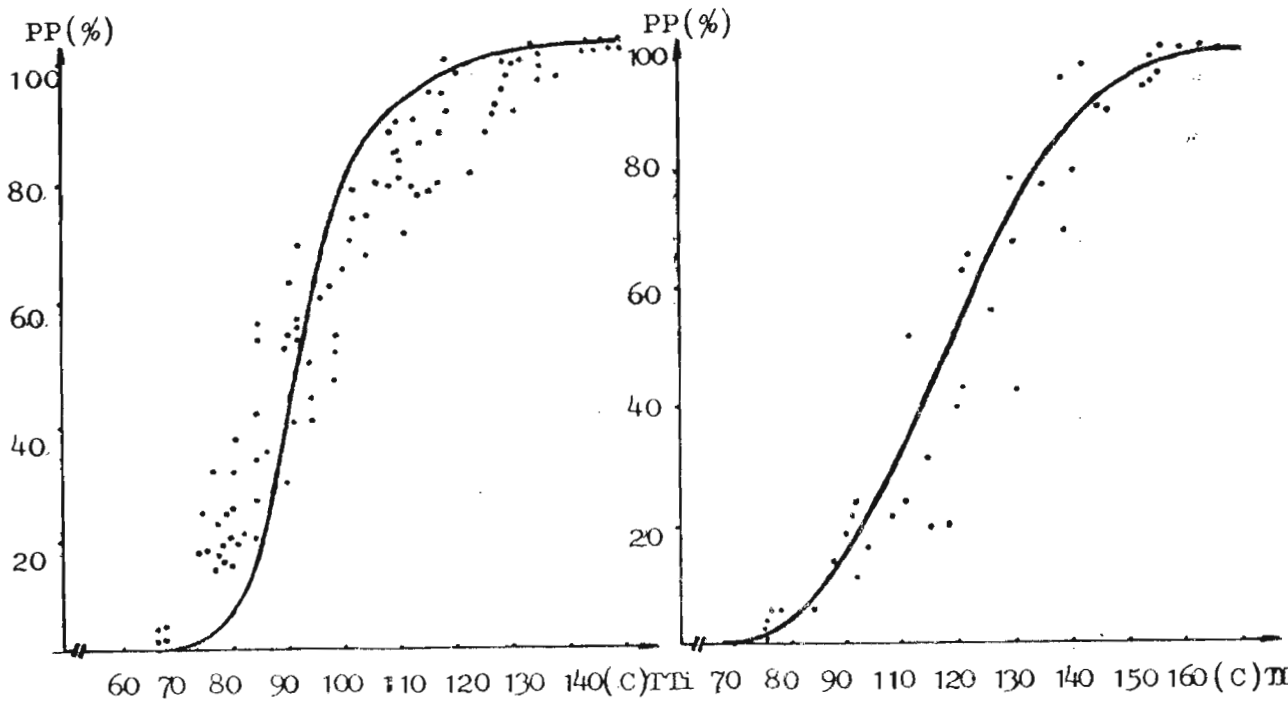


Fig.1Gompertz describes relationship between PPi and TTi of in infected plants during growing stages V1-R2

Gompertz formula :

$PPi1=\exp(-103021.2\exp(-0.1329TTi))$

Fig.2Weibull describes relationship between PPi and TTi of infected plants during growing stages R3-R5

Weibull formula :

$PPi2=1-\exp(-(0.02222(TTi-65))^{2.6})$

According to the stepwise linear regression, the predicting model of the multiple linear regression for the number of aphids landing on per plant canopy area(V)within a day was built of the number of alate aphids per plant(Pt), the average wind speed(W), the average temperature(T), and rainfall(R)per day. The predicting model is :

$$\ln V = 0.5631 \ln Pt - 0.273W + 0.045R + 0.15T - 1.027$$

RL=0.916 ; SY=0.5625

If we get "V" from the green-pantraps, we can calculate "Pt" in the predicting model.

There were higher yield loss and higher seed transmission rate of diseased plants when infected by SMV during the early developmental stages than infected late. Both yield loss and seed transmission rate was very low at the stage R2(see Fig. 3).

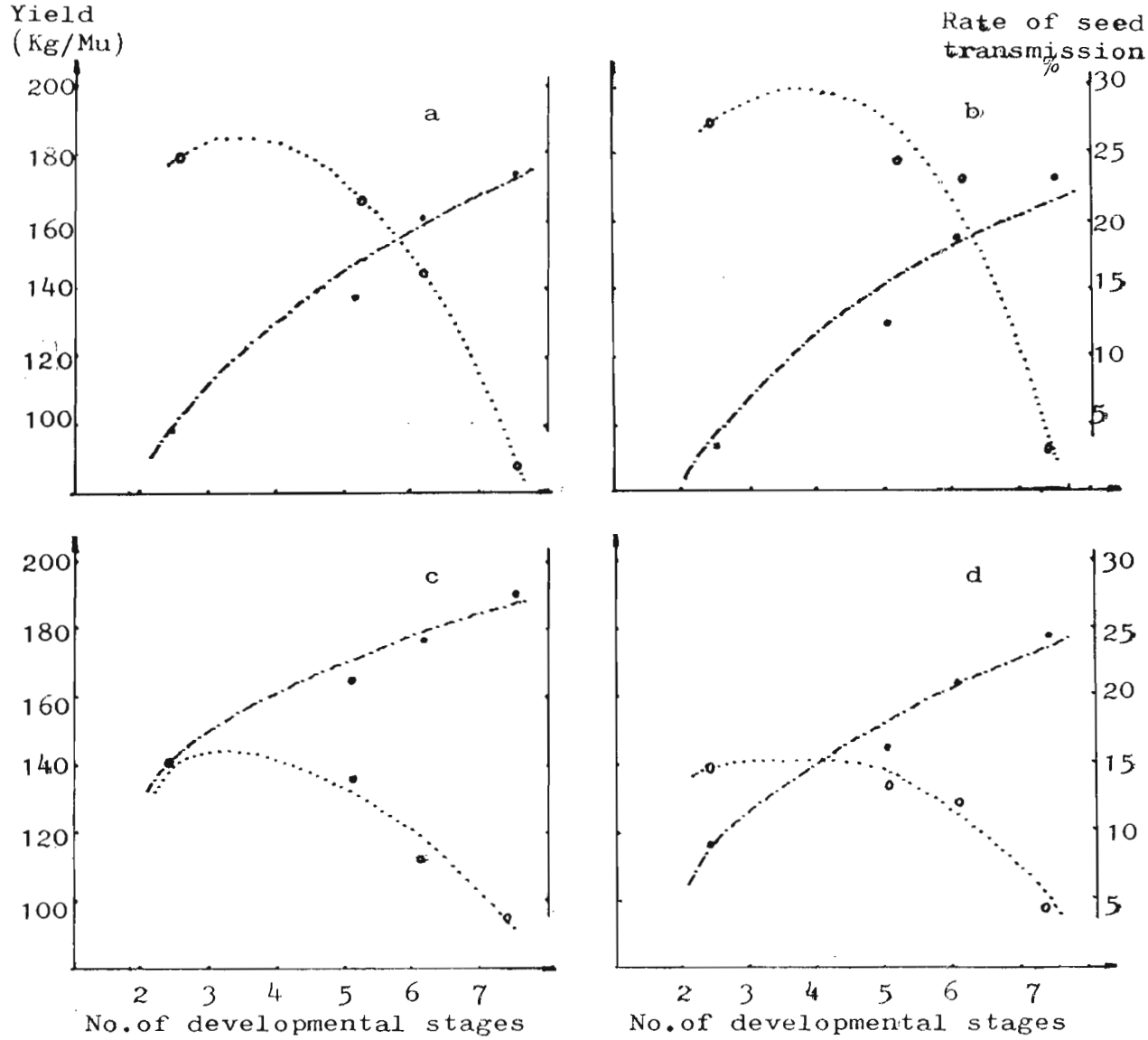
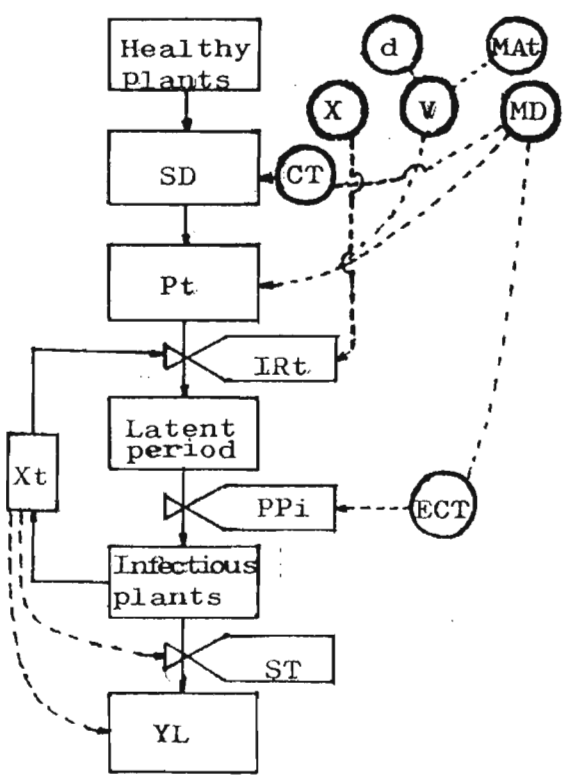


Fig.3 Regression analysis of the effect of infection time on yield and seed transmission rate

Based on the SMV-infected plants at developmental stage R6 without symptom(i.e. supposing no yield loss),the prognostic equations of yield loss and seed transmission rate were built of "No. of developmental stages of diseased plants which the systemic symptom appeared just then. The models of different cultivars were described by following equations:

	Yield loss model	Seed transmission rate model
a, Hefeng23 :	$YL=67.2061\ln(\frac{11}{SD})$ r=0.9781	$ST=9.477+10.767SD-1.571SD^2$ F=2076.55**
b, Dongnong34:	$YL=63.9416\ln(\frac{11}{SD})$ r=0.9451	$ST=3.042+15.083SD-2.020SD^2$ F=19.355*
c, Suinong3 :	$YL=42.5059\ln(\frac{11}{SD})$ r=0.9787	$ST=7.561+4.656SD-0.711SD^2$ F=16.945*
d, Suinong4 :	$YL=54.3079\ln(\frac{11}{SD})$ r=0.9706	$ST=5.854+5.670SD-0.779SD^2$ F=236.55**

We united with the sub-models as above,the simulation model for epidemics and damage of SMV was obtained(see Fig.4). To validate the simulation model,it had excellent valid degree.



Variable name	Description
X	Preliminary source
Xt	Incidence of disease
MAt	Per plant canopy area
V	No.of alate aphids landing on per plant canopy area
d	No.of alate aphids trapped with green-pantraps
MD	Meteorological factors(wind, temperature,rainfall)
CT	Cumulative temperature
ECT	Effective cumulative temperature
SD	Developmental stage of plants
Pt	No.of alate aphids per plant
IRt	Daily infection rate
PPi	Cumulative rate of systemic symptom appearance
ST	Seed transmission rate
YL	Yield loss

Fig.4 Elementary flow diagram of SMV epidemics and damage, variable name and their description

Control of zucchini yellow mosaic virus in cucurbits

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Zucchini yellow mosaic is the most destructive virus in cucurbits, particularly in melons in Israel. Attempts to locate resistance sources in melons where thusfar been futile. The present study reports successful protection using either the mineral oil "Virol" or its combination with pyrethroids. Laboratory experiments indicated that protectants were more effective and significant when alates were allowed free movement from infected to virus free plants in "arena" than in non-choice tests.

The effect of pyrethroids on departure of alates of Aphis gossypii and Myzus persicae was recorded. It was found that most alates will leave treated leaves within 5-7 minutes while few will do so in the control. Sprayers applying high pressure and volume combined with use of blowers were effective for control. The protection was to reduce the proportion of infetion in the plot, to affect the spatial and temporal distribution of infection and to increase significantly the yield of marketable melons. Control of non persistent viruses in dense crops is discussed.

Biological and serological Characterization of a number of potyvirus isolates from Israel

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Potyviruses are economically the most important causes for epidemics in cucurbits in Israel. Four cucurbit potyviruses which have served in this study were collected in the field from the greenhouse in Bet dagan. The primary characterization of the israeli cucurbit potyviruses was done on the basis of host reaction using a number of cucurbits, peas, Umbelliferous and Chaenopodium spp. hosts. Further classification of potyviruses was made by serological methods using antisera against zucchini yellow mosaic virus (ZYMV), watermelon mosaic virus 1 (WMV1) and watermelon mosaic 2 (WMV2). Using enzyme linked immunosorbent assay (ELISA) and serological specific electron microscopy (SSEM) we were able to classify three out of four isolates within the zucchini yellow mosaic virus (ZYMV). The fourth was found to react with an antiserum against Watermelon mosaic 2. Two of the ZYMV isolates were non aphid transmissible. However, one of the non aphid transmissible isolates infects peas while the other does not. Virions and helper extracts were purified from both isolates and both were found to produce helper components capable of assisting the transmission of virions from a transmissible isolate but of their own. The possible reason for lack of transmissibility of these isolates is discussed.

Transmission and retention characteristics of zucchini yellow mosaic virus (ZYMV) by two aphid species

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Zucchini yellow mosaic virus (ZYMV) is a member of the potyvirus group, that causes a disease which produces heavy losses in cucurbit crops in several Mediterranean countries, Central Europe, and the USA (Lisa & Lecoq, 1984). ZYMV is transmitted in a non-persistent manner by at least 8 aphid species (Lisa & Lecoq, 1984, Purcifull et al., 1984; Castle, unpublished). Reports by Nameth et al. (1985, 1986) indicate that this virus is probably the greatest single threat to the cucurbit industry in California. Yield losses are especially important when infection occurs previous to the flowering stage (Blua & Perring, 1989). Symptoms of the disease include mosaic, yellowing, stunting, chlorosis and fruit and seed deformation of zucchini squash (***Cucurbita pepo***), melon (***Cucumis melo***), cucumber (***Cucumis sativus***) and watermelon (***Citrullus lanatus***) (Lisa & Lecoq, 1984).

The short time that non-persistent viruses usually are retained by their vectors may limit the spread of these viruses. Non-persistent viruses have been reported as being retained by their vectors for only a few hours (Harris, 1978), although longer times have been reported (Berger et al.,1987). Retention of ZYMV by its vectors is one of the major factors that can affect the dispersion patterns of the disease.

Our studies have focused on the transmission characteristics of ZYMV by two of the major vectors,(***Aphis gossypii***, Glover, a colonizer and ***Myzus persicae***, Sulzer, a non- colonizer of cucurbits). Specifically we evaluated relative transmission efficiencies after different pre-acquisition starving times (0, 1, 2 and 4 h), acquisition access times (1, 3, 5 and 10 min), and inoculation times (10, 30, 60, and 240 min) for these species. Only young alates from laboratories colonies started from a single virginiparous female were used. Zucchini test plants were inoculated with single aphids, and symptoms were assessed 1-2 weeks after inoculation. An acquisition access period was used instead of the timed probe method because it simulates a natural acquisition more closely than does the timed probe process (Irwin and Ruesink, 1986). The acquisition access method gives a better approximation of vector propensity (*sensu* Irwin & Ruesink), especially if alates are used instead of aptarae.

The highest transmission efficiency (47.5%) of ***A. gossypii*** was obtained after a 1 hour pre-acquisition starving time, although no significant diferences were found

between the 1, 2 and 4-hour treatments. A 4-hour pre-acquisition starving time resulted in the highest transmission rate (35%) for ***M. persicae*** , although no significant differences were found between treatments. A 5-minute acquisition access time resulted in the highest transmission efficiency (65%) for ***A. gossypii.*** The lowest transmission rate (22.5%) was obtained in the 10 min treatment. ***M. persicae*** did not show any significant ($P \leq 0.05$) differences between treatments, although the trends were similar to the ones found for ***A. gossypii*** . No significant differences were found between treatments for the inoculation time study for either species. Also, no correlation was found between ELISA values of the leaves that were used as virus sources for the aphids, and the transmission rate. This resulted in a large degree of variabilty in the transmission efficiency of both aphid species when different virus sources were used.

The effects of different temperatures on the retention time of ZYMV by the 2 aphids also was studied. Alates had access to virus source plants for the time interval which resulted in the highest transmission rate. After virus acquisition access (5 min.), alates were suspended by their wings with single prong curl clips (Morris-Flamingo, Inc., Danville, Ill.), to prevent any kind of contact of their labium with a solid surface. These clips were inserted on plastic frames and introduced into Lexan (General Electric Co., Chicago, Ill.) boxes (12.5 x 15.0 x 15.5 cm). The air inside the boxes was maintained at a constant relative humidity by means of a controlled mixture of dry and moist air. The entire system was placed inside a Conviron growth chamber with fluorescent light (L:D = 16:8) at a constant temperature. Aphids were kept in the chambers for 1h, 3h, 10h, and 20h at 21ºC, and 75% relative humidity (RH) after the acquisiiton access time, and then individually transferred to test plants. Twenty test plants were used for each treatment, and the experiment was repeated twice. The study was repeated at 8ºC and 75% RH.. This temperature was selected because it is close to the flying threshold reported for other aphid species (Cockbain, 1961; Wiktelius, 1981). Aphids were mantained in the chambers for 3h, 5h, 10h, 20h, and 30h to evaluate retention. Twenty test plants were inoculated with single alates for each treatment and the experiment was repeated 5 times.

The results of this experiment for ***M. persicae*** are presented in Table 1. Results for ***A. gossypii*** were not analyzed at the time this summary was written. At 8ºC, ZYMV was retained up to 30 hours by ***M. persicae***. The retention times of ZYMV are much higher than the ones reported in the past for other potyvirus (Sylvester, 1954). According to these results, it is possible that early season infection of ZYMV by viruliferous aphids can come from long distances.

Table 1. Retention time of ZYMV by ***M. persicae*** at two different temperatures.

Time (hours)	% Transmission[1] (at 21ºC)	% Transmission[2] (at 8ºC)
1	33.0	-
3	5.6	11.11
5	0.0	5.10
10	-	2.0
20	0.0	1.94
30	-	1.0

[1] Average values of a total of 40 plants inoculated in two separate experiments
[2] Average values of a total of 100 plants inoculated in five separate experiments

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EVOLUTION OF MUSKMELON VIRUS INFECTION IN OPEN CROPS IN THE EBRO VALLEY.

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The evolution of natural virus infection on muskmelon in the Central Ebro area of Spain was studied in experimental plots during three consecutive years. Weekly observations of plant symptoms were made on 633, 574 and 590 plants in 1985, 1986 and 1987, respectively.

Seedlings were grown in pots and transplanted outdoors the 3rd June in 1985, the 28th May in 1986 and the 1st June in 1987.

Symptoms were first recorded 17 days after transplanting in 1985, 18 days in 1986 and 24 days in 1987.

The infection level reached 95 p. 100 71 days after transplanting in 1985. The incidence was of 100 p. 100, 70 and 66 days after transplanting in 1986 and 1987 respectively.

Samples were taken to determine the causal viruses through biological and serological test on 81 plants in 1985, 75 in 1986 and 50 in 1987. In 1985, 17 p. 100 of the sampled plants showed CMV infection, 72 p. 100 were infected by WMV-2, and 11 p. 100 had a mixed infection. In 1986, CMV was found in 48 p. 100 of the sampled plants, WMV-2 in 5.3 p. 100, and 42.7 p. 100 showed a mixed infection CMV+WMV-2; this year, a few plants (4 p. 100) were infected by MNSV alone, or mixed with CMV. 24 p. 100 of the plants sampled in 1987 appeared infected by CMV, 40 p. 100 by WMV-2 and 36 p. 100 of these plants showed a mixed infection CMV+WMV-2.

It is concluded that in the Central Ebro Valley, CMV and WMV-2 are the most widespread virus diseases.

COMPARISON OF SAMPLING METHODS FOR ALATE APHID POPULATIONS: A STUDY RELATED TO PLUM POX VIRUS SPREAD.

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INTRODUCTION

The plum pox (sharka) disease is the most important fruit tree virus disease in Europe, not only because of the severity of symptoms produced in plums, apricots and peaches, but also because of its frequent spread by aphids (OEPP,1974,1983). Plum pox virus (PPV) was first detected in Spain in 1984 (Llácer et al., 1985a,1985b), mainly in Japanese plum trees cv. Red Beaut. Afterwards, a natural spread of the disease has been observed among Japanese plum trees and apricot trees (Llácer,1987).

PPV epidemiology studies in Spain were initiated in 1986 to determine dominant aphid species . Flight graphs were made on stone fruit tree orchards in diseased areas (Avinent et al., in press). The study was performed using yellow water traps (Moericke,1951).However, in 1987, the group "sharka-apricot" of the EEC AGRIMED program decided to adopt the fishing-line trap method (Labonne et al.,1983) in order to conduct a coordinated study about the PPV epidemiology in several Mediterranean countries.

In this paper we give the results of the first year of alate aphid captures with fishing-line traps placed in apricot tree orchards in Valencia, comparing them to the yellow and green water traps.

METHODS

Three types of traps have been used:

1.- Yellow or Moericke traps (Moericke,1951). These traps were round, 30 cm in diameter and 10 cm deep. The insides were painted yellow (Titanlux synthetic smalt, 529), and they were filled with water, where the aphids will fall down attracted by the colour; this container was placed on a metal stand, 1.5 m high.

2.- Green traps. These have the same characteristics as the traps mentioned above, but the containers were painted green (Titanlux synthetic smalt, 516). We have used this colour because of its similarity to the traps described by Irwin (1980).

3.- Fishing-line traps (Labonne et al.,1983). These consisted of a wooden frame 31 x 28 cm, and transparent polyamide threads (0.4 mm thick) were fixed vertically 3.5 mm apart. The actual capture area (25.5 x 26.5 cm) was sprayed with a sticky product by means of a Souverode aerosol. Two fixed metal stands, placed in North-South (NS), East-West (EW) orientations and 1 m apart, were used to suspend two of these traps from each stand, being the sticky surfaces about 60 and 160 cm above ground level.

These three types of traps were placed for two months in the spring of 1988, in two apricot tree orchards of Alfarp and Carlet (Valencia) where the natural PPV spread is being studied year after year (García et al.,1988,1989). The aphids caught with the traps were collected twice a week and kept in alcohol until identification.

Data was analyzed by a factorial analysis of correspondences (FAC). The clustering method was that of Lance and Williams (1967).

RESULTS AND DISCUSSION

A total of 117 051 aphids, belonging to 29 identified species, was collected, distributed as shown in tables 1 and 2. By observing these tables, and from the FAC we obtained the following results:

- The three types of traps provided different results for some species, mainly the most abundant.

- Aphis spiraecola Patch (=A. citricola van der Goot) was caught in yellow water traps (89.7%) more than in the green traps (56.4%) and in the fishing-line traps (4.0%-21.6%). These results agree with those of Labonne et al. (in press).

- Aphis gossypii Glover was caught in fishing-line traps (68.1%-89.4%) more than in the green traps (37.5%) and in the yellow traps (8%).

- In Carlet (citrus area), T. aurantii Boyer de Fonscolombe was caught in the green trap (8.5%) more than in the yellow trap (2.6%) and in the fishing-line traps (0.3%-1.7%).

- Except for T. aurantii, there were no differences between the captures of different areas using the same trap colour.

- In the fishing-line traps the orientation had no influence either in the number of aphids caught for each species, nor in the number of species caught. This result agree with Labonne and Quiot's (1988). The height does have an influence with certain diferences depending on the species: A. citricola and T. aurantii were consistently more abundant in the traps placed 160 cm high, while A. gossypii was mostly caught in traps placed 60 cm high.

Table 1. Percentage of alate aphids captured in the fishing-line traps.

Species	ALFARP				CARLET			
	NS		EW		NS		EW	
	60cm	160cm	60cm	160cm	60cm	160cm	60cm	160cm
A. gossypii	88.0	68.1	89.4	69.4	84.9	72.4	88.8	76.0
A. spiraecola	8.2	21.6	6.3	18.7	6.1	18.9	4.0	15.1
H. pruni	0.3	0.7	0.3	1.1	5.7	2.5	4.6	4.1
M. persicae	0.2	0.6	0.4	1.1	0.0	0.2	0.1	0.2
T. aurantii	0.0	0.2	0.0	0.3	0.3	1.7	0.3	1.2
Others	3.3	8.8	3.6	9.4	3.0	4.3	2.2	3.4
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Table 2. Percentage of alate aphids captured in the water traps.

Species	Alfarp		Carlet	
	Yellow	Green	Yellow	Green
A. gossypii	8.8	38.7	7.2	36.4
A. spiraecola	89.7	58.8	89.7	54.1
H. pruni	0.0	0.0	0.1	0.1
M. persicae	0.6	1.1	0.1	0.2
T. aurantii	0.2	0.3	2.6	8.5
Others	0.7	1.1	0.3	0.7
Total	100.0	100.0	100.0	100.0

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Plant Mediated Effects of Zucchini Yellow Mosaic Virus Infection on *Aphis gossypii* Alatae Production, Population Development, and Colonization Behavior

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A relatively new virus system has become apparent in cucurbits worldwide over the passed eight years with the establishment of the potyvirus, zucchini yellow mosaic virus (ZYMV). ZYMV causes a disease which severely reduces yield in cultivated squash and melons. It first was reported in Italy and France in 1981, and since has been found in Spain, Germany, Morocco, Israel, Lebanon and the United States (Lecoq et al. 1983, Lesemann et al. 1983, Lisa et al. 1981, Pitrat and Lecoq 1984).

ZYMV is transmitted in a nonpersistent manner by several species of aphids including *Aphis gossypii* Glover (Lisa and Lecoq 1984), which colonizes cucurbits (Blackman and Eastop 1984). Thus, this pathosystem provides an opportunity to study the interrelations between a virus and a vector which share the same host, but have no direct physiological interaction. This investigation was designed to identify interactions between *A. gossypii* and ZYMV on *Cucurbita pepo* (zucchini) which potentially contribute to viral fitness.

Because of their absolute dependency on vectors, selection would favor viruses which adapt to their vectors in ways that enhance transmission. Alternatively, wide-spread viruses could be preadapted to their vectors as a consequence of selection acting on other aspects of fitness. Be that as it may, one can conceptualize two levels in which a nonpersistent virus and an aphid vector may interact to enhance transmission. It is important to keep in mind that such an interaction is necessarily mediated by the common host - the plant - since there is no direct physiological interaction in the nonpersistent mode of transmission. First, an alate aphid and a virus may interact on a behavioral level. In this scenario alatae may be attracted preferentially to virus infected hosts but avoid colony initiation on such a host after probing during sensory evaluation. This probing would result in the aphid becoming viruliferous, whereupon it might fly onto a more suitable, uninfected host. Two components of alatae behavior regarding virus transmission must be considered: landing on a potential host plant, and colony initiation after landing.

Our studies have investigated the roll of ZYMV infection on *A. gossypii* colony initiation. In a field study, 3 alatae, collected while in flight from a laboratory clone,

were placed on one of three outer leaves of zucchini plants which either were uninoculated, or infected with ZYMV for 14 or 28 days. The next morning, each leaf was examined for remaining alatae and colonies established. Out of the 3 original alatae placed on leaves, 2.3 remained on leaves of uninoculated plants and plants infected for 2 weeks. Only 1.6 alatae remained on leaves of plants infected for 4 weeks. These numbers of alatae were significantly different ($p < 0.05$). All alatae remaining on leaves in all treatments produced nymphs, the number of which was not significantly different among treatments.

The second level at which a nonpersistent virus and an aphid vector may interact to enhance transmission is morph development and population increase. Because of their higher mobility, alatae are the most important morphotype for virus transmission. On diseased plants, production of alatae may increase by two ways: (1) indirectly, due to an increase in aphid developmental rate, fecundity or longevity causing rapid crowding leading to alatae formation (Dixon 1985); or (2) due to a change in the nutritional status of the plant in a way the directly increases alatae production. Such an effect never before has been documented but it is well known that alatae production may be related directly to host nutritional status (For review see Dixon 1985).

Our field experiments have shown that morph development of *A. gossypii* is affected by ZYMV-infected plants in a way that may enhance transmission. When zucchini plants were inoculated simultaneously with ZYMV and infested with one newborn *A. gossypii* nymph, the colony produced after 20 days contained an average of over 118 alatae. Colonies on uninoculated plants, and plants inoculated with ZYMV 7, 14, and 21 days prior to aphid infestation averaged significantly less ($p < 0.05$) alatae. Simultaneously inoculated and infested plants also produced a number of apterae which were higher but statistically similar to that of uninoculated plants and plants infested at 7 and 14 days post-inoculation. *A. gossypii* developmental rates were statistically similar in all but one treatment, in which it was lower. In this treatment, infested 21 days post-inoculation, production of apterae and alatae also was lower than the other treatment groups. This suggests that plants infected for a long period are a poor host for *A. gossypii*. It is likely that *A. gossypii* alatae may avoid colony initiation on plants in this condition, but only after probing during sensory evaluation.

In a glasshouse experiment with uninoculated controls and zucchini plants inoculated with ZYMV 0, 7, 14, and 21 days prior to infestation by a single newborn apterae, we found that *A. gossypii* fecundity and longevity was greatest in the two treatments infected for the longest time period. Apterae on plants infected for 14 and 21 days produced and average of 44.2 and 42.9 nymphs respectively, while apterae on the uninoculated plants produced an average of 24.6 nymphs, which was significantly different ($p < 0.05$). Apterae on plants infested 0 and 7 days post-inoculation produced an intermediate number of nymphs. Longevity followed the same trend with apterae on plants infested 14 and 21 days post-inoculation living an average of 17.4 and 15.2 days respectively. Apterae on uninoculated controls lived a mean of 12.0 days which was significantly less ($p < 0.05$). We speculate that plants from treatments infected for 14 and 21 days were physiologically similar to plants

which were simultaneously inoculated with ZYMV and infested with *A. gossypii* in the field experiment, which supports the idea that increased alatae production is due to crowding.

Our investigations have shown the potential for ZYMV and *A. gossypii* to interact in such a way that transmission in enhanced. Behavioral and morph developmental effects may both work in any virus/aphid vector system, and may change throughout the infection process, which should be considered a dynamic event and not a static one.

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EPIDEMIC OUTBREAKS OF CMV AND PVY IN TOMATO CROPS IN SOUTHERN ITALY

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SUMMARY

In the summer of 1988 extensive viral outbreaks were recorded, particularly in canning tomato crops of some southern Italian regions (Apulia, Basilicata, Campania). The three prevailing diseases in the field, each epidemic in scale and largely separated from the others, were characterized by the following symptoms: (i)sudden progressive necrosis and death of the whole plant; (ii) severe deformation of leaves (fern leaf and shoestring) accompanied by stunting of plants and bushy growth; (iii) internal browning of fruits without deformation or discolouration of the foliage.

Regardless of any of the above disease syndromes, plants always had mixed infections of cucumber mosaic cucumovirus (CMV) and an ordinary (non-necrotic) strain of potato potyvirus Y (PVY). In addition, plants affected by lethal necrosis consistently hosted the CMV satellite known as CARNA 5. The biological and structural characterization of this CARNA 5 and its relationship to the necrosis outbreaks is presently under investigation.

A PRACTICAL TEST TO ASSESS THE POTENTIAL OF DISSEMINATION OF A NON-PERSISTENT APHID-BORNE VIRUS FROM AN INFECTED PLANT.

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The virus transmission rate by an aphid can be used to characterize the potential of dissemination of a virus from an infected plant if independent trials can be compared. In this aim we have designed and evaluated a technique to be used in routine test on the model Papaya ringspot virus - Squash - Aphis gossypii.

Aphids proceed from a clone. They are raised to obtain a population of the same age in stable controlled conditions provided by an environmental chamber. The manipulation material is adapted to test a 5 cm2 leaf area.

Experiments have been made to assess the influence of a number of factors regulating the test efficiency, and eventually optimizing them:

- number of aphids used jointly
- number of test plants to be used
- temperature during the test
- apparent probing behavior of aphids
- aphid rearing conditions
- age of aphids
- variation between operators

The variability of the transmission rates measurements has been evaluated on 2 types of experiments:

1) Short time experiments. Successive measurements were done on the same infected leaf area within a one hour period. Each transmission rate was calculated with a group of 20 aphids operated jointly. The measured transmission rates were stable after more than 16 successive measurements: there was no correlation with rank and the coefficients of variation were around 10 %.

2) On a long term period. 8 repeated measurements were done during 2 years using sets of 10 infected plants growing in stable controlled conditions. 30 aphids were used for each source, so that each measurement was calculated from 300 aphids. They also provided stable data: there was no correlation between time and rate of transmission, and the coefficient of variation was 10 %.

This technique has been used to characterize different virus isolates (Quiot et al., this session), the evolution with time of

different strains of virus in plants submitted to defined environmental conditions (Quiot et al., this session), the level of variation of vector efficiency inside the A. gossypii species (Lupoli et al., this session). Combined with the simulation model of Monestiez et al. (this session), it can be a tool to predict potential chance of dissemination of a virus under a defined plant and climate environment.

THE VARIABILITY OF TRANSMISSION EFFICIENCY OF A POTYVIRUS WITHIN THE SPECIES APHIS GOSSYPII (HOMOPTERA: APHIDIDAE).

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Non-persistent aphid-borne viruses are generally spread by numerous species of aphids. Differences in transmission efficiency occur between different species, both in controlled and field conditions. At the intraspecific level although few data are available (Jurik et al., 1980, Sohi & Swenson, 1964, Thottappilly et al., 1977, Upreti & Nagaigh, 1971) inefficient clones of aphid have been found (Lim et al., 1977). We investigate here the range of transmission efficiencies of a Potyvirus, the Papaya ringspot virus, by the aphid Aphis gossypii to assess its importance as an element of epidemics.

Seventy two clones of this aphid have been sampled from 18 different localities in southern France. They were maintained on melon and transferred on squash during 2 generations in controlled conditions before testing their transmission efficiency.

Each trial was performed using 6 clones. For each clone, 4 aphids per test plant were laid on 60 test plants after a 3 mn acquisition period. The transmission test was done with constant and optimal standardized conditions, in order to be able to compare each trial during time (Labonne et al., this session).

The same strain of PRSV has been used all along the time of the trials. Several mecanically infected plants were grown under controlled stable conditions to provide homogeneous source of virus. A clone of A. gossypii was used as a standard in each trial to detect possible variation of the source of virus or of other conditions.

Using these standardized conditions, the variability observed was low: a multiplicative factor of 3 separate maximal differences between transmission efficiencies. Considering the sampling procedure, non-transmitting A. gossypii clone might be rare, if even present, in the field. No relationship have been found between the locality or the host-plant and the transmission rate of the clones.

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Virus de la Sharka : Eléments d'épidémiologie

Répartition spatiale du virus dans un arbre et analyse du développement en verger

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Le virus du Plum Pox (PPV) responsable de la maladie de la Sharka est particulièrement dangereux chez la plupart des arbres fruitiers à noyau. Il provoque, chez les espèces les plus sensibles - Abricotier, Pruniers, Pêcher - de graves anomalies accompagnées de mosaïques sur feuilles. Il rend les fruits impropres à la commercialisation par des déformations épidermiques et altère leur qualité gustative par une réduction des teneurs en sucre et l'apparition de tâches liégeuses.
Appartenant au groupe des potyvirus, il est très efficacement disséminé par de nombreuses espèces d'aphides ce qui rend particulièrement délicate l'élaboration d'une stratégie de lutte contre la maladie et permet notamment de comprendre l'extension prise au cours des dernières décennies.
Observé pour la première fois en 1917 en Bulgarie il a été repéré dans de nombreux pays de l'Europe de l'Est et dans presque toute la zone circumméditerranéenne. En France son introduction remonte aux années 1960. Il a depuis lors fait l'objet d'un suivi et de mesures précises d'assainissement par le service de la Protection des Végétaux sans qu'une éradication ait été pratiquement possible.
L'organisation de la lutte contre cette virose revêt différentes formes :

- l'élaboration de matériels résistants, mais les perspectives restent alors éloignées par le fait que la sélection de nouvelles espèces fruitières prend en moyenne une quinzaine d'années.
- la recherche de stratégies de lutte en verger afin de limiter la progression de la maladie.

De nombreux progès ont été accomplis au cours des dernières années par le développement de méthodes de diagnostic de plus en plus précises (tests sérologiques, hybridation moléculaire). Leur efficacité dépend cependant des progrès attendus en matière d'épidémiologie donc de la connaissance sinon la maîtrise du développement de la maladie tant par sa répartition dans l'arbre que par sa diffusion en verger.

1 Répartition spatiale du virus dans un arbre.

Si l'accès à la répartition spatiale du virus dans un arbre est un élément déterminant dans la mise au point de stratégies d'investigation et de lutte, on se heurte chez les arbres

fruitiers à l'absence de méthodes statistiques et de techniques de visualisation adaptées aux liaisons pouvant exister au sein d'une structure arborscente.
Du point de vue statistique le problème majeur vient du fait que les mesures ou prélèvements sur un même arbre ne peuvent jamais être considérés comme indépendants : il existe une relation spatiale entre les différents "points" d'un arbre. Pour pallier ce problème une méthode est proposée qui s'appuie par sa formalisation sur la structure de dépendance. L'idée de base en est une généralisation du concept de variables régionalisées, utilisé en géostatistique, à un domaine spatial non-classique.
Sa mise en œuvre repose sur deux particularités liées à la structure du support utilisé :

- La définition de fonctions aléatoires sur un support arborescent. La variable qui nous intéresse est définie dans un espace géographique structuré par la morphologie de l'arbre étudié. Conditionnellement au support arbre (définissant l'espace métrique) nous modélisons en chaque point le phénomène étudié par une fonction aléatoire. Cette vision probabiliste permet de mieux prendre en compte la variabilité biologique et de dégager les relations essentielles des fluctuations locales.

- Le choix de distances appropriées dans la structure arborescente. La distance entre deux points d'un arbre est définie par l'intermédiaire d'une métrique. Nous recherchons les plus naturelles et les mieux adaptées à la modélisation des dépendances.

Développée et testée dans un cadre théorique précis et simplifié (arbre binaire), cette méthodologie générale a été appliquée au cas réel des arbres fruitiers, pour la détection de la Sharka sur un abricotier préalablement contaminé.

2 Analyse spatiale du développement en verger.

L'analyse spatiale des épidémies vise à prendre en compte le voisinage des plantes dans le développement de la maladie. Parmi les modèles statistiques possibles, les modèles de Gibbs sont plus particulièrement adaptés à ce but. Ces sont des modèles définis sur des grilles dont chaque noeud représente ici un arbre. Pour chaque point, la probabilité conditionnelle d'être dans un état sanitaire donné dépend de l'état des arbres dans un voisinage donné.
Notons $u = (i,j)$ les coordonnées (en entiers) d'un arbre et $X(i,j,t)$ son état à la date t. $X(i,j,t) = s$ s'il est apparemment sain, $X(i,j,t) = a_k$ s'il est attaqué à une date $k \leq t$, $X(i,j,t) = m_k$ s'il est mort depuis une date $k \leq t$. Le gradient s'il existe est pris dans la direction j. Soit $V_{i,j}$ l'ensemble des quatres voisins les plus proches de u.
Le modèle de Gibbs utilisé sera celui aux plus proches voisins. Il est défini par ses probabilités conditionnelles :

$$P(X(i,j,t) = a_t / X(i,j,t-1) = s) = e^U/(1+e^U)$$

avec

$$U = \alpha + \beta_1 \sum_{u \in V_{i,j}} 1_{\{X(u,t)=a_t\}} + \beta_2 \sum_{u \in V_{i,j}} 1_{\{X(u,t)=m_t\}}$$
$$+\beta_3 \sum_{u \in V_{i,j}, k<t} 1_{\{X(u,t)=m_k\}} + \beta_4 \sum_{u \in V_{i,j}, k<t} 1_{\{X(u,t)=a_k\}} + j\gamma$$

Les paramètres α et γ décrivent plutot la situation générale de la parcelle, alors que les paramètres β nous donnent l'influence du voisinage immédiat.

L'influence du voisinage (taille à prendre en compte) ainsi que de l'état des arbres qui le composent (arbres sains, morts, également malades, à la même date ou précédemment) peut alors être quantifié au travers de l'estimation des paramètres du modèle.
Nous présentons sur quelques exemples la mise en œuvre pratique de tels modèles (paramétrisation, estimation, tests d'hypothèses) ainsi que leurs limites.
Les paramètres α et γ sont souvent significatifs. Ils traduisent l'importance de l'influence régionale, due à la transmission à moyenne et grande distance, dans le développement de l'épidémie. Comparativement l'influence à courte distance est relativement faible et n'a pas toujours été mise en évidence. Elle s'est traduite différemment sur les paramètres β suivant l'intervalle de temps entre rondes d'observation et se révèle de ce fait plus difficile à interpréter.

Isolates from globe artichoke (Cynara scolymus L.) related to Broad Bean Wilt Virus and not transmitted by Myzus persicae Sulz.

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Artichoke virus related to broad bean wilt virus (BBWV) is widespread in Brittany. The three isolates - French artichoke (FA), Italien artichoke (IA) and Brittish artichoke (BA) - are transmitted by the aphid Capitophorus horni **Börner** in a non persistent manner. Artichoke is the only host-plant for C. horni, nevertheless this aphid can successfully transmit the virus to Physalis floridana **Rydb** and Vicia faba **L.** if the inoculum source is artichoke. Under our experimental conditions it has not been possible to transmit these isolates with M. persicae.

These isolates are serologically related to BBWV, but different from serotypes I, II and from Lamium mild mosaic virus (LMMV) and could be a distinct virus in the BBWV group (fabavirus).

Stochastic simulation of spatial contamination in an agricultural plot : a mean to connect virus transmission probabilities, vector behavior and global experimental results.

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Key Words : epidemiology, stochastic models, aphid-borne viruses, non-persistent transmission, simulation methods, numerical experiment.

1 Introduction

To describe the course of epidemics, several methods have been used which relate the proportion of infected plants to the time (Madden & Campbell, 1986) with general models (i.e. logistic, Gompertz).
An other approach was used by Ruesink & Irwin (1986) for the SMV and Sigwald (1986) for the PVY : they relate the proportion of infected plants to vector populations and transmission characteristics by a step by step simulation model using global probability laws applied to the populations of plants and vectors.
The originality of the model we present here is to manage individually each plant and each aphid vector. It results in maps of the spatial patterns of infected plants at each date. Due to the individual management of the vectors, the model is limited for obvious reason of size to non-persistent aphid-borne viruses.

2 Model description

In the model each plant is defined by its state (infected, source, healthy) and its location, and each aphid vector is defined by its state (infectious or not) and its movements during a day.
Each contact between aphid and plant is monitored and can result in a change of state of both aphid and plant.
The model is governed by eleven equations that are presented in the three next paragraphs. Each one concerns a functional part of the model according to the different actors of an epidemic.

2.1 Plants and experimental plot

The agricultural plot is supposed to be a regular grid whose dimensions are given in plant numbers. If the dimensions are $L_1 \times L_2$, we define M_t as the matrix of the values which characterizes the plants infection. M_t is indexed by the time t, the current element is $m_{k,l,t}$ where $k \in [1, L_1]$, $l \in [1, L_2]$, $t \in [1, T]$. T is the experiment duration.

$$m_{k,l,t} = \begin{cases} 0 & \text{the plant } k,l \text{ is healthy at time } t \\ n & \text{the plant } k,l \text{ was infected } n \text{ units of time before} \end{cases}$$

M_0 represents initial conditions of the simulation and M_t will be actualized each day (time unit) with the results of the new infections following the equations :

$$M_t = M_{t-1} + dM_t + M_t^+$$

$$dM_t = \begin{pmatrix} & \vdots & \\ \cdots & dm_{k,l,t} & \cdots \\ & \vdots & \end{pmatrix} \quad \text{where} \quad dm_{k,l,t} = \begin{cases} 1 & \text{if } m_{k,l,t-1} \geq 1 \\ 0 & \text{if } m_{k,l,t-1} = 0 \end{cases} \tag{1}$$

$$M_t^+ = \begin{pmatrix} & \vdots & \\ \cdots & m^+_{k,l,t} & \cdots \\ & \vdots & \end{pmatrix} \quad \text{where} \quad m^+_{k,l,t} = \begin{cases} 1 & \text{the healthy plant } k,l \\ & \text{becomes infected} \\ 0 & \text{otherwise} \end{cases} \tag{2}$$

2.2 Vector flight behavior

At each time unit (day), we consider N vectors which land inside the plot and can have secondary flights. Each individual vector is characterized by its geographical position and by its infection level.
The vector i lands on the plant of coordinates x_i, y_i and have a succession of P local flights (indexed by $j \in [0, P]$) to nearby plants with movement of dx, dy.
All these data are generated by random laws according to the equations :

$$P \sim \mathcal{P}(p) \text{ where } \mathcal{P} \text{ is a Poisson distribution of mean } p \tag{3}$$

$$\begin{cases} x_{i,0} \sim \mathcal{U}(1, L_1) \\ y_{i,0} \sim \mathcal{U}(1, L_2) \end{cases} \quad \text{where } \mathcal{U} \text{ is the uniform discrete distribution} \tag{4}$$

$$\begin{cases} x_{i,j+1} = x_{i,j} + dx \\ y_{i,j+1} = y_{i,j} + dy \end{cases} \quad \begin{cases} \text{where } dx \text{ and } dy \sim \mathbb{E}(\mathcal{N}(0, \sigma^2) + 0.5) \\ \text{where } \mathcal{N} \text{ is a gaussian distribution} \\ \text{and } \mathbb{E} \text{ the integer part of a real} \end{cases} \tag{5}$$

After the secondary flight j, the infection level of the vector i is measured by $e_{i,j}$. The initial state of the vector is $e_{i,0}$.

$$e_{i,0} \sim \delta(a_0) \quad \begin{cases} \text{where } \delta(a_0) \text{ takes the values 1 or 0} \\ \text{with the probabilities } a_0 \text{ and } (1 - a_0) \end{cases} \tag{6}$$

$$e_{i,j} = \max\left(\frac{e_{i,j-1}}{d}, e^+_{i,j}\right) \quad \begin{cases} \text{where } e^+_{i,j} \text{ is the random} \\ \text{result of the virus acquisition} \\ \text{on a source plant and } d \text{ a parameter} \geq 1 \end{cases} \tag{7}$$

2.3 Transmission probabilities between vectors and plants

If the vector i lands after the flight j on the plant with the coordinates $k = x_{i,j}$ and $l = y_{i,j}$, the virus transmission between vector and plant can be reduced to four different cases. It depends on the state of both the plant and the vector, either healthy or infected.

$$\begin{cases} \text{if} \quad e_{i,j} = 0 \\ \text{if} \quad m_{k,l,t} = 0 \end{cases} \Longrightarrow \begin{cases} e^+_{i,j} = 0 \\ m^+_{k,l,t} = 0 \end{cases} \tag{8}$$

$$\begin{cases} \text{if} \quad e_{i,j} > 0 \\ \text{if} \quad m_{k,l,t} = 0 \end{cases} \Longrightarrow \begin{cases} e^+_{i,j} = 0 \\ m^+_{k,l,t} = \delta(a) \\ \text{where } a = a_{max} \times e_{i,j} \end{cases} \tag{9}$$

$$\begin{cases} \text{if} \quad e_{i,j} = 0 \\ \text{if} \quad m_{k,l,t} \geq 1 \end{cases} \Longrightarrow \begin{cases} e^+_{i,j} = \delta(b) \\ m^+_{k,l,t} = 0 \\ \text{where } b = b_{max} f(m_{k,l,t}) \end{cases} \tag{10}$$

The function $f(m)$ is a logistic function of the form: $f(m) = \frac{1}{1+ce^{-hm}}$ with $c = \frac{1-f_0}{f_0}$ and h is proportional to the latent period. a and b are respectively the maximal probability for an infected vector to transmit the virus to an host plant and the maximal probability for a source plant to transmit the virus to a vector.

$$\begin{cases} \text{if} \quad e_{i,j} > 0 \\ \text{if} \quad m_{k,l,t} \geq 1 \end{cases} \Longrightarrow \begin{cases} e^+_{i,j} = \delta(b) \\ m^+_{k,l,t} = \delta(a) \\ \text{where } a \text{ and } b \text{ are the same than previously} \end{cases} \tag{11}$$

2.4 Spatio-temporal running

Under the hypothesis of flight independence (the different flights can be relevant of a same behavior but one does not interact with the others), each succession of vector flights is simulated. For each landing, the random result of virus transmission is calculated according to previous equations.

Each day, the matrix M_t^+ is built with the N vectors of the day using just the matrix M_t as previous information. The simulation process starts with initial conditions defined by M_0 and ends at the date T.

To avoid edge effects in a first time, we adopt the torus convention for the vectors movements (the edge of the plot is connected with the opposite edge and a rectangular plot became equivalent to a torus). If we add some behavior rules when the vectors move out of the plot, edge effects can be studied.

3 Simulation and first results

The model has been run to simulate epidemics of a virus resulting from increasing numbers of vectors. Its simulations were compared to a set of data provided by the study of PRSV-W in squash fields under different vectors pressures. Actual and simulated data are in good agreement.

One of the main interests of this simulation model is its capacity to relate a change in elementary transmission probability to the temporal progress of virus incidence. Measurements

in controlled conditions can be directly used in predictive aims to test new varieties or to estimate potential damages of different virus populations. Numerical experiments can reduce significantly the number of trials.
The maps given by the model can be used to validate or refuse usual assumptions on vector behavior. The actual data of spatial patterns of infected plants are scarcely used because of a difficult interpretation. Generalized to several vector populations, this model gives a tool to analyse complex diffusion resulting of two vectors behaviors.

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THE OCCURRENCE OF ALFALFA MOSAIC VIRUS IN YEMEN ARAB REPUBLIC

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INTRODUCTION

Alfalfa mosaic virus (AlfMV) is seed-transmitted in alfalfa and is also aphid-transmitted in a non-persistent manner (Jaspars and Bos, 1980). It has a very wide host range amongst vegetable and other species and is reported to cause severe diseases in alfalfa (Crill **et al.** 1971), potato, tomato, pepper, legumes, carrot, celery, parsley and dill in many countries (Walkey **et al**, 1989).

The occurrence of AlfMV in the Yemen Arab Republic (YAR) has recently been monitored as a part of a project to survey plant virus diseases in that country (Alhubaishi **et al**, 1987 and Walkey **et al**, 1989).

RESULTS AND DISCUSSION

AlfMV was the most frequent and widespread virus isolated in the YAR (Table 1). It was found in all geographical areas, from the Tihama desert (300m) bordering the Red Sea, through the Central Highlands (2700-3700m) and down to the Eastern Desert regions (1300m) bordering the Empty Quarter of the Arabian Desert. It was common wherever susceptible vegetable and herb crops were grown in close proximity to alfalfa forage plantings. The virus was also isolated from weeds (**Datura** sp.) showing mosaic symptoms and from a legume shrub (**Parkinsonia aculeata**) showing chlorotic mottle leaf symptoms, growing adjacent to infected alfalfa.

AlfMV was frequently isolated from tomato plants showing necrotic symptoms and severely infected plants of the cv. Roma VF and local Yemen cultivars often had necrotic lesions on the fruits. The virus was regularly isolated from potato with vivid yellowing symptoms and from stunted sweet and chilli peppers with chlorotic mottle leaf symptoms.

Chlorotic mottle and leaf reddening symptoms were common in coriander plants infected with AlfMV. The virus was also isolated from leaves of dill, parsley and carrot with chlorotic blotch or veinal chlorosis symptoms and from French and broad beans with mosaic symptoms. AlfMV has also been isolated from Okra, local cultivars of cowpea (**Vigna sinensis**) and **Cassia occidentalis.**

TABLE 1. Occurrence of alfalfa mosaic virus in various crops in Yemen Arab Republic

Host species	Isolations	Location isolated
Tomato (**Lycopersicon esculentum**)	7	Sana'a - Central Highlands Al-Masajid - Central Highlands Ma'bar - Central Highlands
Potato (**Solanum tuberosum**)	1	Al-Masajid - Central Highlands
	6	Amran - Central Highlands
Sweet pepper (**Capsicum annuum**)	1	Haddah - Central Highlands
Chilli pepper (**C. annuum**)	1	Wadi Sheih - Western Escarpment
Okra (**Hibiscus esculentus**)	1	Haddah - Central Highlands
Carrot (**Daucus carota**)		
Dill (**Anethum graveolens**)	1	Haddah - Central Highlands
Parsley (**Petroselinum crispum**)	1	Haddah - Central Highlands
Coriander (**Coriandrum sativum**)	3	Al-Masajid - Central Highlands Haddah - Central Highlands
Broad bean (**Vicia faba**)	1	Dhamar - Central Highlands
Dwarf bean (**Phaseolus vulgaris**)	3	Haddah - Central Highlands
Datura sp (yellow flowered)	1	Taizz - Southern Uplands
Parkinsonia aculeata (Shrub)	1	Marib - Eastern Desert
Cassia occidentalis	1	Medinat Ash Shirq - Western Escarpment
Cowpea (**Vigna sinensis**)	1	Tihama - Red Sea Coast

Every alfalfa crop sampled, in all topographical areas, was infected with AlfMV (Table 2). Leaf symptoms in alfalfa were variable, ranging from mild chlorotic streaks and mottles to vivid chlorotic blotches. No other virus has yet been isolated from these alfalfa crops. The proportion of plants showing symptoms in recently planted crops, is much lower than that in longer established crops many of which were grown for 7 years or longer.

Table 2. Occurrence of alfalfa mosaic virus in alfalfa in Yemen Arab Republic

Host species	Isolations	Location isolated
Alfalfa (**Medicago sativa**)	2	Dhamar - Central Highlands
	4	Amran - Central Highlands
	3	Qa Al Bawn - Central Highlands
	1	Dawran - Central Highlands
	2	Wadi Altishliel/Central Highlands
	2	Jabal an Bani Shu'ayb/C Highlands
	1	Al-Masajid - Central Highlands
	1	Ibb - Southern Uplands
	3	Wadi Sheih - Western Escarpment
	7	Sa'dah - Northern Highlands
	5	Marib - Eastern Desert

Table 3. Occurrence of alfalfa mosaic virus infection in different batches of alfalfa seed in Yemen Arab Republic

Sample No.	Source	Cultivar	% virus infection
1.	Dhamar Souk	Bahadhi*	1
2.	Dhamar Soul	Kauli*	1
3.	Research Authority,Dhamar	Bahadhi*	12
4.	Research Authority,Dhamar	Kauli*	2
5.	Research Authority,Dhamar	Diablo Verde	1
6.	Research Authority,Dhamar	CUF. 101 (Nickerson)	3
7.	Research Authority,Dhamar	Hunter River	0
8.	Amran Souk	Raimani*	11
9.	Sana'a Souk	Raimani*	0
10.	Raydah Souk	Reili*	1
11.	Souk Al Amar (Nr Sa'dah)	Bahadhi*	1
12.	Souk Al Amar (Nr Sa'dah)	Local Yemeni	2
13.	Souk Al Amar (Nr Sa'dah)	Turkish cv. (?)	0
14.	Sadah Souk	Unknown cv.	3
15.	Souk Al Talh (Nr Sa'dah)	Saudi Arabian cv. (?)	3
16.	Sadah Souk	CUF. 101 Nickerson	3

* local Yemeni cultivars

Commercial stocks of alfalfa seed from various areas of the YAR usually carried seed-borne AlfMV. Seed transmission rates varied from 1 to 12% (Table 3).

The frequent infection of vegetable and alfalfa forage crops by AlfMV in the YAR is attributable to the high levels of seed transmission of the virus in commercial seed stocks, and to the traditional farming practices used in villages in which many small areas of mixed vegetable and alfalfa crops are grown side by side. In the Yemen, the combination of a high incidence of seed transmitted virus in commercial alfalfa crops and a climate that favours high populations of the aphid vectors, results in rapid spread of the disease in newly-established alfalfa crops and subsequently to adjacent vegetable crops. The alfalfa is also a 'reservoir' host of the virus allowing it to overwinter and survive the dry season when surrounding vegetable crops and alternative weed hosts are absent. The longer the alfalfa crop remains in the ground, the greater the overall infection level in the crop and the greater the 'reservoir' of AlfMV for transmission to newly planted vegetable crops in the next growing season.

The objectives of control measures to avoid AlfMV infection should be twofold; firstly to avoid infection in the vegetable hosts and secondly, to decrease infection in alfalfa to improve its yield and quality. Separation of susceptible vegetable crops from the alfalfa crops would largely achieve the first objective. In some areas, social division of

the land into small units may make this control measure difficult to implement but one possible solution may be to grow all alfalfa crops in one section of the village, separated from vegetable crops.

The second control objective of eradicating the virus from alfalfa crops, would result in improved fodder yields and quality. Since the major initial source of infection in the alfalfa crop is from seed-borne AlfMV, the use of virus-free seed stocks would be the most effective control procedure. The virus could be eliminated from seed stocks by growing seed crops from a nucleus of virus-free seed in isolated areas and then controlling subsequent multiplication of these healthy stocks. Seed should be collected only from one-year old plants and crops for seed production should be inspected and indexed for seed infection.

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Epidemiology of Yam mosaic virus : importance of aphid transmission.

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SUMMARY

Yam mosaic virus (YMV), a member of the Potyvirus group, causes a severe disease of yam, *Dioscorea* spp., mainly in Africa and the Carribean. There have been no data available hitherto on the quantitative effects of the disease on crop yield, due to difficulties in detecting the presence of the virus in the plant.

By using ELISA tests to identify healthy yam material prior to and after planting, reliable estimates of crop losses due to YMV were obtained through serial field trials over a three years period. Fresh tuber weights obtained from diseased *Dioscorea alata*, cv. Florido averaged 15% lower than those obtained from healthy plants grown under similar conditions. There was no difference however in the number of tubers formed.

Disease contamination by aphid vection during crop growth was found to be relatively slight compared to disease levels in the initial seed tubers. This suggests that selection of virus-free tubers could rapidly eradicate yam mosaic disease.

Yam mosaic disease is the major problem in yam cropping and the *D. cayenensis-rotundata* complex is particularly susceptible to it. The Phytovirology laboratory at Adiopodoumé (near Abidjan, in southern Ivory Coast), was amongst the first to identify and characterise the causal agent, Yam mosaic virus or YMV. It is a filamentous virus 750 nm in length, is aphid-borne in the non-persistent manner and is related to the Potyvirus group (Thouvenel & Fauquet, 1977, 1979). The virus has been purified and a specific antiserum prepared. As a result it was discovered that the area of distribution of the disease is very large and extends beyond Africa, for we have found diseased plants in the Carribean and in the Pacific (Thouvenel & Fauquet, 1986).

No other viruses or mycoplasmas have been identified from yam during 15 years of surveys in Ivory Coast, nor in neighbouring countries (Thouvenel & Fauquet, 1986).

Following the development of a diagnostic method for detecting the disease, based on immunoserological techniques, epidemiological studies were recently begun in Ivory Coast (Thouvenel & Fauquet, 1980, 1982). The initial results of this study are presented in this paper.

The cultivar used in these trials was *D. alata* cv. Florido, a variety widely grown in central Ivory Coast and considered to be tolerant to yam mosaic disease.

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Evaluation of yield losses due to Yam Mosaic Virus

Experimental conditions : Seed-tubers were collected from plants subjected to ELISA test to determine sanitary state before harvest.

4 fields of 240 plants each were planted (12 rows of 20 plants) with healthy and infected seed-tubers, disposed in Fisher block where 1 block comprised 3 rows of 5 plants. Detection of YMV was achieved by an other ELISA test on the leaves during crop cycle.

1) Yield of presumed healthy and diseased plants based on ELISA tests before planting.

	Number of plants	Mean yield per plant (g)	Losses
Healthy	480	6150*	
Diseased	480	5350*	13%*

* *highly significant (P< 0.002).*

2) Yield of true healthy and diseased plants based on ELISA tests during cropping

	Number of plants	Mean yield per plant (g)	Losses
Healthy	550	6670 *	
Diseased	328	5630 *	15%*

* *highly significant (P< 0.001).*

It can be seen that diseased plants showed decreases in yields of about 15% (highly significant).

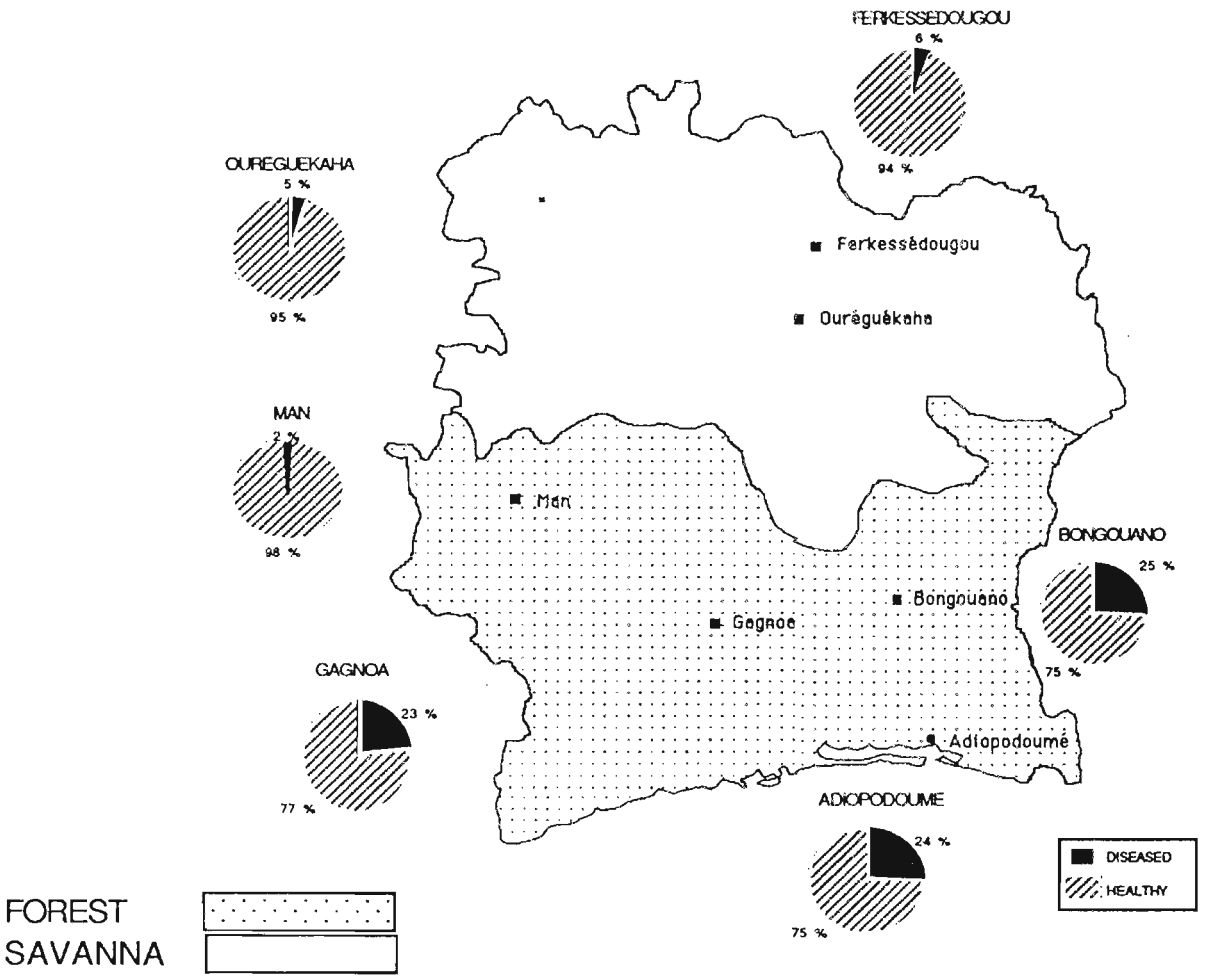
Based on the results of ELISA tests before planting and before harvest, it appears that 480 plants were presumed planted using healthy seed-tubers, but 550 healthy plants were found during crop cycle.

This fact seems to indicate that an infected seed-tuber can produce healthy plants. This point is further investigated in our laboratory. First results seems to confirm it.

This could be an important factor for the recovering of a healthy plant material from infected tubers. It would be a simple way of sanitation.

Epidemiological study of YMV in the Ivory Coast

Experimental conditions : Presence of the virus was determined by ELISA test on leaves before seed-tubers harvest. Fields of 120 healthy plants were planted in 6 different places of the Ivory Coast under usual local cropping conditions. After harvest tubers of each place were planted in pots under insect-proof greenhouses at Adiopodoumé and ELISA tests were performed on leaves.



The disease incidence is only attributable to insect vector contamination that range from 25% in south Ivory Coast to about 6% in savanna region (north). The presence of yam fields which could be a potential source of contamination near our trials, was not took in account during this study.

CONCLUSION

From the above results, the following conclusions may be drawn:

1) Yam mosaic disease may cause yield losses of 15% in *D. alata* cv. Florido despite the fact that this cultivar is considered to be tolerant to the disease.

2) Yam mosaic disease does not affect the whole number of tuber-seed pieces produced by a diseased tuber. This observation has implications for the selection of planting material.

3) Rates of transmission of the disease by aphid vectors seems very slow during crop growth in the savanna region ; determination of disease transmission in relation with insect vector will be very useful in the near future to choose the propagation sites for yam cropping.

Other experiments are now in progress to determine the effects of environmental conditions on the patterns of disease incidence and spread.

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Daniels, J. and Campbell, R. N.

Epidemiology of cucumber mosaic virus (CMV) in the southern Santa Clara Valley of California

Epidemiology of cucumber mosaic virus (CMV) in the southern Santa Clara Valley of California

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CMV was the predominant virus in the epidemics that severely damaged pepper crops in 1986-87 near Gilroy, Santa Clara county, CA. In March and April of 1988 and of 1989 the vegetation in the area was tested to identify the overwintering hosts of this virus. More than 400 samples belonging to about 30 species of winter annuals and perennials were tested for CMV by ELISA. Positive ELISA results were obtained with 111 samples from only 5 plant species: Vinca major, V. minor, Hirchfeldia incana, Urtica sp, and Cucurbita sp. V. major may play an important role on CMV epidemiology in that region. It occurs as escaped, naturalized plants along streams, as well as in landscape plantings. Although the incidence of CMV in V. minor was high, it has been found only in landscape plantings. The role of the other species is less clear. The positive ELISA reactions for H. incana and Urtica sp. were false positives because CMV not recovered by sap transmission. Nicotiana glauca and Datura meteloides, common virus reservoir hosts in other agricultural regions of California, have not been found in this area. Virus infection of peppers in 10 fields was monitored in 1988, a year in which there was drouth in late winter-spring in contrast to the previous two years. CMV incidence remained low for the entire summer in all fields except the field located nearest to stands of infected V. major.

COMPARATIVE EPIDEMIOLOGY AND ECOLOGY OF NON PERSISTENT APHID BORNE VIRUSES INFECTING CUCURBITS IN FRANCE.

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A cucumovirus (Cucumber Mosaic Virus, CMV), and three potyviruses (Papaya Ringspot Virus type W, PRSV-W, Watermelon Mosaic Virus 2, WMV2, and Zucchini Yellow Mosaic Virus, ZYMV) are commonly found infecting Cucurbit crops in France. These four viruses are transmitted non persistently by several aphid species, and have been associated occasionally with very severe yield losses.

CMV epidemiology and ecology have been extensively studied in southern France (Quiot et al., 1980). The increase in frequency of potyviruses infections pointed out the need for a better understanding of the epidemiology and ecology of these viruses, in order to develop appropriate control measures.

In a first approach, virus epidemics were studied in melon (**Cucumis melo** L. var. "Védrantais") plots from 1981 to 1987, and in Zucchini Squash (**Cucurbita pepo** L. F1 hybrid "Diamant") plots from 1982 to 1986. Plots of approximatively 600 m^2 were planted with 225 plants of each species every year. Plants were observed individually for mosaic symptoms every 2 or 3 days. At weekly intervals, 30 samples were collected from plants developing mosaic symptoms in each plot and their virus content was characterized using SDS immunodiffusion technique (Purcifull and Batchelor,1977) and antisera against CMV, PRSV-W, WMV 2, and ZYMV. In separate experiments, this technique proved to be as efficient to detect these viruses in field samples as DAS ELISA, but much less time consuming. The percentage of plants infected by a virus was deduced from the percentage of plants found infected by this virus within the sample analysed serologically and from the total number of plants with mosaic in the plot.

In melons, epidemics of CMV and WMV 2 (Fig. 1 and 2) occurred every year, soon after planting. In contrast ZYMV (Fig. 3) was observed only in 1983,1985 and 1987 and its occurrence was later in the season. PRSV-W was found only in 1986, late in the growing season (Fig. 4).

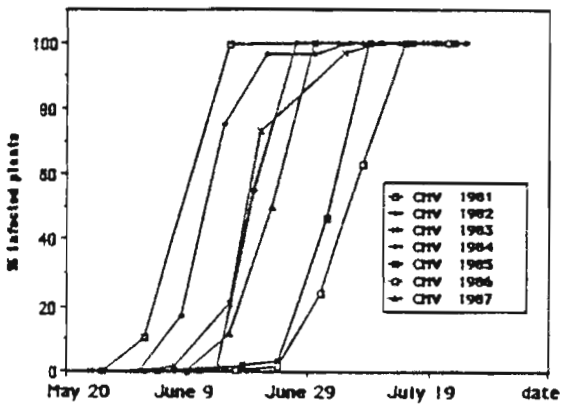

Figure 1: Development of CMV epidemics in melon var. "Védrantais" in Montfavet, France, 1981-1987.

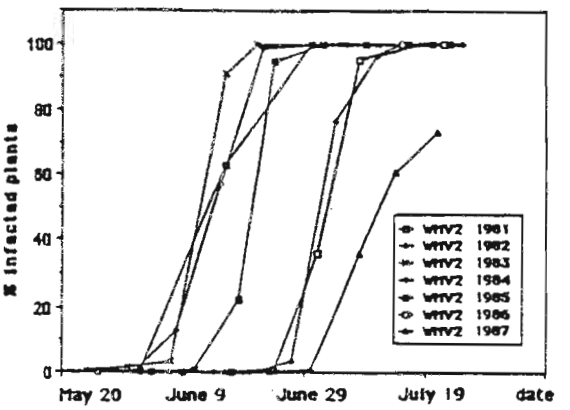

Figure 2: Development of WMV2 epidemics in melon var. "Védrantais" in Montfavet, France, 1981-1987.

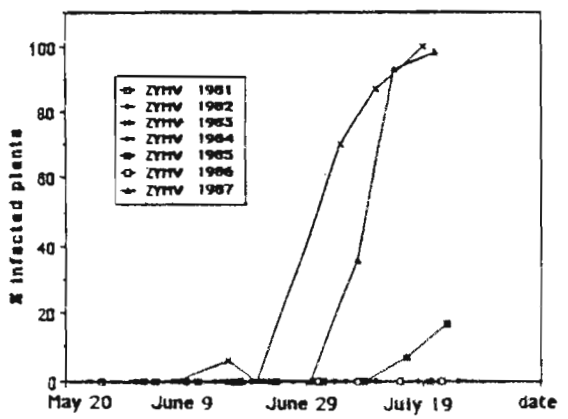

Figure 3: Development of ZYMV epidemics in melon var. "Védrantais" in Montfavet, France, 1981-1987.

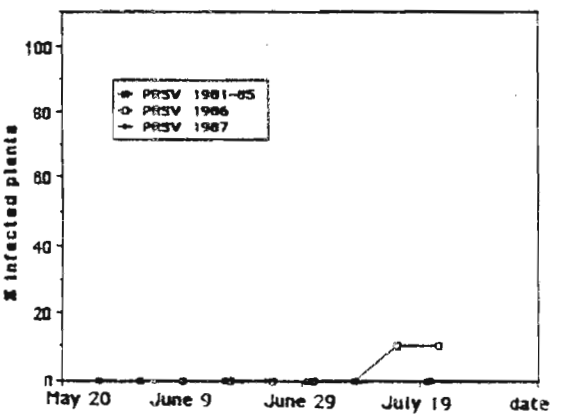

Figure 4: Development of PRSV-W epidemics in melon var. "Védrantais" in Montfavet, France, 1981-1987.

The epidemic curves have similar "S" shapes with a steep slope, except when the first infections are late in the season. Generally all plants became infected by a virus 2-3 weeks after 5% of the plants were found infected.

In zucchini squashes, WMV 2 followed a very similar pattern of spread. For ZYMV and PRSV-W epidemics were also very similar to those observed in melon, except that they may develop a bit further due to the longer growing period of this crop (4 to 6 weeks more than melons). In particular some late infections by ZYMV were noticed in 1982 and 1984 while no ZYMV infection was detected in melons. In contrast CMV epidemics developped later and

slowlier in zucchini squashes than in melons and generally did not reached a 50% infection rate by the end of the crop. This suggests a lower "field" susceptibility to CMV in zucchini squash than in melon.

CMV has a number of alternative hosts among weeds growing around vegetable fields in southern France (Quiot et al., 1979); This abundance of reservoirs has been pointed out as a major reason for the earliness of CMV epidemics in melon crops. The similarity observed in the spread of CMV and WMV2 in melons (Fig. 1 and 2) prompted the search for WMV2 overwintering hosts.

Weeds were left growing freely in plots where zucchini squashes had been cultivated, during the winters 1985-86, 1986-87, and 1988-89. Common weeds known to be susceptible to WMV 2 following mechanical inoculation (Lecoq et al., 1981) were collected at random from November to May and analysed using biological tests and SDS immunodiffusion or DAS ELISA. Four new natural hosts of WMV2 were identified: **Capsella bursa-pastoris** (L.) Medik (28 plants found infected out of 254 tested), **Fumaria officinalis** L. (4 out of 108), **Lamium amplexicaule** L. (13 out of 225), and **Senecio vulgaris** L. (8 out of 261). Some individual plants of **C. bursa-pastoris** and **L. amplexicaule** were found infected by WMV2 in november and were still alive the following early May when new Cucurbit plantings were made. Aphids allowed to probe on these plants readily transmitted WMV2 to test plants demonstrating the potential importance of these weeds as natural reservoirs of WMV2. Interestingly these weed species are different from those reported in the arid climate of southwestern Arizona (Nelson and Tuttle, 1969) or the subtropical climate of central Florida (Adlerz, 1969). This suggests the capacity of WMV2 to adapt itself to different ecological conditions.

Several of these plants were found co-infected by CMV, but none was infected by PRSV-W or ZYMV, although some ZYMV isolates are known to infect **L.amplexicaule** following mechanical inoculation (Lecoq et al.,1981).

The earliness and the regularity of the CMV and WMV2 epidemics, and the identification of natural reservoirs for these viruses suggest that these two viruses can readily overwinter in France. In contrast PRSV-W, which occurs only occasionnaly, is more likely to be introduced by aphids from more meridional regions, where it can overwinter in cultivated Cucurbits. As for ZYMV the irregularities of the epidemics suggest either a similar situation than for PRSV-W, or that the overwintering hosts for this virus are present but uncommon in our regions.

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Une mosaïque du NIEBE (Vigna unguiculata) transmise par pucerons au Burkina Faso : Quelques données épidémiologiques.

G. Konate

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Le NIEBE (Vigna unguiculata) est une légumineuse alimentaire riche en protéine. Il est largement cultivé en Afrique Sahélienne et Soudanienne.

Nous avons identifié une contrainte majeure à la production de cette légumineuse. Il s'agit d'une mosaïque sévère transmise par les pucerons, notamment par Aphis craccivora, et par les semences.

Les symptômes de cette maladie varient considérablement d'une variété à l'autre.

L'agent pathogène responsable de la maladie a de nombreuses caractéristiques communes avec un virus que l'on retrouve au NIGERIA, le Cowpea Aphid-borne Mosaïc Virus (CABMV).

En effet, il est reconnu très facilement par un antisérum préparé contre ce virus par l'IITA et a la même gamme d'hôtes

Les études épidémiologiques réalisées durant les années 1987-1988 et 1988-1989 nous ont fourni les informations suivantes :

- la maladie est présente sur l'ensemble du pays, depuis la zone sahélienne (400-500 mm de pluie) jusque dans la zone Nord Guinéenne (900-1200 mm de pluie). Mais l'importance de la maladie décroît avec la faiblesse de la pluviométrie.

- les taux d'infection des champs peuvent atteindre 100 %.

- La maladie se propage dans les champs rapidement.

- Les pertes de rendement peuvent atteindre 50 %.

- Les taux d'infection sont fonction des dates de semis. Les semis précoces sont plus touchés que les semis tardifs.

- Bien que nous n'ayons pas identifié de source de résistance à cette maladie, on constate que toutes les variétés ne sont pas attaquées au même degré.

WEEDS, HOSTS OF PHYTOPATHOGENIC VIRUSES IN BASILICATA AND APULIA (SOUTHERN ITALY)

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A survey on plant viruses infecting weeds was carried out in Basilicata and Apulia in the last two years.

Cucumber mosaic virus (CMV) was isolated from the following species: Amaranthus retroflexus L., Cirsium arvense Scop., Chenopodium album L., Clematis vitalba L., Portulaca oleracea L., Erygeron canadensis L., and Solanum nigrum L. The CMV isolate from A. retroflexus, mechanically inoculated to tomato seedlings (cvs Bandera, Indo) under controlled environmental conditions (22-24 °C) caused severe necrosis of stems and leaf petioles followed by plant death.

Pelargonium zonate spot virus was isolated from symptomless Diplotaxis erucoides D.C. and from Chrysanthemum coronarium L. showing leaf mosaic, deformation and stunting.

Two flexuous elongated viruses, morphologically and cytopathologically resembling closteroviruses, were isolated from Sonchus oleraceus L. and Conium maculatum L. exhibiting vein clearing and yellow mosaic on the leaves, respectively. Both the viruses have restricted host ranges and are serologically unrelated to the following closteroviruses: citrus tristeza virus, lettuce infectious yellows virus, carnation necrotic fleck virus, two closteroviruses associated to grapevine "legno riccio" and leaf roll, apple chlorotic leaf spot virus, and Heracleum latent virus.

Campbell, R. N. and Daniels, J.

Mosaic viruses in pepper crops and some weed hosts in California

Mosaic viruses in pepper crops and some weed hosts in California

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Mosaic viruses are the main phytopathological problem in pepper production in California. To determine the most important viruses occurring in peppers, a survey was done by ELISA using antisera of eight viruses commonly reported in peppers. A total of 767 pepper samples with mosaic virus symptoms, representing 80 fields in 12 counties of California were tested. The frequency of detection in 1987-88 was: cucumber mosaic virus (CMV) 55%, alfalfa mosaic virus (AMV) 24%, tobacco etch virus (TEV) 10%, pepper mottle virus (PeMV) 9%, tomato spotted wilt virus (TSWV) 9%, potato virus Y (PVY) 6%, tobacco mosaic virus (TMV) 3%, and potato virus X (PVX) 0 %. Multiple infections by 2 to 4 viruses were common. Datura meteloides DC. and Nicotiana glauca Graham occur widely and are known as reservoir hosts for viruses. From 43 samples of D. meteloides tested, 56% were infected by TEV, 37% by PeMV, 12% by PVY, 12% by TMV and 9% by CMV. From 36 samples of N. glauca tested, 78% were infected by PVY, 58% by CMV, 19% by TMV, 11% by AMV and 11% by TSWV. Multiple infections were the rule for both hosts.

Comparison of Epidemics of Two Aphid Transmitted Potyviruses in Muskmelon Fields

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The co-occurrence of two or more viruses within a crop offers the ideal opportunity to directly compare epidemics of each virus. Individual differences and/or similarities between rates of increase become more apparent when comparisons are made within the same field and growing season. The factors (aphid densities, crop development, climate, etc.) that influence the course of epidemics are most similar within a single field in which viruses are compared. A set of single field comparisons that reveal consistent differences in the rates of increase of viruses make possible deduction of critical factors that affect the behaviors of epidemics.

Cucurbit potyviruses often occur in mixed infections in many parts of the world (Lisa and Lecoq, 1984). In the Imperial Valley of California (USA), watermelon mosaic virus 2 (WMV 2) and zucchini yellow mosaic virus (ZYMV) are prevalent in cucurbit plantings and frequently cause substantial yield losses. The present study reports findings from 6 muskmelon fields that were intensively studied in order to measure WMV 2 and ZYMV incidences and aphid landing rates. Differences in the timings of the initial infections and rates of increase of each virus are interpreted with respect to vector densities, transmission efficiencies and putative differences in inoculum pressures.

METHODS AND MATERIALS

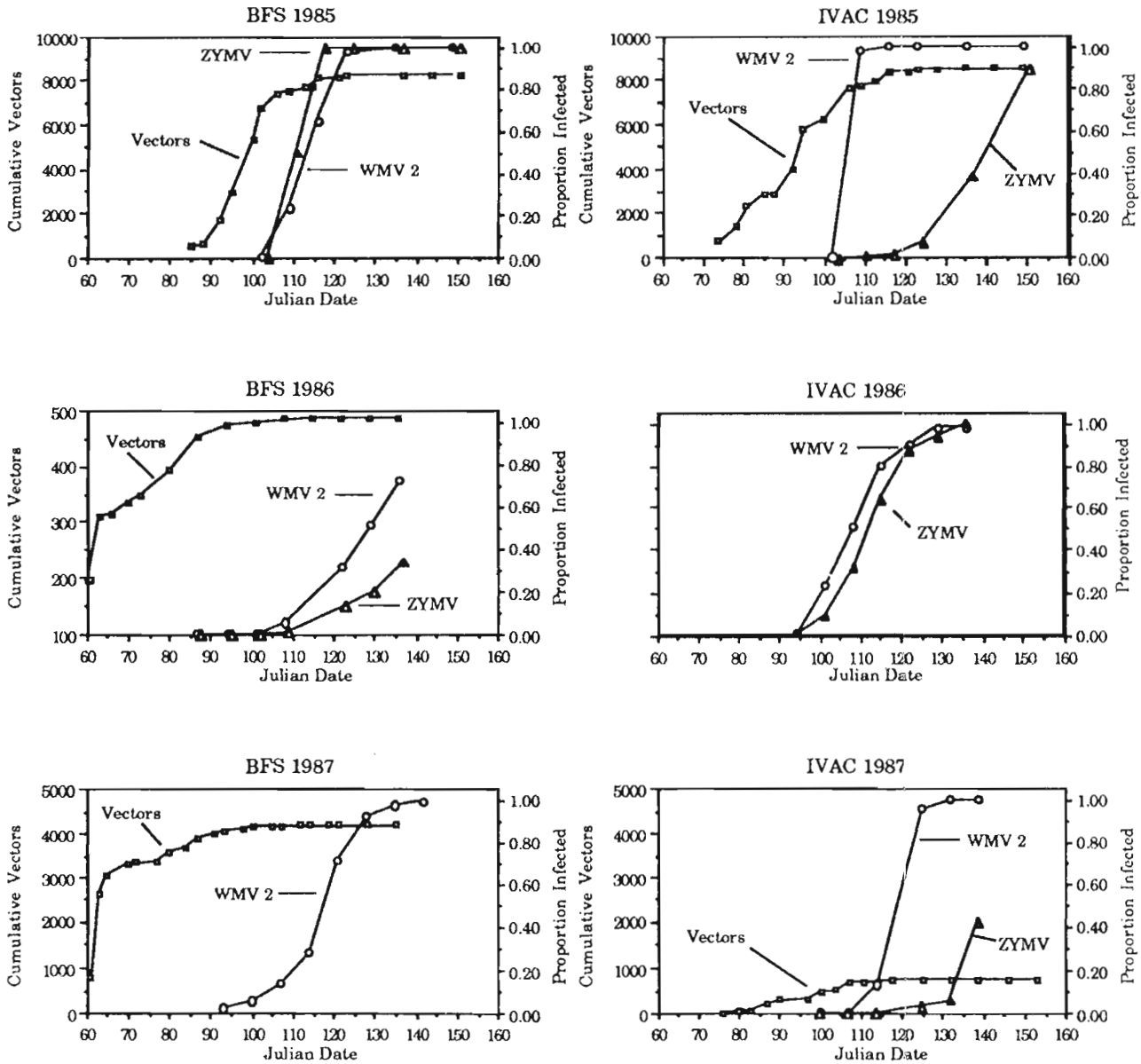
All 6 fields were planted on or between 30 January-10 February at two locations (IVAC and BFS) each year during 1985-87. Aphid landing rates were monitored in 5 of the 6 fields throughout the growing season with a set of 6-10 green tile traps in each field. Following thinning of the fields, cohorts of 100 randomly selected plants were tagged in each field and sampled at weekly intervals for virus infection. All leaf samples (which had been frozen) from a plant were evaluated at one time on the same microtitre plate using indirect DAS ELISAs. This permitted quantitative estimates of the relative titres of WMV 2 and ZYMV on each sampling date. Disease progress curves in each field were plotted together with cumulative vector numbers.

Aphid transmission from source plants singly or multiply infected with WMV 2 and/or ZYMV was performed to evaluate the relationship between transmission efficiencies and growth of epidemics. A series of 4 transmission tests were conducted at weekly intervals beginning 10 days post aphid-inoculation. All positive test plants from multiply-infected source plants were tested by ELISA to identify the infecting virus(es).

RESULTS

Rates of increase of WMV 2 and ZYMV were equivalent in only 2 of 6 fields (BFS '85, IVAC '86) (Fig. 1). In 3 of the 6 fields, an earlier entry and greater rate of increase

Figure 1. Disease incidence curves for WMV 2 and ZYMV (right vertical axis) in 6 different muskmelon fields over a three year period. ZYMV did not occur at BFS 1987. Also presented in each graph (except IVAC 1986) are cumulative numbers of aphid vectors (left vertical axis) trapped throughout the melon growing season. Scaling of cumulative vectors is the same within years but varies between years.

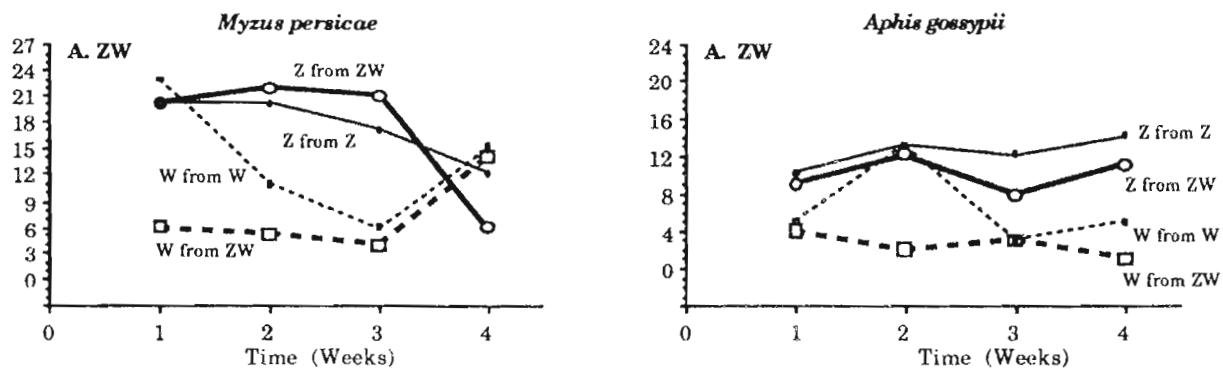


of WMV 2 was observed (IVAC '85, BFS '86, IVAC '87), while in one field (BFS '87) ZYMV was absent (Fig. 1). The incidence of ZYMV at BFS '85 increased from 0-100% in a two week period, while a similar rate of increase was observed for WMV 2 at IVAC '85. Cumulative vectors varied dramatically between years, but were similar between locations within a year. The apparently large difference in

numbers of vectors between BFS and IVAC locations in 1987 was due to the later placement of green tile traps at IVAC that missed recording the large flight peak observed at BFS between Julian days 60-75.

Transmission of ZYMV was usually higher than WMV 2 from single and double-infected sources by both *Myzus persicae* and *Aphis gossypii* (Fig. 2). Results from the *M. persicae* transmission series especially suggest the dynamic actions of viruses in plants and their relationships to aphid transmission efficiencies. Transmission of ZYMV by *M. persicae* from both single and double infected sources declined in week 4 while transmission of WMV 2 from single and double infected sources increased in week 4 (Fig. 2). Transmission efficiency of WMV 2 from the single-infected source plant by *M. persicae* varied each test. Transmission efficiencies of WMV 2 and ZYMV from all sources by *A. gossypii* were consistently lower and less variable than *M. persicae*.

Figure 2. Comparison of the transmission rates of WMV 2 and ZYMV from single and double infected source plants by *Myzus persicae* and *Aphis gossypii* at weekly intervals post-inoculation. Vertical axes represent the number of positives out of 27 trials for *M. persicae* or 24 trials for *A. gossypii* from each source plant. Heavy lines (solid and broken) in each graph represent transmission of each virus from the double infected source (designated ZW); light (solid and broken) lines represent transmission of the respective viruses from single-infected sources. Letters representing each virus (Z=ZYMV; W=WMV 2) indicate the virus transmitted and the source plant (single- or double-infected) from which it was acquired by the aphid.



DISCUSSION

Because WMV 2 and ZYMV are dependent on aphids for movement to new plant hosts, it is reasonable to expect that a field of susceptible plants would be at greater risk of infection with greater numbers of aphids present. However, the data presented suggest that a simple relationship between virus incidence and numbers of aphids does not exist, at least in the cucurbit potyviruses pathosystem in the Imperial Valley. If there was a simple relationship, one would expect a consistently higher incidence of ZYMV than WMV 2 due to the higher aphid transmissability of ZYMV(Fig. 2; Adlerz, 1987). Instead, WMV 2 infections increased more rapidly than ZYMV in 5 of 6 fields .

Clearly, sources of virus must be present if aphids transmit viruses to healthy plants in the field. The absence of ZYMV from the BFS 1987 field and its delayed entry in the IVAC 1987 field suggests that fewer sources of ZYMV were available for acquisition by aphid vectors than in previous years. In contrast, the high incidence of ZYMV in the IVAC 1986 field during a year of lower vector numbers (as estimated by data from the BFS 1986 field) suggests that sources of ZYMV were available for aphid acquisition and transfer to the melon field.

More inoculum sources proximal to a muskmelon field would increase the probability of an early virus infection. Subsequent spread to neighboring plants within the field may proceed quickly even with comparatively few vectors present. Realistic analyses of virus epidemics require that equal consideration be given to the number of inoculum sources in a region in addition to numbers of vectors present. Surveys of wild hosts in the Imperial Valley failed to reveal any natural infections by either WMV 2 or ZYMV.

The time during muskmelon season when initial infections of WMV 2 or ZYMV occur appears to be of critical importance to the particular behaviour of each epidemic. Because WMV 2 has been endemic in the Imperial Valley for much longer than ZYMV, there may be more alternate hosts infected with WMV 2 than with ZYMV. With higher inoculum potential in the region, more fields may become infected earlier with WMV 2 than ZYMV, resulting in a faster rate of increase and higher final incidence of WMV 2. However, as observed in this study, ZYMV increase in a field may at times be comparable to WMV 2, perhaps because the timing and number of primary introductions of ZYMV to a field is equivalent to that of WMV 2.

Numbers of *M. persicae* caught in green tile traps greatly exceeded all other aphid species each year even though fluctuations of 1 to 2 orders of magnitude among years were observed in *M. persicae* numbers. *M. persicae* frequently represented 90-95% of the total aphid catch through the season in the sets of green tile traps. *M. persicae* also was the most efficient vector of WMV 2 and ZYMV among 6 aphid species that were compared. The relative abundances and vector efficiencies of *M. persicae* clearly make it the most important vector species in the muskmelon pathosystem in the Imperial Valley, CA.

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EVOLUTION DU RISQUE DE VIROSE SUR LES CULTURES MARAICHERES DU ROUSSILLON : L'EXEMPLE DE LA SALADE

Increase of epidemiological risks of viruses in salad crops of Roussillon

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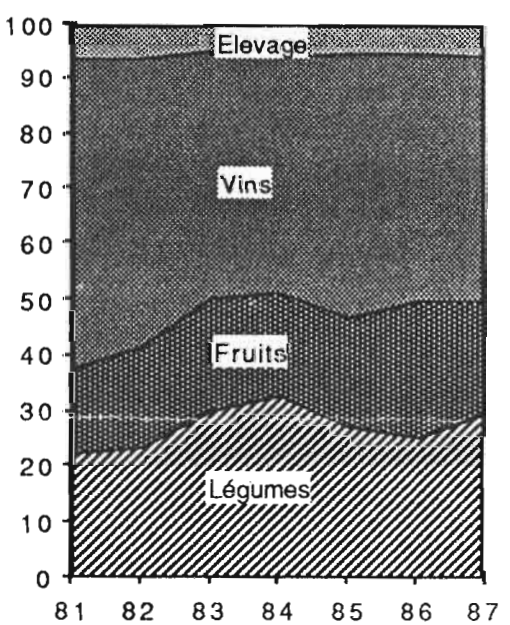
Les dernières campagnes salades en Roussillon ont été marquées par des niveaux de pertes causées par des viroses LMV, CMV, BWYV exceptionnelles, certaines parcelles étant infestées à plus de 80 %.
Devant la gravité des problèmes constatés en particulier depuis 1987 à Saint-Estève, il nous a paru opportun de rassembler différents éléments économiques et techniques susceptibles d'illustrer les conséquences et les facteurs explicatifs probables de ce risque virus.

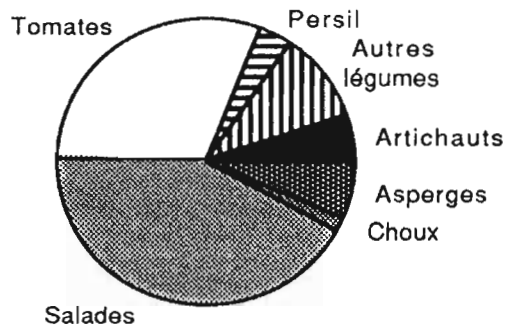
Au niveau du maraîcher, les critères économiques sont essentiels pour déterminer la marge de plus en plus étroite laissée par une spéculation. Aussi, nous commencerons par rappeler l'importance économique de la salade en Roussillon.

LA SALADE : UN ATOUT IMPORTANT POUR LE ROUSSILLON



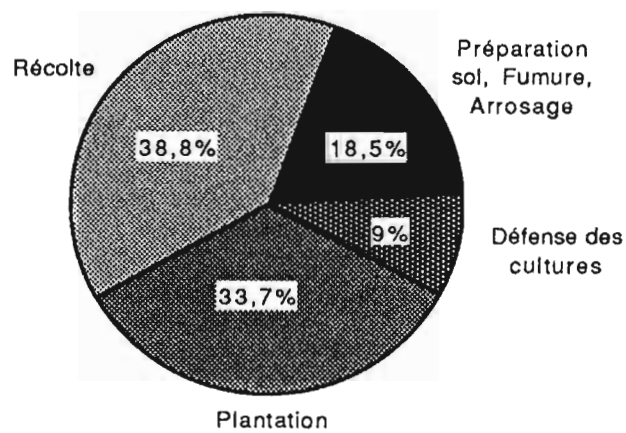
Les statistiques les plus récentes des Services de la DDAF et de la Chambre d'Agriculture révèlent l'importance croissante de la part des cultures maraîchères dans l'économie agricole départementale au détriment de la viticulture.

Fig. 1



Production Agricole Finale
Léaumes des P.O en 1987

Au sein des cultures maraîchères, la salade représente un atout traditionnel pour le Roussillon et qui se maintient en importance relative avec 30 % de la P.A.F.* (Production Agricole Finale), soit 251 000 000 Frs en 1988. **Fig. 2**



Coût de Production d'une Laitue de Plein champ en Roussillon (1988) par poste de travail.

Pour mieux cerner l'incidence économique de cette spéculation, il convient de rappeler à quel coût de production annuel correspond pour les producteurs du Roussillon la mise en culture d'environ 3 000 hectares de salade.

Le coût hectare (48821 Francs en 1988) pour une laitue de plein champ permet facilement d'apprécier la perte nette qui résulte de la récolte partielle d'une culture virosée à plus de 50 %.

Fig. 3

Lorsque ces pertes sont localisées géographiquement sur un petit secteur, leur caractère spectaculaire est encore accentué.

DES DEGATS CATASTROPHIQUES EN 1987 A SAINT-ESTEVE

3 000 000 Francs de perte pour **70 producteurs**, ce qui correspond à uen zone touchée de 150 hectares où les dégâts de viroses ont pu être estimés à 50 % minimum, sur la base de 1 Franc 50 du pied, prix payé au producteur en moyenne pendant la saison.

Les indexages réalisés à cette occasion par l'Inra Montfavet ont montré la présence quasi systématique de LMV associé le plus souvent au CMV dans toutes les espèces et variétés cultivées dans cette zone.
Le pourcentage de pieds virosés était le plus important pour les espèces et variétés sensibles : frisée, scarole, laitue Verpia, batavia, feuille de chêne, lollo rossa ...
Certains des lots de graines testés par Elisa concernés ont montré un taux de contamination supérieur à 0,1 % (LOT, 1988).

Depuis la campagne 1987, nos observations ont permis de confirmer la présence de cas de viroses aussi graves en pourcentage d'infection mais plus dispersé dans les différentes zones maraîchères du département.

On peut sans doute considérer que l'influence d'une contamination anormalement élevée de certains lots de graines a pu jouer un rôle dans l'évolution récente de ce risque virus.
Cette possibilité a d'ailleurs déterminé l'interprofession à une action de responsabilisation des maisons grainières.
Il est également probable que d'autres facteurs ont pu jouer aussi un rôle déterminant.

Essayons de les passer en revue.

DES FACTEURS DE RISQUE POTENTIEL

L'évolution des techniques culturales

Le Roussillon est une région maraîchère de vieille tradition avec jusqu'en 1985 un créneau très précis pour la salade de plein champ:**La production hivernale**.
Depuis, les tentatives de diversification ont amené certains producteurs à étaler dans le temps leur période de plantation et à expérimenter diverses variétés de salades.

On rencontre dans les parcelles à côté de la laitue beurre classique des variétés plus ou moins colorées comme la batavia, la feuille de chêne, la lollo rossa, la chiaggia. Le créneau des chicorées frisées et scaroles est également exploré.
Toutes ces espèces et variétés, à l'exception de quelques variétés de laitue beurre sont sensibles au LMV.
Il n'est pas rare qu'un même producteur fasse plusieurs rotations successives sur des parcelles voisines : on trouve à proximité immédiate des jeunes plantations et des fins de récolte souvent à l'abondon ...
L'intensification du maraîchage avec le développement important des abris, en particulier, s'accompagne d'une augmentation du nombre de friches ...

Une présence permanente de pucerons vecteurs

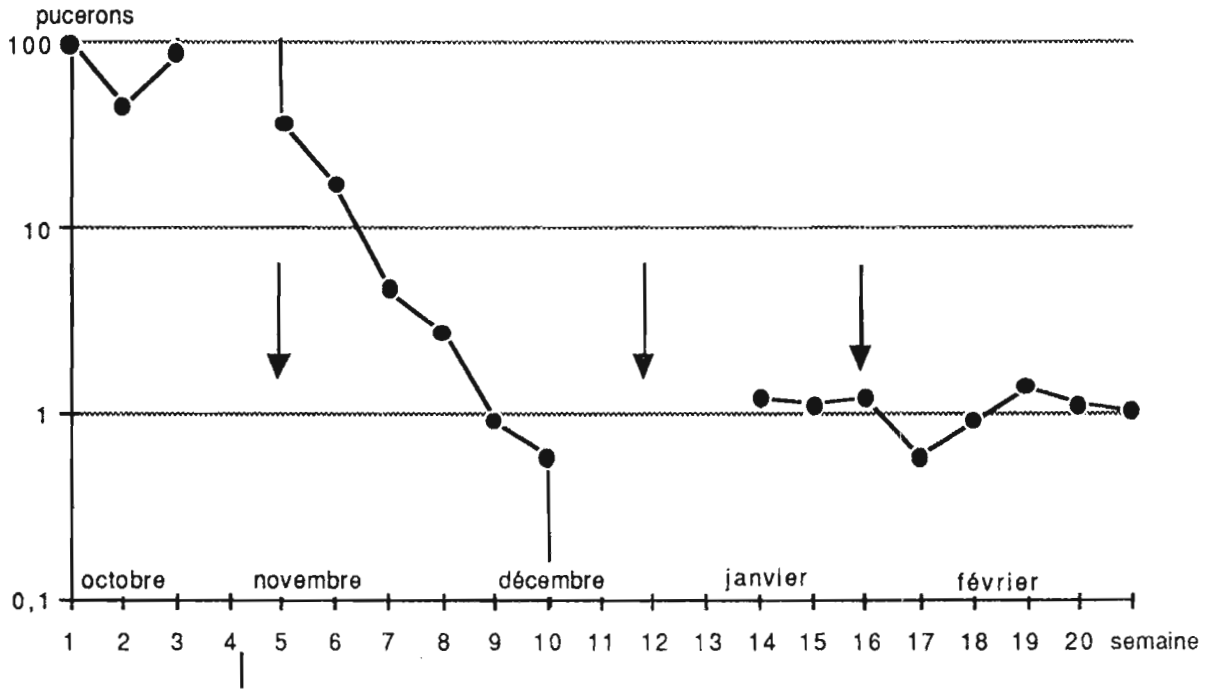


Fig. 4: Nombre de pucerons/piège (moyenne de 9 pièges) dans la période du 8/10/88 au 2/3/89 à Perpignan.

D'octobre à mars 1989, un piégeage par fil englué au niveau d'une parcelle plantée en salade proche de Perpignan, a montré la présence permanente à un niveau important de pucerons vecteurs potentiels.
L'échantillonnage pratiqué pendant les premières semaines de piégeage révèle une forte proportion d'*Aphis spp.* , soit 54 %.
Sur le nombre total d'espèces piégées, 90 % sont des vecteurs potentiels de virose pour la salade (LABONNE, communication personnelle).

Des adventices réservoirs de virus et hôtes de pucerons virulifères
Depuis 10 ans, l'utilisation intensive de la propyzamide en désherbage de la salade a entraîné sélectivement une inversion de flore à base de composées (séneçon, matricaire, galinsoga ...).
Ces composées sont des plantes réservoirs connues pour certains virus.
Aujourd'hui, en Roussillon, il n'existe pas de parcelle indemne de la présence de ces espèces de mauvaises herbes.

CONCLUSION

L'évolution du risque de virose sur salade en Roussillon nous semble réelle.
L'exacerbation des problèmes pendant les deux dernières campagnes peut être mise sur le compte de la douceur hivernale propice aux vecteurs.
Cependant, d'autres facteurs moins conjoncturels comme la diversification des variétés cultivées, l'échelonnement des plantations doivent être pris en considération. Le développement économique espéré avec le marché de la "4ème gamme" passe par une prévision de ces risques et, si possible, l'atténuation de leurs conséquences.

VIRUS TRANSMIS PAR PUCERONS/ APHID TRANSMITTED VIRUSES

VIRUS PERSISTANTS/ PERSISTENT VIRUSES

COOPERATIVE DE RECHERCHES ET EXPERIMENTATIONS AGRICOLES DES PYRENNEES-ORIENTALES
19, Avenue de Grande-Bretagne - 66025 PERPIGNAN - Tél 68-34-80-21

C. MARTIN, R. MARIO et L. SCHOEN

EVOLUTION DU RISQUE DE VIROSES SUR LES CULTURES MARAICHERES DU ROUSSILLON

Depuis 1987, le risque de dégâts liés à des maladies virales classiques sur salades de plein champ (LMV associé ou non à du CMV) sur cucurbitacées (CMV), sur tomates (PVY, CMV, Rhabdovirus) s'est accru dans des proportions très inquiétantes pour les maraîchers, au point de remettre en cause la rentabilité de certaines spéculations.

Différents éléments d'explication sont avancés, liés à des phénomènes de pullulation de vecteurs potentiels, à la présence d'adventices réservoir de virus, et à l'échelonnement des cultures dans le temps.

Influence of weed density in sugar beets on aphid populations and incidence of virus yellows disease

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INTRODUCTION

The presence of weeds may influence the habitat for beneficial insects in a positive way and may also change the microclimate in a way that promotes Entomophtorales fungi on aphids. To study the effect of weed density on the incidence of sugar beet pests and diseases, a field experiment with two different weed densities was established.

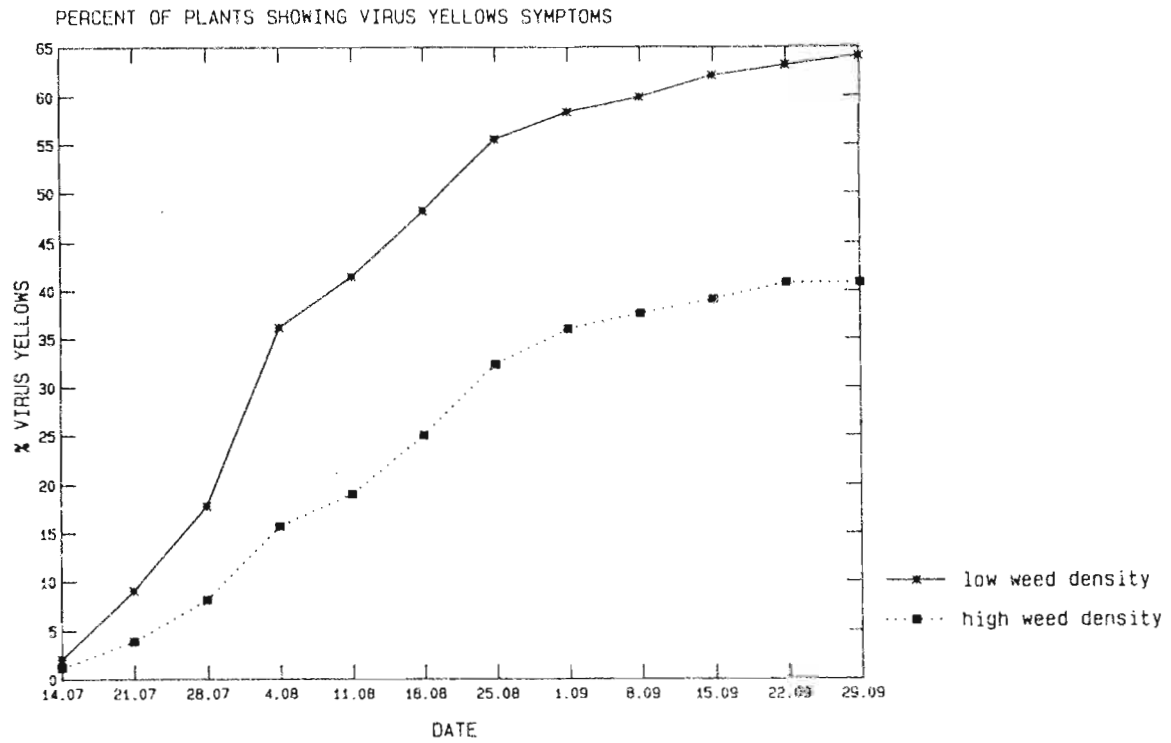
METHODS

Treatment design. In two adjacent plots each 3600 m2 large and designed for pairwise comparisons, the following treatments were made.

a) "Low weed density": Herbicide application before and after emergence to achieve a sugar beet stand as clean as possible.
b) "High weed density": Weeds were removed only when yield reducing competition was expected (Ammon and Kunz, 1982). In order to obtain this density, herbicide application was restricted to a band of 20 cm over the beet rows. Between the rows, mechanical weed control by hoeing at the 6-8 leaf stage of beets was carried out.

Artificial virus infection. In the center of each observation spot, 10 beet plants were infected with beet mild yellowing virus by viruliferous aphids.

Assessement of pests and diseases. Many parameters concerning the occurrence of pests, diseases and beneficial organisms were determined. Among them the following are referred to in this paper:
Aphids Aphis fabae
Myzus persicae
Virus yellows caused by beet mild yellowing virus.



RESULTS

In 1988, the first year of experimentation, striking differences between the two treatments were recorded. The occurrence of Aphis fabae in the high weed density treatment was significantly reduced to about 30 %. Myzus persicae was diminished to about 60 %, although this reduction was not significant.

Results concerning the virus yellows disease are shown in the figure. With the exception of the first set of records on 14th July, the reduction of the disease was significant over the rest of the observation period. In terms of "infected plant weeks" (Heathcote et al. 1971), which corresponds to the proportion of disease present during the observation period, the values obtained are 602 i.p.w. for "low weed density" and 353. i.p.w. for "high weed density".

More aphids were killed by Entomophtorales and more carabids and spiders were observed in the plot "high weed density". The corresponding figures will be published later.

In 1989, the experiment was repeated and it seems that the results of the year before are being confirmed.

DISCUSSION

The positive effect of beneficial organisms in the reduction of virus vectors and possibly on virus yellows disease has been described (Thornhill 1988). El Titi (1986) showed that late removal of weeds can reduce the incidence of Aphis fabae. Our experiments confirme these effects.

To our knowledge it is the first time that significant reduction of virus yellows incidence is shown by an indirect measure like late removal of weeds. As shown by the difference in "infected plant weeks", it can be estimated that sugar yield was increased in this way by ca. 7 % (Häni, 1979). This increase would correspond roughly to the benefits of virus yellows disease reduction after chemical aphid control.

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IV INTERNATIONAL PLANT VIRUS EPIDEMIOLOGY WORKSHOP

TITLE : Improving the forecasting of Barley Yellow Dwarf Virus high risk conditions in autumn-sown cereals

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ABSTRACT

Methods of identifying high risk infection by BYDV of autumn-sown cereals in western areas where winter crops are interspersed with ryegrass, and where mild autumns promote aphid survival are being evaluated and compared to the existing Infectivity Indexing Scheme (IIS).

The IIS was devised in 1983 based on proposals from Rothamsted Experimental Station, and has been in operation at Auchincruive from September to November of each year since. It must be emphasised that the IIS is intended to assess the extent of **primary** infection with RPV/PAV strains of BYDV transmitted by flying migrant bird-cherry and apple-grass aphids (*R. padi and R. insertum*) in the autumn.

IIS was not intended to assess the risk of the following methods of virus spread:-

(a) Aphids walking from ploughed-in grass or weedy stubbles to following cereal crops. In these situations the risk appears to be high irrespective of 'migrant' pressure, and a delay between ploughing and sowing and desiccation

of swards or weed grass offers the best method of prevention. This approach is now recommended to farmers in the west of Scotland, and would apply equally elsewhere in the U.K.

(b) Offspring of migrant aphids causing secondary spread by walking within cereal crops in the autumn. Here the risks follow upon high early IIS figures combined with early sowing, but assessment of the need for insecticidal treatment should be based on confirmatory crops inspections for aphids.

(c) Aphids overwintering in exceptionally mild winters. In most years it can be reasonably assumed that aphids will fail to survive beyond February, and so will never build up in sufficient numbers to cause secondary spread in the spring.

The method of calculating the infectivity index has been called into question during our work. Even though the IIS is fairly arbitrary it is important to standardise procedures, if not interpretation. We have opted finally for calculation of IIS for each vector species independently on a weekly basis. An overall score can be calculated by accumulating species scores.

The serious BYDV problem experienced by many farmers in Scotland in 1989 was caused almost exclusively by the MAV strain, transmitted by *S. avenae*. Although, very importantly, several very severe outbreaks in the coastal areas of south west Scotland resulted from infection by *R. padi*-transmitted RPV (and PAV). These high risk situations were not predicted by the IIS calculated at Auchincruive. Although the MAV strain is regarded as relatively mild in its effects on cereals, it has been associated with plant death and stunting in the west of Scotland, and also in previous years in Yorkshire. The tower trap does not catch significant numbers of *S. avenae* population, nor take into account local movement of aphids from grasses. A survey of grass crops has confirmed the enormous extent of symptomless BYDV infection of ryegrass. The tolerance of ryegrass to infection often by all three strains of BYDV, means that it is a constant source of virus. The number of aphids in grass in 1988 did not appear to build up during the season, mainly due to constant removal of top growth by cutting and grazing. Furthermore, during the winter, even in crops with adequate top growth, there were relatively few aphids compared to those found in cereal crops. Although the numbers of aphids migrating or

walking out of grass in the autumn may be sufficient to initiate the BYDV problem in autumn sown cereals, the role of the weed grasses in and around fields, in hedgerows and headlands need full evaluation. This type of habitat may be more important than cultivated grasses as an anholocyclic overwintering site because it offers shelter from wind, rain and frost, and is perhaps less prone to waterlogging.

The possibility of a computer model for predicting by 1 September the size of the migration of *R. padi* offers an attractive method of obtaining an early indication of BYDV risk. The work of A'Brook conducted at Aberystwyth indicated an association between high aphid populations and high summer rainfall. This association, he suggested, resulted from high rainfall promoting grass growth. However, there can be a large variation in aphid numbers in years of similar summer rainfall and temperature which grass growth alone cannot explain. Factors which affect the populations of aphid predators and aphid disease need evaluation.

The use of the amplified ELISA technique to detect virus directly in aphids undoubtedly provides a valuable tool for improving methods of forecasting. Although in our tests so far it has proved somewhat variable and difficult to interpret, this is probably a matter of perfecting the technique. The advantages of amplified ELISA are that it provides a method of testing large numbers of aphids quickly (2 days instead of 2-4 weeks with biological methods). However, more data is required to establish a clear relationship between the number of aphids carrying virus and the number which would have transmitted the virus to cereal plants.

In summary, the prediction of BYDV high risk years is essential if farmers are to avoid serious disease problems, and blanket insecticide spraying. The IIS provides a valuable indication of the risk of infection from viruliferous Rhopalosiphum spp., but it is not adequate to predict problems arising from local spread of *R. padi* and in particular the threat created by *S. avenae* in areas of mixed grass and cereal cropping. Detailed knowledge of strain incidence in defined geographical areas, the use of strategic aphid monitoring of grass and cereals from August onwards, the rapid tsting of aphids by amplified ELISA to determine the proportion emerging from grass carrying virus, and the development of computer prediction models may provide a

reliable alternative or supplementary method of forecasting. Careful structuring will be required to make the operation logistically and financially viable.

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INTEGRATED CONTROL OF BARLEY YELLOW DWARF VIRUSES

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INTRODUCTION

The viruses that cause barley yellow dwarf (BYD) are probably the most widespread and may be the most damaging in the world. As the area of susceptible arable crops grown has increased so has awareness of the disease. Part of the reason for this has been the availability of rapid diagnostic methods, such as ELISA, which have allowed symptoms that might otherwise have been associated with nutrient imbalance, drought, waterlogging or other physiological disorders, to be accurately diagnosed as virus induced. With this awareness has come concern about the yield lost to infection and much effort has been devoted to attempts to prevent infection or minimise its effects. Broadly control methods can be classified as avoidance, chemical control and resistance.

AVOIDANCE

As the barley yellow dwarf viruses are only transmitted by aphids the avoidance of infection is equivalent to the avoidance of the aphids that spread the disease. In some regions, such as much of Europe, this can be based on a sophisticated monitoring system using a network of suction traps. Elsewhere other trapping systems such as colour based or impaction (sticky) traps can be used. However, even in the absence of a monitoring system, avoidance can be exploited by using knowledge based on the infection of crops sown at different times. Although much of 'traditional' agricultural practice was, unknowingly, exploiting such opportunities, it was in New Zealand that the concept of the earliest safe sowing date was first formulated (Lowe, 1967), i.e. the date after which infection is unlikely. Clearly to be effective such a control depends upon a period free of aphid activity, usually during winter, and at a time when cereal cultivation is possible. In reality the pressures on the farmer to sow crops in the most favourable conditions and the yield penalties that often result from sowing crops later, are such that while avoidance can be exploited in a minor way, e.g., fields known to be most at risk because of aspect or shelter can be sown last, it is rarely the principal strategy used.

Nevertheless, it does seem valid in regions where crops are autumn sown, but the risk of spring infection remains. Autumn-sown crops can be infected in the following spring either from sources of aphids and virus established in the crops the previous autumn, or from aphids migrating into

cereals in the spring. The relative importance of these two sources differs between regions and years but in general spring migrants rarely cause much virus infection on autumn-sown crops and the infection that does occur is usually much less damaging then autumn infection because of the plants advanced growth stage. Spring sown cereals, on the other hand, can be severely infected and, in contrast to autumn sown crops, it is the latest sown crops that are damaged most. Indeed, late sown crops often appear more attractive to migrant aphids, possibly because of the soil/plant mosaic presented to flying aphids, than early sown crops that have produced a complete green cover by the time aphids migrate (Jenkyn & Plumb, 1983). For spring crops there is a further incentive to sow early as the earliest sown crops usually have the greatest yield potential.

Other influences on the usefulness of avoidance are aphid infectivity and the morph of the migrant species. Thus holocyclic aphids, those with a sexual cycle, produce sexual generations in autumn which will often feed only on hosts immune to BYDV. The production of sexual forms is daylength and temperature dependent but where data is available its timing is quite consistent. Therefore potential populations of vectors can be qualified by the likelihood of them infecting cereals and infection can be avoided if the majority of aphids are sexual forms.

So far avoidance has been discussed only in terms of the risk posed by migrant vectors. Sources of aphids and virus from ploughed-in grassland, cereal regrowths or volunteers can be equally important sources. Here there is an overlap with the section on chemical control as hygiene is the most effective way of avoiding this source of infection. Volunteers, grass or stubbles can be destroyed by herbicides or cultivated thoroughly before a new crop is sown, so that they do not act as a 'green-bridge' allowing direct transfer of aphids onto the newly sown crop.

As a practice that underlies much cereal cropping, avoidance of infection is probably quite widely practised. In areas of intensive agriculture and potentially large yields it is less economically acceptable and in these regions reliance is mainly on chemical control or resistance to ameliorate BYDV infection.

CHEMICAL CONTROL

In the continuing absence of any direct chemical effect on the virus, chemical control is entirely directed at the aphid vectors. There is no shortage of effective insecticides, although some countries restrict the use of some materials to particular times of the year. There is also no evidence of insecticide resistance in any of the BYDV vectors. Thus the restrictions on their use are timing, economics and, increasingly, environmental considerations. In the UK, chemical control of aphid vectors is the standard method of control and much evidence has accumulated that the optimal time for treatment of autumn-sown crops is at the end of the autumn aphid migration, usually the end of October or the beginning of November. The relative cheapness of the chemical and the perceived risk of infection are such that many farmers treat their crops routinely as an insurance. Such an attitude has been encouraged by two successive, milder than usual, winters during which virus has spread more than usual. Their enthusiasm for spraying is tempered by attempted

guidance based upon an integration of aphids caught in the suction traps and their infectivity, in an infectivity index (Plumb, Lennon & Gutteridge, 1983) and they recognise that it is the earliest sown crops that are likely to benefit most. Thus there is a partial integration of avoidance and chemical control.

Chemical control of aphid vectors in spring-sown crops is rarely practiced and most farmers rely on early sowing. There is also much less evidence than for autumn crops, as to when aphicides should be applied to spring crops, thus if they are used their timing is probably dictated by other activities such as the application of herbicides or fungicides and in consequence the effects are very variable.

While chemical control of aphicides on cereals is used in many regions to control their direct feeding damage, its use to control BYDV appears a predominantly European activity. This has no doubt been encouraged by the economics of cereal production, a larger than world price for the produce, and a large, and therefore relatively cheap, market for aphicides. There seems little prospect of changing this reliance on toxic pesticides in the near future and, if the 'greenhouse effect' of the last two winters is here to stay, their use will probably increase. However, in 5-10 years non-toxic materials may partially replace them. Experiments using plant derived products such as the antifeedant polygodial (Dawson *et al.*, 1986) have given encouraging results but its short persistence and the need for repeated applications make it unacceptable in its present form. However, enhanced activity, integration with pesticides to increase the effectiveness of the latter, and the incorporation of their production in cereals by genetic manipulation may all contribute to control in the future.

RESISTANCE

It could be argued that many, indeed most, of our cereals show some degree of resistance as few of them are killed when infected, and there are continuing discussions on the designation of resistance or tolerance. The most widely exploited gene has been the Yd_2 gene in barley, the presence of which clearly ameliorates the effects of BYDV infection, but certainly does not prevent it. The gene is used, especially in 'Mediterranean'climates, where rainfed cereals have a short growing season. In California yield benefits of 30-40% result from the use of this gene. However, when varieties incorporating the gene are grown in different regions the relative performance of varieties with and without the Yd_2 differ. More detailed research using defined isolates of BYDV has shown that, whether in spring or winter genetic backgrounds, the Yd_2 gene is very effective against the PAV- and MAV-like isolates (BYDV-1) but has little effect against the RPV-like isolates (BYDV-2) (Herrera & Plumb, 1988, 1989). Thus the successful use of such varieties depends upon the frequency of occurrence of the different BYDV strains and this is known in detail for few regions. Where PAV and MAV isolates predominate, and they are the most commonly encountered in a world survey (S.M.D. Forde & I. Barker, personal communication), then the Yd_2 gene is an effective control, especially where the economics of growing preclude the use of insecticide. More broadly based control will depend on the identification of yet unrecognised sources of resistance or the incorporation of resistance mechanisms by genetic

transformation. No single genes have been identified in oats or wheat giving comparable effects to Yd_2 but differences do exist and may be exploitable in future.

Resistance to the aphid vector has not been attempted as a control for BYDV although changes during growth in susceptibility to colonisation may confer some benefit. The possibility for transferring antifeedant properties has been mentioned above.

As more is learnt of the molecular biology of the barley yellow dwarf viruses the prospect of coat protein insertion, anti-sense RNA, ribozymes, the possibility of satellite viruses and other exciting prospects become closer to reality, but, even assuming that effective nucleic acid insertion techniques and regeneration methods are developed for cereals, there seems little likelihood of them influencing control methods before the end of the century and even when insertion, regeneration and expression are achieved much 'conventional' plant breeding will be essential to produce plants with acceptable characteristics for other purposes.

CONCLUSIONS

Current methods of control are likely to continue to be the most important. Yd_2-containing barleys may be deployed more widely where BYDV-1 is present, but control of BYDV-2 seems likely to continue to be by chemicals. Much of the future control strategy and the extent to which different methods are integrated depends on the economics of cereal cultivation in each region. However, the exploitation and correct use of current and future control strategies will continue to depend on knowledge of the biology and phenology of the vectors and their interaction with the BYDV strains.

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Epidemiological studies of Barley Yellow Darf Virus (BYDV) in Kenya.

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Until recently, Barley Yellow Dwarf Virus (BYDV) was considered a minor disease of cereal crops in the Eastern Africa region (Torres, 1984). However, recent epithytotics associated with the virus have occured within the major barley and wheat growing areas in Kenya. The incidence has been high and in many cases very severe making BYDV an important disease of small grain cereals in the country.

Cereal fields were surveyed over a three year period (1986-1988) for the occurence and severity of BYDV and the abundance of different species of cereal aphids. The study covered all the major barley and wheat producing areas, all of which lie between 1850-3000 m above sea-level. Detailed surveys were carried out in the Mau Escarpment area (Mau-Narok, East and West Mau), where over 70% of the barley crop is grown, and where BYDV incidences have been very severe.

Aphids in randomly selected fields were monitored by frequent inspection of the crop and by use of sweep net.

The aphid species that were found to be widely distributed in the areas where wheat and barley are grown included Rhopalosiphum padi, R. maidis, Schizaphis graminum, Sitobion avenae, S. fragariae and Metopolophium dirhodum. All these species are known vectors of BYDV (Rochow, 1970). R. padi was the most common species found in Nakuru district and Mau Escarpment while Metopolophium dirhodum and Sitobion avenae were the dominant species in East Mau and Timau, respectively. Sitobion avenae and R. padi were the most abundant species in Trans Nzoia district while M. dirhodum was the dominant species in Uasin Gishu district.

The incidence of BYDV in Nakuru District assessed through direct inspection of the crop during the period of this study was moderate. The disease symptoms on the crop were observed in small pockets which did not spread extensively. This could be attributed to the dry weather in 1987 and the excessively wet season in 1988, conditions that had adverse effects on aphid populations.

In the Mau Escarpment, the infestation by Rhopalosiphum padi and R. maidis on the crop was noted to occur at early seedling stage, followed by M. dirhodum and Sitobion avenae.

The BYDV symptoms appears early on the crop as isolated patches which later coalesced throughout the field. The disease was observed in every field that was sampled in 1987 and 1988, the extent of spread ranging between 10% - 100%. In most of the fields where the crop was attacked at a early growth stage there was a total crop failure.

The incidence of the disease in Mt. Kenya region (Timau) was very severe in the second season of 1986 (planted September-November) and the first crop season of 1987 (planted February-April). The aphid populations on the crop in the two seasons were very high, the most dominant species being Metopolophium dirhodum. Large numbers of S. avenae and R. padi were also observed.

Surveys in Uasin Gishu (Eldoret) and Trans-Nzoia (Endebess) districts where 40% of wheat is grown was done in 1988. Symptoms of BYDV were observed in most of the fields sampled.

The aphid populations were high, the dominant species being M. dirhodum in Uasin Gishu and S. avenae and R. padi in Trans-Nzoia.

At the end of 1986 and early 1987, few natural enemies of aphids were identified in Timau. The population of Syrphids (several species) and coccinellids (Adonia variagator) predators and hymenopteran parasitoids (mainly Aphidius species) increased at a lower rate than the aphids.

During the first crop season of 1987 (February-August) the population of the coccinellid predators increased to large numbers. There was a high population of syrphids at the end of 1987 season and the numbers increased further in the 1988 season.

Leaf samples that were selected at random from the cereal fields were air-dried and sent to Rothamsted Experiment station for serological tests, where three BYDV isolates have been identified. PAV-like isolate detected in material from the districts of Nakuru, Uasin Gishu, Trans Nzoia and the Mau Escarpment ; MAV-like isolate from Timau region, and the RPV-like isolate was traced in plants from Mau Escarpment.

Rainfall distribution in Kenya which varies from region to region dictates the time of cereal planting which start in Mt. Kenya region and lower Narok (February-April) ending in Mau Escarpment in June-September. The result of such a cropping system is that there is a green crop of cereals in the field throughout the year, which allows for aphid survival round the year and facilities their prolonged local movements as well as providing source of aphids that colonize green crops in other areas after long distance migration.

Local cereal aphids have also been found to survive through the dry season on other gramineous alternate hosts of the virus (Fargette et al., 1982). It is also probable that aphids causing primary infection come from neighbouring countries that grow cereals, especially Ethiopia to the north and Tanzania to the South where BYDV has also been reported (Torres, 1984 and Agranovsky et al, 1985).

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Assessing the risk of primary infection of cereals by the barley yellow dwarf virus in autumn in the Rennes basin of France.

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In the Rennes basin, Rhopalosiphum padi is anholocyclic and represents more than 90% of suction trap catches of potential vectors of barley yellow dwarf virus (BYDV) during autumn. From 1983 to 1987, relationships between temperature, suction trap catches of alate R.padi at 12.2m and aphid infestation and BYDV infection of batches of barley test seedlings (sampling units) exposed each week near the trap, were investigated.

Highly significant positive correlation coefficients were found between temperature and alate aphid catches. The correlation was best when the weekly mean temperature and weekly accumulated catches of alate R.padi, less males, were compared. Weekly catches of R.padi, less males, were also highly correlated with the percentage infestation of barley sampling units and the proportion infected with BYDV.

However, whilst the ratio of infested sampling units to numbers of trapped alate R.padi, less males, was similar for all years, that of infected sampling units to either numbers of trapped alate R.padi less males or infested sampling units was similar in 1983, 1984, 1985 but much smaller in 1986. In 1987, few aphids were caught resulting in low infection.

These results demonstrate that a good understanding of the biology of aphid vectors and changes in virus sources is a prerequisite for improving assessments of the risk of primary infection by BYDV.

ROLE OF MAIZE CROPS IN THE EPIDEMIOLOGY OF BARLEY YELLOW DWARF IN WESTERN FRANCE

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Barley yellow dwarf is recognised as an important disease of winter cereals in western France. Primary infection of cereals occurs in autumn and is mainly due to alate aphids landing on the crops. Transmission of BYDV by alate aphids caught at this period can be very high (Gillet *et al.*, 1989). One of the only hosts available for cereal aphids in summer is maize. Occurrence of three species of cereal aphids (*Metopolophium dirhodum*, *Sitobion avenae* and *Rhopalosiphum padi*) and of barley yellow dwarf virus (BYDV) was assessed by observation and by enzyme linked immunosorbent assay respectively from 1984 to 1987 in maize crops in western France. The results of this study show that there is a good correlation between the number of aphids caught in the suction trap of Le Rheu and the fluctuations of aphid populations on maize crops. On the other hand, maize could be infected by BYDV in certain years. Infection of maize seems to be due to the first aphids which land on these crops. These aphids are *Sitobion avenae* and *Metopolophium dirhodum*.. Secondary infection of maize seems to be very low. In autumn, *Rhopalosiphum padi* developed high populations on these crops in certain years and could then be responsible for the transfer of the virus from maize to winter cereals. PAV was the most common strain found on maize during the three years studied.

EPIDEMICS OF RPV- AND PAV- LIKE BARLEY YELLOW DWARF VIRUSES IN CEREALS IN SPAIN

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The epidemiology of RPV- and PAV-like barley yellow dwarf viruses (BYDV) was studied in Central Spain by monitoring its incidence and distribution by indirect ELISA using monoclonal antibidies to PAV and RPV. Samples were collected periodically on a fixed grid pattern in a winter barley field during two crops; aphid populations were also quantified in this grid. Aphids and BYDV viruses were also monitored in perennial weeds around the fields, in volonteer plants and in nearby maize fields during these two crop periods. The analysis of the obtained data indicate that fall/winter infection is the critical stage in RPV epidemics, possibly from local inoculum sources. On the other hand, the dispersion of the more prevalent PAV is associated with high late spring populations of Sitobion avenae and Metopolophium dirhodum, possibly from long distant sources. Data on the distribution of RPV and PAV on the main cereal-growing areas of Spain seem to confirm this hypothesis.

BARLEY YELLOW DWARF IN PORTUGAL.
I. INCIDENCE OF BYDV ISOLATES AND APHID-FLIGHTS IN THE NORTH REGION.

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INTRODUCTION

Barley yellow dwarf (BYD) is the main world-wide aphid-transmitted disease of *Gramineae* caused by luteoviruses. From the five aphid specific isolates of barley yellow dwarf virus (BYDV) known in North America (7) only MAV, PAV and RPV isolates were identified in Europe (4).

For the past years BYD symptoms were observed in the South of Portugal (Alentejo). Surveys of aphids showed the presence of BYDV vectors (2). A PAV-like isolate, Plumb's "Type B", was detected in 1987 by Enzyme-linked immunosorbent assay (ELISA) in samples from Alentejo (1). BYD symptoms were also found in the North (Tras-os-Montes), an important growing region for rye, wheat and triticale. Identification of BYDV isolates and monitoring of aphid flights in two locations of Tras-os-Montes are presented.

METHODS

Monitoring aphid-flights. Aphids were caught in Moericke traps continuously operating at cereal level at climatic distinct regions of Tras-os-Montes (Vila Real and Mirandela), collected three-times a week, identified, and counted.

The use of bait plants to assess aphid-infectivity. BYDV spread was obtained by exposing bait plants (*Avena sativa* L. cv. Clintland 64 and cv. Blenda) near cereal plots. Every fortnight several pots containing four oat seedlings were placed alongside the cereal plots in Vila Real and Mirandela. After the exposure period the pots were sprayed with insecticide and grown in an aphid-protected area for further 10-15 days. Plants from each pot were then harvested and frozen until tested for BYDV.

ELISA. Double antibody sandwich ELISA (DAS-ELISA) was done as described by Lister and Rochow (3) with polyclonal IgG from several sources (R. Lister, R. Plumb, and BIOREBA AG, Switz.), specific for PAV, MAV, and RPV isolates. IgG concentrations varied from 2.5 $\mu g.ml^{-1}$ to 1 $\mu g.ml^{-1}$, according to source. Alkaline phosphatase conjugates were used at 1/400 and 1/1000, respectively. For indirect ELISA (8), the plates were also coated with polyclonal IgG. Monoclonal antibodies (ADAS, Harpenden Lab.), specific for PAV (Mac 91), RPV (Mac 92) and MAV (Maff 2) isolates, were used at 1/500. Samples were extracted 1:10 (w/v) in 0.01 M phosphate-buffered saline, pH 7.4, containing 0.05% Tween 20 and 2% polyvinylpyrrolidone, and tested in duplicate wells in Immulon M129A plates (Dynatech, Ltd.). Absorbance at 405 nm was read in a Titertek Multiskan Plus MK II spectrophotometer. ELISA values higher than twice the mean for healthy plants were considered positive.

RESULTS AND DISCUSSION

Aphid-flights. The main BYDV vectors are present in Tras-os-Montes (Fig. 1). *Rhopalosiphum padi* was predominant followed by *Sitobion avenae* and *R. maidis*. *S. fragariae* was trapped in very small quantities. The four species were caught at the two regions but aphids were present earlier and in higher number at Mirandela than at Vila Real. Flights of BYDV vectors at Tras-os-Montes and other regions of Europe (5; 6) have similar seasonal activity. However aphids appeared sooner in Spring at Tras-os-Montes and the usual fall peak for *R. padi* was not observed in 1988, eventually due to intense rainfall.

Aphid-infectivity. Infectivity of flying aphids in Spring 1988 was different at the two regions (Table 1). The first infective aphids appeared at Mirandela by the end of March and were found up to the end of May. The BYDV isolate was "Type B". At Vila Real infective aphids were only present by the end of April, but were still found infective by late-June. "Type B" predominates, but aphids transmitting "Type F" (MAV-like) were also present.

TABLE 1. BYDV IN OAT BAIT PLANTS EXPOSED NEAR CEREAL PLOTS AT TWO LOCATIONS IN TRAS-OS-MONTES, FROM MARCH TO JUNE 1988, AS INDEXED BY ELISA

LOCAL	EXPOSURE PERIOD	Nº POSITIVE SAMPLES*/TOTAL Nº SAMPLES	
		ELISA-POSITIVE FOR:	
		TYPE B#	TYPE F+
VILA REAL	23 MAR-6 APR	0/3	0/3
	6 APR-21 APR	0/4	0/4
	21 APR-5 MAY	3/12	1/12
	5 MAY-18 MAY	5/12	0/12
	18 MAY-15 JUN	7/10	0/10
	15 JUN-30 JUN	7/12	0/12
MIRANDELA	16 MAR-30 MAR	0/2	0/2
	30 MAR-20 APR	3/5	0/5
	20 APR-11 MAY	5/12	0/12
	11 MAY-24 MAY	7/12	0/12
	24 MAY-8 JUN	0/9	0/9
	8 JUN-23 JUN	0/11	0/11

* Each sample (one pot) consisted of four Clintland 64 oat plants.
\# DAS-ELISA with commercial polyclonal Ig "Type B" (BIOREBA AG, Switz.).
\+ DAS-ELISA with polyclonal Ig "Type F" (R. Plumb, Rotham. Exp. Stat., England).

BYDV isolates in cereal plots as indexed by ELISA. Distribution of BYDV isolates was different at the two sites (Table 2). At Vila Real predominates the MAV-like isolate in all cereals tested. At Mirandela the PAV-like isolate was the most frequent. RPV-like was only present, alone, in triticale, at Vila Real. Mix infections were also detected at Vila Real and Mirandela. Oats were the most susceptible to BYDV infection and rye the least. A total of 30% BYDV infection for cereals in Tras-os-Montes is high. However, samples were taken from plots with frequent margins for aphid activity. Besides annual variation, a survey of BYDV in cereal fields of Tras-os-Montes may present lower level of infection.

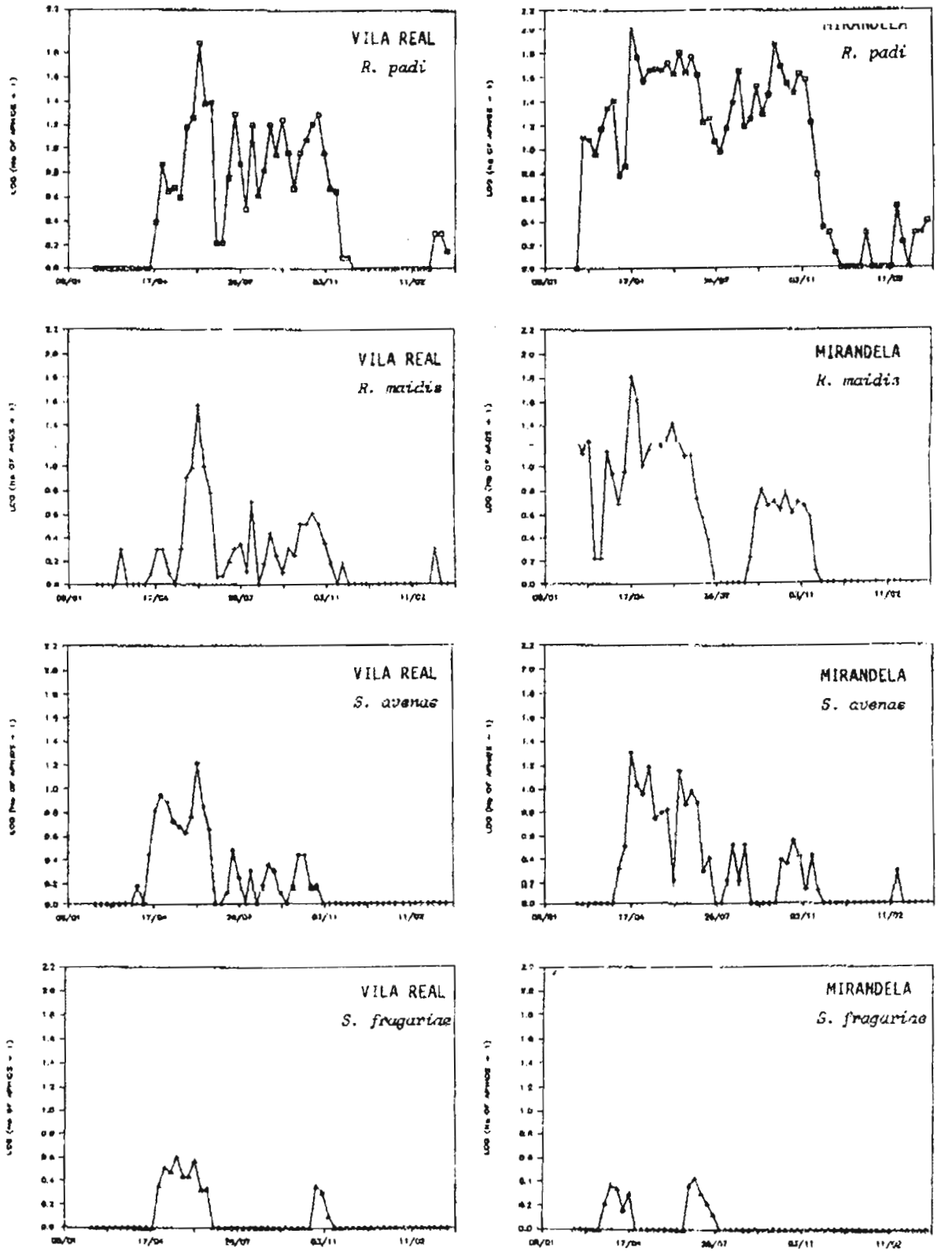

Figure 1. Total weekly catches of cereal aphids in yellow traps at Tras-os-Montes from February 1th, 1988 to March 31th 1989

TABLE 2. BYDV IN SAMPLES FROM CEREAL PLOTS AT TWO LOCATIONS IN TRAS-OS-MONTES, DURING THE GROWING SEASON 1987-88, AS INDEXED BY ELISA*

LOCAL	CEREAL	NUMBER OF SAMPLES	VIRUS INFECTIONS (%) ELISA POSITIVE FOR: PAV	MAV	RPV	PAV+MAV	PAV+RPV	TOTAL
VILA REAL	WHEAT	150	4	33	0	0	0	37
	RYE	150	5	11	0	0	0	16
	TRITICALE	150	13	18	1	3	1	36
MIRANDELA	WHEAT	150	10	5	0	2	1	18
	OATS	50	52	2	0	4	2	60
TOTAL		650	11	15	0	1	1	30

* Leaves of individual field plants were chopped, stored at -20°C, and tested in duplicate by DAS-ELISA or Indirect ELISA. One-gram samples were powdered in liquid nitrogen and homogenized in 10 ml 0.01 M phosphate buffered saline, pH 7.4, with 0.05% Tween 20 and 2% PVP (MW 40,000). DAS-ELISA using polyclonal Ig from P.PAV, RPV(2) and MAV antisera (R. Lister, Purdue Univ., USA). Indirect ELISA using rabbit polyclonal Ig for coating and rat monoclonal antibodies (MAC 91, MAC 92 and MAFF 2, from ADAS Harpenden Lab.) as second antibody.

CONCLUSIONS

Cereals in Tras-os-Montes are winter varieties. They are sown from mid-October to late-November, overwinter as young tillered plants and harvested in July. According to the 1988 seasonal flight-activity of the main BYDV vectors, the probability of BYDV infections at Vila Real and Mirandela can be minimized sowing as late as possible. However, because aphids return early in Spring, cereals will be very sensitive and can be infected. Studies on the seasonal flight of cereal aphids by suction traps at 2 m as well as their "infectivity index", an extensive survey of BYDV in all major cereal fields, and purification of BYDV isolates are in progress for Tras-os-Montes.

Acknowledgements. Polyclonal antisera from Profs. R. Lister and R. Plumb and monoclonal antibodies from ADAS. C. Martins, C. Rodrigues and P. Castro for expert technical assistance. Work supported by grant 81/87 from JNICT, Portugal.

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Annual variation in isolates of barley yellow dwarf virus in cereals, in the Toluca Valley of Mexico.

M. Mezzalama and P. Burnett

During the past 5 years most isolates of barley yellow dwarf virus (BYDV) detected in Mexico have been MAV-like. However most sampling was carried out late in the growing season. In 1987 a higher proportion of PAV-like isolates was found in winter (Dec-July) sown cereals than in summer (May- October) sowing when tested by ELISA.

In February 1988, 180 plants were marked, from a spaced wheat population sown in December. Leaf samples for detecting MAV-like, PAV-like and RPV-like isolates of BYDV, were collected 5 times at monthly intervals from February 9th, until June 6th. The sample was increased by 50 plants for each successive sampling date. The incidence of MAV-like isolates of BYDV increased throughout the cycle. However the incidence of PAV-isolates and RPV-like increased until April and then decreased. Approximately 50% of the 280 plants sampled in April were positive for BYDV. Fifty seven plants contained MAV-like isolates, 46 plants PAV-like isolates, 20 plants RPV-like isolates and the remaining 20 positives contained mixtures of isolates. Approximately 60% of the 324 plants sampled in June were positive for BYDV. One hundred and fifty seven plants contained MAV-like isolates, one plant PAV-like isolates, three RPV-like isolates and the remaining 40 positives contained mixtures of isolates.

During the summer of 1988 we marked 150 barley plants and 180 wheat plants in spaced populations and were sampled twice, in July and

September. Of the July barley sample twenty nine plants were positive for BYDV. Twenty six plants contained MAV-like isolates, one a PAV-like isolate, one a RPV-like isolate and one a mixture of PAV and MAV-like isolates. In wheat only forty nine plants were positive, plants contained MAV-like isolates, nine plants PAV-like isolates and three a mixture of PAV- and MAV-like isolates. Of the September barley sample 123 plants were positive for BYDV with 110 containing MAV-like isolates alone and eleven contained PAV-like and MAV-like isolates together. In wheat, only 63 were positive, all being MAV-like isolates.

It appears that the proportion of PAV like isolates is higher in the winter.

This study confirms the variation in isolates of BYDV in the field throughout the year. This may be important if germplasm is being selected under natural infection.

Cereal aphid transmission of barley yellow dwarf virus in the high valleys of Mexico.

M. Mezzalama and P.A. Burnett

Since 1987 at El Batan we have been captured live aphids, daily, with a 2 m. high Rothamsted suction trap. Since 1988, an additional Rothhamsted trap caught live aphids in the Toluca valley, for two days per week.

The follow cereal infesting aphid species have been caught at both sites Metopolophium dirhodum, Rhopalosiphum maidis, R. padi, Rhopalosiphum spp., Diuraphis noxia, Sitobion avenae and Schizaphis graminum.

In 1987 the most prevalent aphid caught at El Batan was M. dirhodum while in 1988 it was R. padi. However, in both years the majority of isolates of barley yellow dwarf virus (BYDV) detected by feeding live aphids on oat plants were MAV-like and were transmitted by M. dirhodum.

In Toluca in 1988 the most common cereal aphid species captured was R. maidis. The majority of BYDV isolates were MAV-like, transmitted by M. dirhodum.

Barley yellow dwarf virus particles contain readthrough protein.

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The PAV serotype of barley yellow dwarf virus (BYDV-PAV) has been sequenced and the coat protein open reading frame (ORF) shown to end with an amber termination codon (NAR 16:6097). Following the amber codon there is an ORF that could encode a readthrough protein of an additional 50 Kd. This readthrough ORF, minus its first codon, and the coat protein ORF were each subcloned into a GEX expression vector (Gene 67:31-40). Fusion proteins were produced, and purified by affinity adsorption on glutathione-agarose beads. In Western blots, BYDV-PAV antiserum prepared to whole virus particles reacted with both the readthrough and coat protein fusion proteins.

Immunoglobulins from BYDV-PAV virion antiserum were affinity purified using readthrough fusion protein. They reacted with the readthrough fusion protein but not with the coat protein, suggesting that the readthrough protein is present in purified virus and distinct from the coat protein. Immuno-precipitation, immuno-labelling with colloidal gold and tryptic digestion experiments demonstrated that the readthrough protein is on the surface of the particle. We propose a model for the involvement of this protein in aphid transmission.

ROLE OF A SATELLITE RNA IN GREEN AND CHLOROTIC FORMS OF GROUNDNUT ROSETTE

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Two main forms of rosette disease of groundnut (Arachis hypogaea) have been described (Hayes, 1932; Hull & Adams, 1968): chlorotic rosette, in which the leaves have extensive yellowing; and green rosette, in which the leaves are dark green or show a light green/dark green mosaic and are much reduced in size. In both forms there is stunting of the plants and this is especially severe in green rosette.

Rosette symptoms in naturally infected groundnut are associated with infection by groundnut rosette virus (GRV). GRV is manually transmissible but no virus-like particles have been seen in plant extracts; infectivity is associated with a single-stranded RNA of molecular weight 1.5×10^6. Plants infected with GRV contain abundant double-stranded (ds) RNA (Reddy et al., 1985), with prominent electrophoretic species of 4.6 kbp (dsRNA-1), 1.3 kbp (dsRNA-2) and 900 bp (dsRNA-3). Murant et al. (1988) showed that dsRNA-3 is the replicative form of a satellite RNA of GRV; they found that a satellite-free isolate of GRV caused no symptoms in groundnut but that reintroduction of the satellite restored the ability of the virus to induce symptoms, suggesting that the satellite is the actual cause of rosette disease.

In the present study three satellite-free isolates of GRV were used: (i) isolate G96 of Murant et al. (1988), which was derived from a GRV culture obtained from a chlorotic form of rosette from Nigeria, (ii) isolate MC1, derived from a GRV culture obtained from a chlorotic form of rosette from Malawi, and (iii) isolate GG1, derived from a GRV culture from a green form of rosette from Nigeria. Isolates MC1 and GG1 were made by electrophoresing dsRNA preparations made from GRV-infected Nicotiana benthamiana in 0.9% low-gelling-temperature agarose gels, excising the dsRNA-1 bands, boiling the gel slices for 5 minutes in 0.5 ml TE buffer (10 mM-tris, 1mM-EDTA, pH 7.4), then rapidly cooling and inoculating the melted dsRNA to N. benthamiana in the presence of bentonite. All three satellite-free isolates induced no symptoms, or only transient chlorotic mottle, in groundnut under glasshouse conditions in Dundee.

When RNA-3 derived in the same way from the Malawi chlorotic rosette culture, or RNA-3 from the Nigerian green rosette culture, was reintroduced into the satellite-free cultures in homologous and heterologous combinations, symptoms of chlorotic rosette were induced in groundnut by each of the cultures containing RNA-3 from Malawi chlorotic rosette, whereas symptoms of green rosette were induced by each of the cultures containing RNA-3 from Nigerian green rosette.

The symptoms induced by the homologously and heterologously reconstituted cultures were slightly milder than those induced by the cultures from which the RNA-3 was derived, suggesting that the satellite may have become attenuated after passage in N. benthamiana. The results indicate that the different forms of groundnut rosette disease are determined by different forms of the satellite RNA.

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"Synergistic interaction between the symptom inducing agent of the groundnut rosette virus disease and its assistor virus". O.A. ANSA*, C.W. KUHN, S.M. MISARI, J.W. DEMSKI, R. CASPER and E. BREYEL. Institute for Agricultural Research, Zaria, Nigeria, Department of Plant Pathology, University of Georgia, and BBA, Brauschweig West Germany.

Experiments were conducted to determine the effects on growth and yield of susceptible groundnut lines when each component of the rosette disease acted alone or in combination with its partner in the disease complex. Groundnut seedlings were subjected to four infection treatments namely GRV alone, GRAV alone, GRV + GRAV and 0.1M PO_4 pH 7.4 buffer alone. GRV was obtained from a mechanically maintained culture of green rosette. GRAV was maintained in healthy seedlings using the vector aphid and confirmed with the use of ELISA. A combination of GRV and GRAV was maintained using aphids fed on doubly infected plants. Plants subjected to the four treatments were maintained in a screenhouse over a period of about 110 days. The growth and yield parameters measured included plant height, dry weight of the roots and shoots and the numbers and weights of pegs, pods and seeds formed. Results indicate that GRAV acting alone shows no symptoms in groundnut plants and there are no significant differences between plants carrying GRAV alone and non-infected plants with respect to number of pods produced, plant height and dry weight of shoots. There were significant differences, however, with respect to the other parameters. There were significant differences with respect to pod and seed numbers and weight between plants infected singly with GRV and doubly with GRV and GRAV. When non-infected plants or plants infected with GRAV alone were compared to plants infected with GRV alone or in combination with GRAV, significant differences were obtained with respect to all parameters measured. It would appear that GRV acting alone produces a much less dramatic effect on measured growth and yield parameters. GRAV appears to act in a synergistic manner in combination with GRV to produce the dramatic losses in yield experienced from the rosette disease. All groundnut lines irrespective of level of resistance incorporated appear to be susceptible to GRAV.

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SESSION 10

VIRUS TRANSMIS PAR ALEURODES/ WHITEFLY - BORNE VIRUSES

A DETAILED INVESTIGATION OF THE FLIGHT ACTIVITY OF THE WHITEFLY BEMISIA TABACI (GENN.) ON CASSAVA IN COTE D'IVOIRE.

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INTRODUCTION

The whitefly, Bemisia tabaci (Genn.), is the only known insect vector of the virus that causes African Cassava Mosaic Disease. This disease, which has been recorded throughout sub-Saharan Africa where cassava is grown, can cause crop losses of up to 95% and so is of major importance.

Although much attention has been focused on the epidemiology of the disease (e.g. Fargette et al., 1987; Fauquet et al., 1988), there have been very few detailed studies on the biology of the vector, for example, little is known about the flight activity of Bemisia tabaci. In particular, it is not known whether the insect can disperse over long distances and so transmit the virus to cassava in different areas. The effects of enviromental conditions and crop development on flight behaviour are also unknown. The aims of the present study were therefore to:

1. Investigate the diurnal flight activity of the insect.

2. By means of various trapping methods, determine the height and direction of flight and from this interpret the potential of Bemisia tabaci to disperse over long distances.

3. Examine the relationship between wind speed, wind direction, ambient temperature, light intensity, relative humidity, rainfall and crop age on the flight activity of the insect.

MATERIALS AND METHODS

All experiments were conducted on the experimental farm at IIRSDA, Cote d'Ivoire.

Diurnal flight activity

Attractive sticky traps were used to catch Bemisia tabaci in flight. Each trap consisted of a 4 m-high piece of cylindrical PVC tubing, 10 cm in diameter. 10 cm-wide yellow sticky strips

were placed around the tube at 20 cm intervals along its length. The bottom strip was located 25 cm above the soil surface and the top strip at 2.95 m. Each yellow band was marked off into eight sections corresponding to the eight major compass points. Six such traps were placed in a 1/2 ha cassava plot.

The traps were placed in position just before sunrise of each experimental period. Then at two-hourly intervals the tubes were replaced and the number of whiteflies caught in each compass section at each band height was recorded per trap. This procedure was repeated throughout the day until sunset, when the traps were again replaced and left until sunrise of the following day. The number of whiteflies caught during the night was then recorded.

Throughout the experimental period detailed meteorological data were obtained. Wind speed and wind direction were monitored at different heights above and below the crop canopy. Measurements of ambient temperature and relative humidity were also obtained at corresponding heights using thermohydrographs.

The experiment was repeated once a week for nine weeks. Each week measurements of plant height, width, number of new leaves etc. were taken from 50 cassava plants to give a measurement of crop development.

Precise take-off times

A second trap type was used to determine the time of day at which the insects took-off from the plants. Once a week a plant was surrounded by non-attractive sticky screens. Each screen was made from a wooden frame (2 m high x 50 cm wife) and covered in mosquito netting. Sticky cellophane strips were used to completely surround the plant so that any insects taking-off from the plant would fly onto the screens and be caught. Insects taking-off vertically from the plant were trapped with a 'roof' screen. The numbers of Bemisia tabaci caught every two hours from sunrise to sunset were recorded. The total number of whitefly trapped throughout the night was also recorded.

Meteorological data were collected during the experiment as previously described. The experiment was repeated weekly for seven weeks.

RESULTS AND DISCUSSION

Diurnal flight activity

Three relationships can be established from the data. (i) The relationship between the number of whitefly and the time of day. (ii) The relationship between numbers of whitefly caught and enviromental conditions and (iii) the relationship between numbers caught and the age and development of the cassava.

(i) When the cassava was young (less than three months old) the majority of whiteflies were caught within the first 3-4 hours after sunrise. Relatively fewer were caught after 10 a.m. Virtually no whiteflies were caught during either the dusk or night period.

(ii) The pattern of flight activity can be at least partly explained by enviromental conditions. The greatest number of whiteflies were caught during early morning when temperature and wind speeds were low. Rainfall greatly reduced the numbers of Bemisia tabaci in flight.

(iii) As the age of the cassava increased, the total number of whitefly caught increased, reaching a maximum when the cassava was approximately three months old. On cassava older than three months the total numbers flying decreased. The time of day at which insects flew also varied with crop age. When the cassava was older than three months a higher proportion of whiteflies flew in the day and during the night. It is possible that this change in diurnal flight activity with crop age represents the onset of migratory flight.

Precise take-off times

The times at which Bemisia tabaci took-off from the cassava were very similar to the times that the greatest number of whitefly were caught on the attractive traps. As the crop aged, larger numbers of Bemisia tabaci took-off from the plants later in the day and during the night. Again it seems possible that this may reflect the onset of migratory flight.

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ECOLOGICAL STUDIES ON THE IMMATURE STAGES OF THE WHITEFLY *BEMISIA TABACI* ON CASSAVA

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Résumé

La mouche blanche *Bemisia tabaci* (Homoptera: Aleyrodidae) est le seul insecte connu vecteur de la mosaïque africaine du manioc. Des essais aux champs sont en cours en basse Côte d'Ivoire pour étudier l'écologie des populations aux stades immatures sur le manioc. Des données préliminaires sur la vitesse de développement et le taux de mortalité de chaque stade, la distribution à l'interieur de la plante et l'évolution des populations dans le temps, sont présentées ici. Les mensurations morphomètriques ont révelé qu'on peut distinguer entre les sexes au dernier stade larvaire.

Introduction

The whitefly *Bemisia tabaci* Genn. (Homoptera: Aleyrodidae) is the only known insect vector of African Cassava Mosaic Virus. This disease and the rôle played in its epidemiology by *B. tabaci* have been the subject of a series of studies in southern Côte d'Ivoire, West Africa (*e. g.* Fargette *et al* 1985, Fauquet *et al* 1988). Hitherto work on the vector has concentrated largely on the adult (Fargette 1985, Fishpool *et al* 1988): preliminary findings from a continuing field study of the population ecology of the immature stages are presented below.

The work is being carried out on an experimental farm at Adiopodoumé (05°19'N 04°08'W), 20km W of Abidjan in the forest zone of Côte d'Ivoire. The Kenyan cassava variety Kasimbidgi Green has been used, which is largely resistant to the disease, and is grown in plots of 1ha. Studies have concentrated on the first 4-5 months in the life of a cassava crop as it has been shown that infection with the disease after this period results in little yield loss (Fargette *et al.*, 1988).

Developmental Times

Direct observations in the field, at average mean temperatures of 26-28°C and average minimum relative humidities 63-65%, have shown that mean developmental times of the immature stages are as follows:

Immature stage	Mean duration in days	
Egg	6.1	
I instar	3.5	
II instar	2.3	
III instar	2.7	
IV instar	3.5	
'Pupa'	2.7	
Total	20.8	(Range 18-23, n=46)

Population Curves

Population curves of nymphs within a cassava crop over the first five months after planting are shown in Figs. 1 & 2. It can be seen that once the crop has been colonised there is a rapid build up in numbers to a maximum at 7-10 weeks, whence there is a marked decline. Similar shaped population curves are seen for adult numbers in cassava of the same age (Fishpool *et al* 1988 and unpublished observations). While with a developmental time of about three weeks from egg to adult some four to five generations are possible during this period, the degree of overlap between generations is such that it is not possible to distinguish them.

Distribution within plant

Adult *B. tabaci* feed and oviposit preferentially on the youngest open five to seven

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leaves of a cassava shoot (Fargette 1985). This, coupled with the rapid growth of cassava during this period (one leaf per 1,2 days) and the fact that *B. tabaci* nymphs are sessile, results in a stratification by age of instars down the cassava plant, as can be seen in Fig. 3. This has implications when devising sampling programmes for nymphs (Abisgold & Fishpool in prep.) and explains the choice of leaves sampled in Fig. 2.

Mortality

Preliminary estimates of mortalities of immature stages in the field have been obtained:

Developmental Stage	Mortality
Egg	negligible
I instar	35-50%
II instar	5-15%
III instar	5-15%
IV instar + 'Pupa'	30-40%
Survivorship to adult	2-10%

The main causes of mortality seem to be the failure of I instar nymphs to establish themselves after eclosion and death through parasitism and predation of the IV instar. The main predators recorded are mites of the genus *Euseius* (Acari: Phytoseiidae), while the parasitic wasp *Encarsia transvena* (Hymenoptera: Aphelinidae) is responsible for mortalities ranging from 10 to 60% of the IV instar and 'pupa' (Limberg & van Lingen 1988, and unpublished observations).

Morphometrics of IV instar nymphs

Morphometric measurement of the sizes of IV instar nymphs and 'pupae' revealed them to be bimodally distributed. The smaller size class, (length 0,55-0,63mm, width 0,35-0,43mm), gave rise uniquely to male adults while the larger class, (length 0,67-0,76mm, width 0,44-0,5mm) produced only females imagines. This is being used to investigate possible variations in the sex ratio of the population with time; *B. tabaci* is able to reproduce parthenogenetically with unfertilised eggs deveoping into males (Mound 1983).

Studies into the population ecology of immature *B. tabaci* continue and the results will be integrated with those from concurrent monitoring of adult populations in cassava, investigations of associated flight behaviour, and disease epidemiology.

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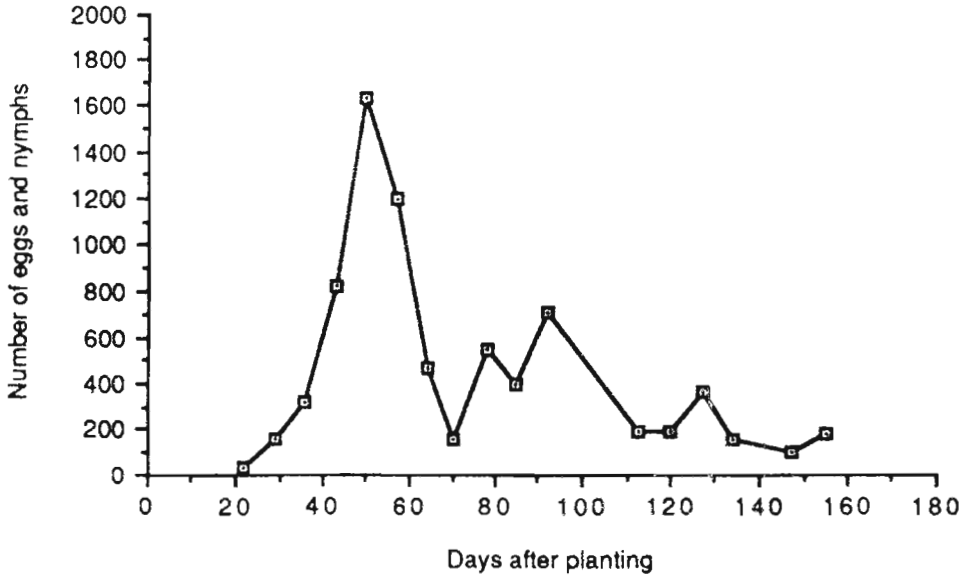

Fig. 1 Change with time in combined numbers of all living immature stages of *Bemisia tabaci* in a cassava crop over the first five months of growth (Dec. 88 - May 1989). Each data point represents the total recorded from one complete plant, except for the first three which are the means from five plants.

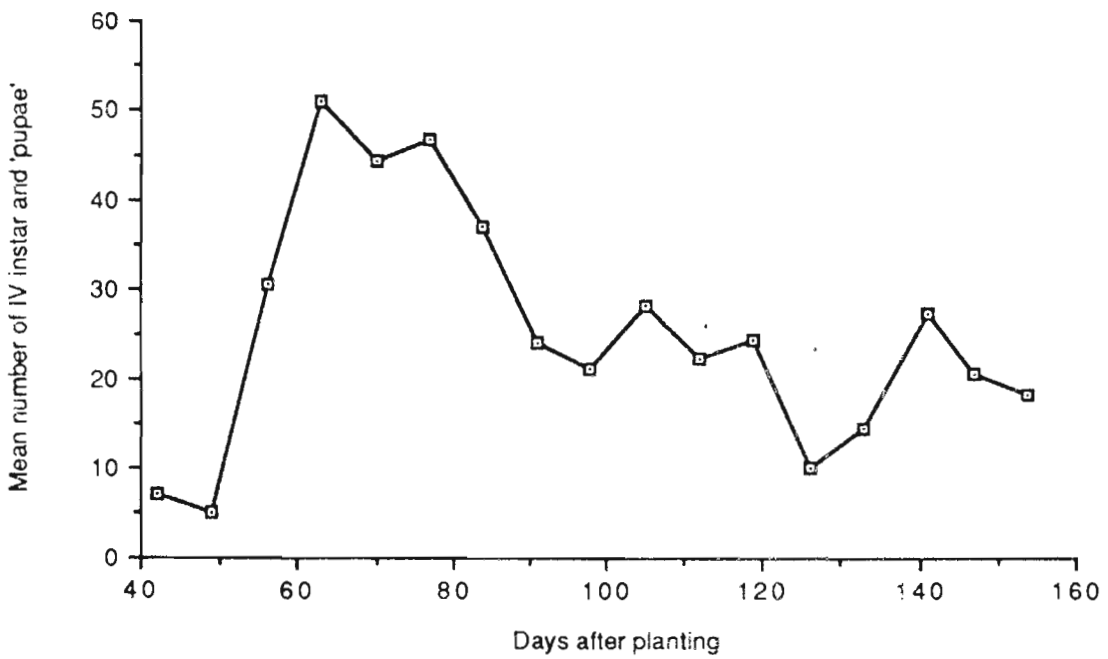

Fig. 2 Change with time in numbers of living IV instars and 'pupae' of *Bemisia tabaci* in a cassava crop over the first five months of growth (Dec. 88 - May 1989). Each data point is the mean number recorded from leaves 10 to 17 from a sample of 49 plants, where leaf 1 is the youngest (apical) open leaf of a stem.

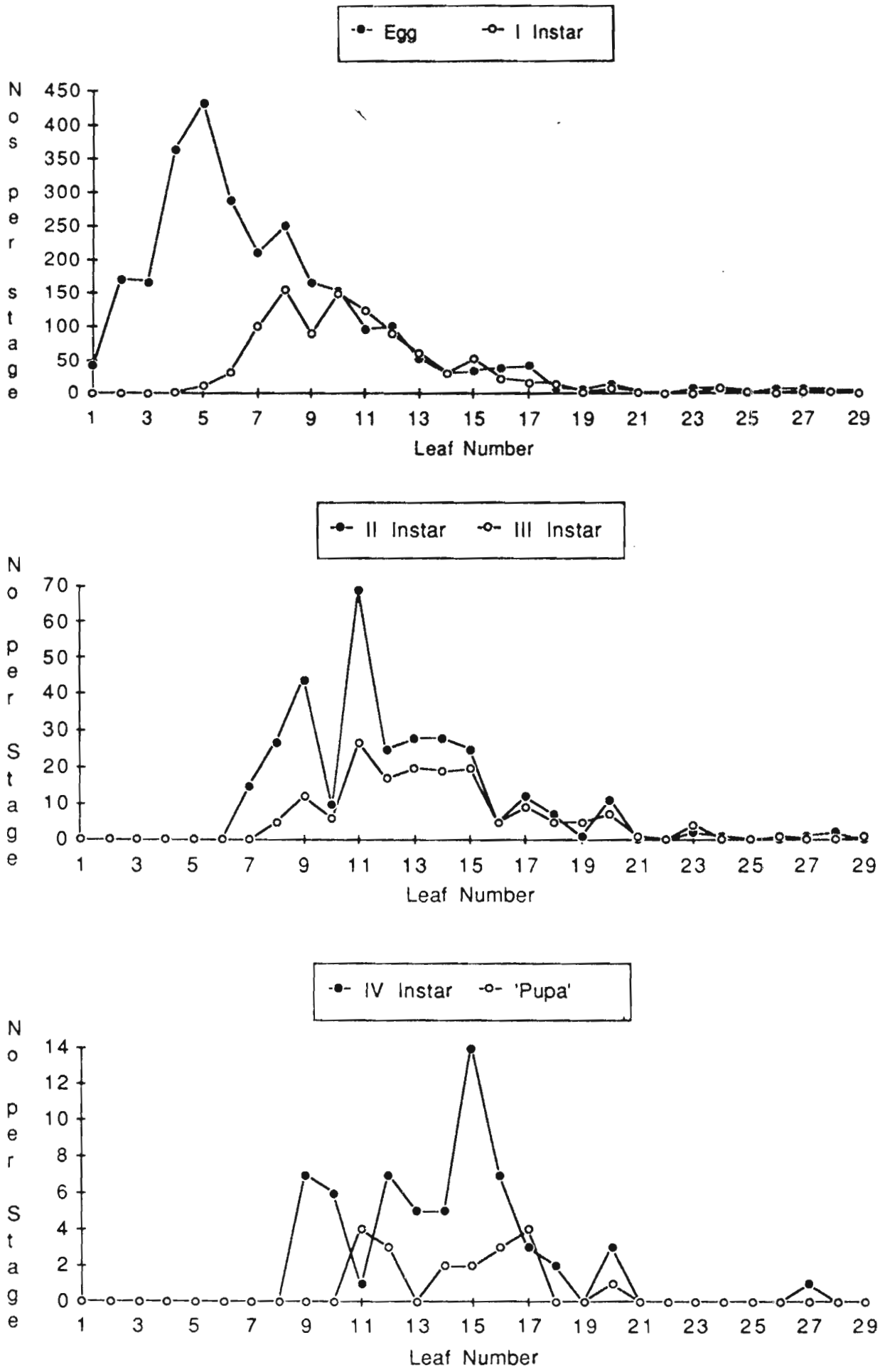

Fig. 3 Distribution in two month old cassava of all immature stages of *Bemisia tabaci* in relation to leaf age. Figures are combined totals from five complete plants counted in early Feb. 1989. Leaf 1 is the youngest (apical) open leaf of a stem.

CARACTERISATION DES POPULATIONS DE *BEMISIA TABACI* EN FONCTION DES PLANTES HOTES : RECHERCHE DE MARQUEURS ELECTROPHORETIQUES ET TRANSFERTS D'HOTES

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SUMMARY

The whitefly *Bemisia tabaci* is a well known vector of African Cassava Mosaic in tropical countries. By using isozyme electrophoresis (esterase patterns) and host-range studies, two types of *B.tabaci* were characterised: one breeding mainly on cassava, the other breeding on all plants other than cassava. Each types shows a different esterase pattern.

La mouche blanche du tabac, *Bemisia tabaci* Genn. (Homoptera: Aleyrodidae) est connue dans les régions tropicales comme espèce vectrice de la Mosaïque Africaine du Manioc (Harrison et al., 1977). Elle possède une grande gamme de plantes hôtes, plus de soixante espèces ayant été dénombrées en Basse Côte d'Ivoire. Le rôle joué par la dynamique des populations de *B.tabaci* dans l'épidémiologie de la Mosaïque Africaine du Manioc a été largement étudié sur manioc (Fargette et al., 1985 ; Robertson 1988). Nous nous sommes intéressés aux relations établies par *B. tabaci* avec ses plantes hôtes.

Cette étude a été réalisée à partir d'insectes capturés sur le site d'Adiopodoumé, en Basse Côte d'Ivoire. Les insectes sont prélevés au stade pupal sur leur plante hôte, et l'adulte est étudié après émergence. Dans un premier temps, nous avons caractérisé les populations de *B.tabaci* selon leurs hôtes au moyen de marqueurs enzymatiques. Puis nous avons examiné le comportement de *B. tabaci* vis-à-vis de différentes plantes hôtes par des essais de transferts d'hôtes en conditions contrôlées.

RECHERCHE DE MARQUEURS ENZYMATIQUES

La recherche de marqueurs enzymatiques susceptibles de caractériser les populations de *B. tabaci* en fonction de leur plantes hôtes est réalisée par électrophorèse des isozymes. Nous avons retenu les estérases dont les résultats sont les plus concluants. L'électrophorèse est effectuée sur gel vertical d'acrylamide à 7% avec un tampon Tris-Glycine de pH 8,3, sous un potentiel de 100 volts pendant 30 mn, puis de 200 volts pendant 1 h 30 (Debret et al., 1983). Chaque individu est broyé dans un tampon Trudgill à 10% de saccharose (Babaut, 1986). La révélation des estérases se fait en employant de l' alpha- et du bêta-naphtyl acétate comme substrat (Shaw & Prasad, 1970).

Deux types de zymogrammes ont été obtenus, l'un à une bande, l'autre à deux bandes (Fig. 1).

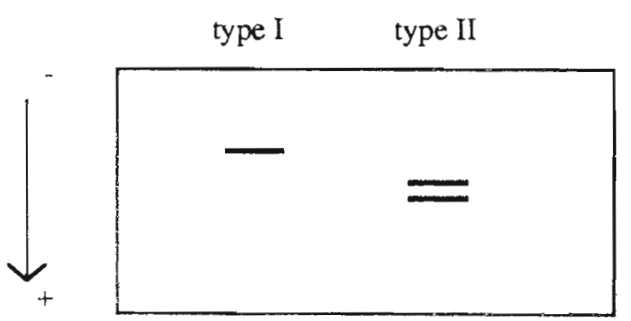

Fig 1. Zymogramme des esterases de *Bemisia tabaci*

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Le type de zymogramme est directement lié à la plante hôte d'origine de l'insecte. (Tab. 1).

Tab 1. Zymogrammes des estérases de *Bemisia tabaci* selon l' espèce de plante hôte

plante hôte	nombre d' individus testés	ZYMOGRAMME	
		type I	type II
Manihot esculenta (manioc)	20	0	20
Manihot glaziovii	1	0	1
Albemoschus spp. (gombo)	30	30	0
Sida rhomboïdea	12	12	0
Ipomea involucrata	5	5	0
Eupatorium odoratum	6	5	0
Centrosema pubescens	5	5	0
Pueraria phaseolides	7	7	0
Borreria ocymoides	1	1	0

B. tabaci capturé sur des plantes du genre Mahinot possède donc un pattern electrophorétique, au niveau des estérases, différent de celui de *B. tabaci* capturé sur toutes les autres plantes étudiées (Test du X^2, significatif, $P < 0.001$).

TRANSFERTS ENTRE PLANTES HOTES

Les essais de transferts, effectués en salle d'élevage, concernent la variété Kasimbidgi Green de manioc et Clemson Spineless de gombo. Les autres plantes utilisées sont issues de graines récoltées dans les environs d'Adiopodoumé.

L'hôte à tester (manioc ou gombo) est placé dans une cage d'élevage et présenté à un seul couple d'adulte issu de larves obtenues sur une plante hôte donnée. Cette expérience a été répétée 20 fois, 10 couples étant transférés sur une espèce hôte nouvelle, les 10 autres, servant de témoin, étant transférés sur la même espèce hôte. La descendance est observée 4 semaines plus tard en notant la taille de la descendance, et en procédant à des zymogrammmes. Le cas échéant, on note la présence d'une reproduction parthénogénétique, révélée par une descendance uniquement mâle (Mound, 1983) (Tab. 2 & 3).

Tab. 2 Transferts d'hôtes à partir d'un couple provenant du manioc

	manioc - manioc										manioc - gombo
répétitions	1	2	3	4	5	6	7	8	9	10	10 répétitions
nombre de descendants	0	0	0	0	0	45	0	33	0	0	aucune descendance
reprod. parthenogen.	-	-	-	-	-	+	-	+	-	-	
type de zymogramme	-	-	-	-	-	II	-	II	-	-	

Tab. 3 Transferts d'hôtes à partir d'un couple provenant du gombo

	gombo - gombo										gombo - manioc
répétitions	1	2	3	4	5	6	7	8	9	10	10 répétitions
nombre de descendants	89	108	122	70	102	65	137	121	91	76	aucune descendance
reprod. parthenogen.	-	-	-	-	-	-	-	-	-	-	
type de zymogramme	I	I	I	I	I	I	I	I	I	I	

Les changements d'hôtes dans les deux sens entre le manioc et le gombo n'ont pas donné de descendance. Une descendance bisexuée à toujours été observée dans le cas des transferts témoins gombo-gombo. Pour les transferts témoins manioc-manioc, la présence d'une reproduction parthénogénétique, indique que les femelles n'ont pas été fécondées.

Nous avons alors repris l'expérience en augmentant le nombre d'insectes par plante, présentant à un nouvel hôte non pas le couple parental, mais toute ou une partie de sa descendance (Tab 4).

Tab. 4 Essais de transferts à partir d'une descendance

	gombo - gombo	gombo - manioc	manioc - manioc	manioc - gombo
nbre de répétitions	5	5	5	5
nbre de descendances de seconde génération	5	0	3	0
reprod. parthenogen.	0	-	1	-
type de zymogramme	I	-	II	-

Les résultats obtenus vont dans le même sens que les précédents, et tendent à montrer l'impossibilité d'effectuer, dans nos conditions expérimentales, des changements d'hôtes entre le manioc et le gombo. Il faut noter également des difficultés quand à l'élevage des insectes sur manioc.

Nous avons entrepris en dernier lieu une expérience en présentant simultanément plusieurs plantes hôtes d'espèces différentes à des adultes d'origine donnée. Des plantes de *Manihot esculenta, Euphorbia heterophylla, Albemoschus* sp., *Sida rhomboïdea, Sida carpinifolia, Crotalaria* sp., *Centrosema pubescens, Pueraria phaseloides, Eupatorium odoratum, Lycopersicon esculentum, Solanum nigrum* ont été placées dans deux salles d'élevage. Dans la salle d'élevage n°1 ont été introduits des adultes de *B. tabaci* capturés sur gombo, dans la n°2, des insectes provenant du manioc.

Après six semaines, les insectes avaient colonisé l'ensemble des plantes hôtes dans la salle n°1; seules les plantes de manioc ne portaient pas de larves, bien que la présence d'adultes ait été notée sur les feuilles. L'analyse électrophorétique a révélé, chez les adultes après emergence, un zymogramme estérasique de type I, quel que soit l'hôte considéré. Dans la salle n°2, la population de *B. tabaci* était plus réduite, les insectes ne se trouvant que sur le manioc, avec des zymogrammes estérasiques de type II.

De telles différences de comportement selon les plantes hôtes considérées ont été décrites au Costa Rica, où l'on distingue deux races de *B. tabaci* selon leurs plantes hôtes: l'une se développe uniquement sur *Jatropha*, et l'autre sur de nombreux hôtes, mais pas sur *Jatropha* (Bird , 1957). Au Nigeria, des différences ont été notées selon les hôtes considérés, lors d'expériences de changements d'hôtes chez *B. tabaci* (Mound , 1981).

CONCLUSIONS

L'ensemble des résultats obtenus tant à démontrer qu'il existe en Basse Côte d'Ivoire deux types de *B. tabaci* ayant des comportements différents vis-à-vis de leurs plantes hôtes. Un premier type se développerait sur un grand nombre de plantes hôtes, mais pas sur le manioc; un second type serait inféodé au genre *Manihot*. Cette différence se reflète au niveau biochimique par la présence d'isozymes différentes au niveau des estérases. Le degré de différenciation des deux types mis en évidence doit être précisé par une étude du polymorphisme enzymatique des populations à plus vaste échelle, et par des essais de croisements.

Il semble, de plus, que le principal réservoir de *B. tabaci* vecteur dans l'épidémiologie de la Mosaïque Africaine du Manioc soit le manioc lui-même.

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Epidemiology of Whitefly-Vectored Lettuce Infectious Yellows Virus in Southern California.

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INTRODUCTION

Fall lettuce growers in the Imperial Valley of southern California have experienced severe yield losses due to disease caused by lettuce infectious yellows virus (LIYV), which is vectored by the sweetpotato whitefly, **Bemisia tabaci** (Gennadius) (Duffus and Flock 1982, Johnson et al. 1982, and Duffus et al. 1986). Several studies have determined the importance of cotton and melons in the development of LIYV epidemics (Brown and Nelson 1986, Coudriet et al. 1985, Duffus and Flock 1982, Duffus et al. 1986, Johnson et al. 1982, and Youngman et al. 1986). The present study was undertaken to evaluate further the interacting effects of cotton and cucurbits in the development of LIYV epidemics in lettuce. Our objectives were to describe the temporal and spacial distribution of **B. tabaci** on cotton, melons and lettuce, and define the interaction among seasonal whitefly densities and LIYV infections in melons and lettuce.

METHODS AND PROCEDURES

Whitefly Trapping

Studies were conducted in 1987 and 1988 in the Imperial Valley, an approximately 140,000 ha irrigated agricultural region. We trapped whiteflies using a 3.75 sq. cm. yellow sticky card which was stapled to a 30 cm wooden stake and placed so that the trap was approximately 25 cm from the ground. Two cards were placed back-to-back so that both sides of the trap were coated with adhesive. In 1987 traps were placed on the edges of the fields, while in 1988 they were placed among plants in the fields.

In 1987, we chose 220 trap sites, uniformly placed every 8 km. From these data, we determined which of the traps were located next to cotton, melons, lettuce and alfalfa. These crop types were of interest because we hypothesized that whitefly populations colonized and increased in cotton, moved to melons in the early spring where they reproduced and transmitted LIYV before migrating to the lettuce to which they transmitted virus. The interest in alfalfa was from the standpoint that, since alfalfa is not a host

for the whitefly, it would serve as an indicator of the general population of whiteflies migrating throughout the Valley.

These analyses showed that our uniform trap selection in 1987 resulted in 32 sites next to cotton, 16 sites next to melons, 11 sites next to lettuce, and 53 sites next to alfalfa. For 1988, we conducted early surveys of the Imperial Valley and determined field sites that were destined for each of the various crop types. Using this information we selected 23 sites in cotton, 11 sites in melons, 12 sites in lettuce, and 17 sites in alfalfa, all sites adequately placed to represent the entire Valley.

Trapping was initiated in cotton and alfalfa on 30 June 1987 and 14 June 1988. Trapping in melons and lettuce could not be done until fields of each crop type were planted. Thus traps were placed in melons on 11 August 1987 and 10 August 1988 and in lettuce on 29 September 1987 and 13 October 1988.

Disease Evaluation

LIYV distributions were characterized in melons and lettuce by surveying all fields in the Valley. With the assistance of the Imperial Valley Agricultural Commissioner we were able to locate and map all of the melon and lettuce fields in the Valley. Melons were considered important to the epidemic in lettuce because whiteflies breed and transmit LIYV to and from melons.

We surveyed melons on 1 October 1987 (153 fields) and 5 October 1988 (122 fields). Lettuce surveys were conducted on 5 November 1987 (106 fields) and 16 November 1988 (91 fields). Surveys consisted of counting 100 randomly selected plants in each of the southeast and northwest corners of the fields and recording the number of plants showing LIYV symptoms. Since very young lettuce does not produce a strong symptom that is recognized easily in field surveys, we omitted fields that were not at least in a "prehead" condition. This was defined as the point in time at which leaves began wrapping to form the lettuce head.

Data Analyses

Data analyses consisted of computing the average number of whiteflies per trap collected each week at fields with like crop types. Thus the weekly number of whiteflies on cotton, melons, lettuce and alfalfa could be plotted and compared. Virus data were categorized into 3 categories, based on the average percent infection of the 2 corners in each field. Fields were considered in the low category if they had an average of fewer than 33% of the plants infected. The moderate category was used for fields

with 34% - 66% infections and those fields 67% and over were classified in the high category. Descriptive statistics were used to draw correlations of whitefly and virus data collected in the two growing seasons (1987 and 1988).

RESULTS AND DISCUSSION

Whitefly numbers virtually were undetectable until early July in both years of the study. At this time whitefly densities began to increase in cotton, peaking near the end of September. When melons were planted, whiteflies moved into them from the cotton, and increased at approximately the same rate as those in cotton. Whitefly numbers in melons also peaked near the end of September. It should be restated that melons not only provide excellent hosts for the whitefly, but also serve as reservoirs for LIYV. Thus the whiteflies feeding in melons have a high likelihood of being viruliferous. When lettuce emerged in early October whitefly populations in cotton and melons were declining, primarily because cotton was being dessicated and defoliated and melons were nearing harvest. This caused emigration of whiteflies from cotton and melons throughout the Imperial Valley and lettuce seedlings experience high B. tabaci populations as they emerged. Our data indicate that this is how the devistating epidemics that have characterized the Valley in recent history occurs.

Disease surveys in melons and lettuce, coupled with information on whitefly distributions add to our knowledge of the LIYV epidemic. Surveys in melons in 1987 indicated that 57% of the fields were in the low virus category. The other 43% were moderately or highly infected. In 1988, nearly 71% of the fields were in the low category, while only 30% were in the moderate or high category. Thus our survey indicated that there was more inoculum source in melons available in 1987, which should have resulted in wider distribution of the virus in lettuce in 1987. Our lettuce surveys indicated that this was the case. In 1987, there were 26% of the lettuce fields in the low category while 74% were moderately or heavily infected. This compared to 39% of the fields in the low category in 1988, an indication that there was less virus, on a valley-wide basis in the second year. This also agreed with the whitefly information obtained for the two years in alfalfa in which there were fewer numbers of whiteflies in the valley in 1988, the year in which virus disease incidence was less.

These data help explain the interacting roll played by whiteflies, which can be estimated adequately using our trapping system in alfalfa, and virus disease incidence estimated in melons, which leads to the LIYV epidemic in lettuce. Our future research will focus on this interaction in an attempt to provide information on

the importance of melons in the epidemiological cycle which leads to crop loss in lettuce.

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IMPLICATIONS OF THE PRESENCE OF A MIGRATORY MORPH IN THE SWEETPOTATO WHITEFLY, *BEMISIA TABACI*

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It is well known that the sweetpotato whitefly, *Bemisia tabaci*, is of considerable economic importance worldwide, primarily because it vectors a number of important viral pathogens. The ability of *B. tabaci* to vector these pathogens is enhanced by the fact that subsets of the population are constantly moving among a series of crop and weed plant hosts.

This makes *B. tabaci* unique among members of the family Aleurodae since most whiteflies are oligophagous or monophagous. Because they often find themselves in ephemeral habitats, there is a likelihood that *B. tabaci* has selected for dispersal dimorphism. That means that there will be certain individuals within populations that will engage only in trivial flight (Southwood, 1962). Others will engage in flights of longer distances whose principal goal is migratory.

Identifying this phenomenon in *B. tabaci* proved to be difficult since whiteflies are among the smallest insects for which flight mechanisms such as wingbeat frequency and wing loading have been characterized (Byrne et al., 1988).

We began by collecting animals from within the boundaries of a robustly growing field of common beans. It was our assumption that animals found within such a field would likely be trivial fliers, not wanting to pay the price involved in leaving a perfectly suitable habitat. To capture animals which we thought would be putative migrators, we placed trap plants at varying distances from these fields.

Males and females of both the trivial fliers and the migrators were selected at random from a larger sample. Forewings and hindwings were removed and floated on ethanol above a glass slide. The pictures of these wings were then taken using a scanning electron microscope. From the photomicrographs, anatomical landmarks and helping points (Bookstein et al., 1985) were electronically converted to Cartesian coordinates using a digitizing pad.

Characters were chosen for their repeatability of measurement, minimal distortion through handling, and equitable distribution across the form. Distances were converted to natural logarithms to equalize variance, preserve elementary relationships among characters and produce a scale covariance matrix (Jolicoeur, 1963). The total wing form was partitioned into seven characters.

Character size was examined initially by means of a conventional univariate character examination. Multivariate statistical procedures were also applied to the data. The first was principal components analysis and the second multivarate procedure was discriminant function analysis. This is an optimization technique which extracts from the character set the linear combination of characters which best separates (discriminates) predefined groups, i.e., trivial fliers and the migrators.

Morphological differences were found to exist in the characters comparing migrating and trivial flying morphs. Differences exist in both sexes, but male trivial flyers are more distinctly different from there migrating counterparts than are female trivial flyers (Tables 1 and 2). Nevertheless, with careful examination, trivial fliers of both sex can be differentiated using these techniques.

Applications of morphometric techniques enabled us to detect very subtle differences between the two whitefly morphs. We hypothesize that whiteflies have acquired their ability to respond to changing conditions relatively recently and that we are seeing incipient separation of morphs.

Our statistical techniques confirm the discovery of Ihaegwam (1977) who found behavioral differences in the seasonal morphs of *Aleyrodes protella*, the cabbage whitefly. The winter females of this whitefly are much more willing to fly than the summer females. They are also more positively phototactic.

With the recognition of wing dimorphism in *B. tabaci*, further experimentation will allow us to examine the influence of various environmental factors on the production of the migrating morph.

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Table 1. Mean differences, in microns, among females for the seven wing characters measured. Coefficients of variation (C.V.) are expressed as percentages.

Variable	Forewing N	Mean ± Std Dev	C.V.	Hindwing N	Mean ± Std Dev	C.V.
		Migrators				
Length anterior margin	10	1147.11 ± 35.52	3.1	10	959.04 ± 36.09	3.8
Length posterior margin	10	1189.52 ± 59.82a	5.0	10	1030.04 ± 45.96	4.5
Length of radial sector vein	10	1017.62 ± 30.05	3.0	10	869.97 ± 32.47	3.7
Centroid-wing base	10	531.64 ± 23.17	4.4	10	464.80 ± 15.73	3.4
Centroid-wing margin	10	472.54 ± 13.58	2.9	10	402.72 ± 20.51	5.1
Area of anterior wing "cell"	10	348.79 ± 17.86	5.1	10	306.28 ± 16.58	5.4
Area of posterior wing "cell"	10	443.56 ± 19.75a	4.5	10	362.13 ± 17.46	4.8
		Trivial Fliers				
Length anterior margin	10	1136.04 ± 47.34	4.2	10	930.44 ± 29.65	3.2
Length posterior margin	10	1030.94 ± 55.18a	5.4	10	996.81 ± 51.04	5.1
Length of radial sector vein	10	985.45 ± 44.32	4.5	10	841.86 ± 33.79	4.0
Centroid-wing base	10	512.67 ± 24.12	4.7	10	445.88 ± 25.48	5.7
Centroid-wing margin	10	456.33 ± 22.87	5.0	10	394.26 ± 14.56	3.7
Area of anterior wing "cell"	10	341.42 ± 26.35	7.7	10	297.76 ± 18.12	6.1
Area of posterior wing "cell"	10	425.11 ± 18.68a	4.4	10	350.07 ± 13.60	3.9

a Significantly different from corresponding character in opposing morph ($P < 0.05$)

Table 2. Mean differences in microns among males for the seven wing characters measured Coefficients of variation (C.V.) are expressed as percentages.

Variable	Forewing N	Mean ± Std Dev	C.V.	Hindwing N	Mean ± Std Dev	C.V.
		Migrators				
Length anterior margin	10	797.98 ± 27.66	3.5	10	654.17 ± 22.50b	3.4
Length posterior margin	10	817.03 ± 27.65	3.4	10	710.93 ± 12.00b	1.7
Length of radial sector vein	10	706.89 ± 18.63	2.6	10	599.78 ± 17.25b	2.9
Centroid-wing base	10	371.96 ± 10.49	2.8	10	320.62 ± 12.56	3.9
Centroid-wing margin	10	327.45 ± 14.61	4.5	10	277.53 ± 8.18a	2.9
Area of anterior wing "cell"	10	234.46 ± 8.17	3.5	10	202.84 ± 10.21	5.0
Area of posterior wing "cell"	10	296.79 ± 5.91	2.0	10	243.11 ± 6.99	2.9

continued next page

Variable	Forewing N	Mean ± Std Dev	C.V.	Hindwing N	Mean ± Std Dev	C.V.
			Trivial Fliers			
Length anterior margin	10	809.52 ± 31.85	3.9	10	685.45 ± 29.89**b**	4.4
Length posterior margin	10	822.27 ± 26.05	3.2	10	732.77 ± 20.40**b**	2.8
Length of radial sector vein	10	708.82 ± 21.43	3.0	10	621.89 ± 15.35**b**	2.5
Centroid-wing base	10	368.77 ± 10.39	2.8	10	329.31 ± 13.12	4.0
Centroid-wing margin	10	331.65 ± 13.09	3.9	10	290.48 ± 7.34**a**	2.5
Area of anterior wing "cell"	10	241.68 ± 9.64	4.0	10	209.78 ± 8.27	3.9
Area of posterior wing "cell"	10	302.24 ± 9.65	3.2	10	247.66 ± 8.58	3.5

a Significantly different from corresponding character in opposing morph ($P < 0.05$)
b Significantly different from corresponding character in opposing morph ($P < 0.01$)

Development of non-radioactive, sulfonated probes for the detection and identification of whitefly-transmitted geminiviruses.

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Diseases caused by whitefly (**Bemisia tabaci** Genn.)-transmitted (WFT) geminiviruses have become recognized as serious threats to crop production in tropical, subtropical, and fringe subtropical areas throughout the world. Numerous fiber and vegetable crops including bean, cassava, cotton, cowpea, cucurbits, eggplant, jute, kenaf, pepper and tomato are affected by these diseases (Bird & Maramorosch 1975; Duffus, 1987). Symptoms typically associated with these diseases are leaf curling and distortion or bright yellow and/or green mosaics.

WFT geminiviruses are characterized by a morphologically unique, paired particle (20 X 30 nm) and a circular single-stranded bipartite DNA genome, Harrison (1985). The genomes of several well-characterized WFT geminiviruses have been cloned and sequenced, and varying degrees of nucleotide sequence homologies have been demonstrated. Because the DNA sequences corresponding to the viral coat protein and the putative viral polymerase genes are relatively highly conserved in those cases examined thus far, Stanley (1985), clones of the viral A component (containing the coat protein and polymerase genes) are potentially useful as generic probes in hybridization assays for the detection of WFT geminiviruses. Likewise, the B components (exhibiting minimal sequence conservation between viruses) could be useful as virus-specific probes for virus identification. In this study described herein, clones representing the A and B components of five WFT geminiviruses were utilized to develop either virus-specific or broad spectrum sulfonated probes (Table 1). Fourteen WFT geminivirus isolates representing a wide geographic range were tested in crude sap dot blot hybridization assays to determine relative levels of cross-reactivity between probes and virus isolates, and thus, the efficacy of the probes as reliable, sensitive indicators of WFT geminivirus infection in a variety of hosts. The feasibility of virus diagnosis and detection using non-radioactive probes was investigated in anticipation of the utility of a diagnostic method in which probes would be both relatively easy to label under less sophisticated conditions, and convenient to provide to other researchers, thus, minimizing the difficulties associated with enzymatic labelling systems, and with isotopically labelled materials, respectively.

MATERIALS AND METHODS

Probes were made by sulfonating DNA clones (Table 1) as directed by the manufacturer (ChemiProbe, FMC BioProducts, Rockland, ME 04841). Crude sap preparations of virus infected material and healthy controls (Table 2) were alkaline denatured and neutralized, Robinson, et al. (1984), dotted (3 ul) onto nylon membrane strips, and baked for 2 hr at 68C. Strips were prehybridized for at least 1 hr and hybridized overnight with sulfonated probes at 42C. Post-hybridization washes were done at low and high stringencies according to the manufacturers instructions. Bound probe was detected by labelling with an antisulfone group alkaline phosphatase-conjugated monoclonal antibody and precipitation reactions were visualized following addition of AP substrates. The relative intensity of the reactions was rated as - = negative rxn, + = faint, + + moderate, and + + + high.

RESULTS AND DISCUSSION

The results of the hybridization assays with probes made from viral A components are summarized in Table 3. The homologous virus could always be identified by a strong reaction (+ + +) to its own probe. Probes cross-hybridized with non-homologous virus preparations to varying degrees, depending on the virus isolate and the stringency of the post-hybridization wash. (In most cases, the advantage of the high stringency wash was a reduction in background and not a loss of signal.) In all cases, where a geminivirus was known to be present, individual or cocktail (mixtures) probes made with A components resulted in a signal which ranged from faint (+) to moderate (+ +) for heterologous systems, and high (+ + +) for homologous systems. Thus, each of the fourteen geminivirus isolates was detectable in one of eight different host plant species using individual and/or cocktail probes. Hybridization assays using the same virus isolates, but probes made from B components, resulted in a signal only when the homologous virus was present (data not shown). These preliminary results

indicate the potential viability and reliability of sulfonated, chromogenic probes for the detection of geminivirus infections and for the identification of geminiviruses when a homologous probe is available. The feasibility of testing samples which have been applied to nylon prior to exchange between researchers remains to be tested. However, probes and dotted strips are likely to be stable for long periods of time, providing extreme conditions can be minimized during shipping. An additional goal is to further optimize this system using a wider range of virus clones and subclones as probes and to incorporate virus isolates representing additional geographic locations. Currently, the development of a viable means for quantifying the results of chromogenic dot blot assays is also under investigation.

TABLE 1. Virus clones used in dot blot hybridization assays.

Virus Clones	Geographic Origin	Contributor
Bean Golden Mosaic Virus (BGMV)	Puerto Rico	A. J. Howarth
Chino del Tomate Virus (CdTV)	Sinaloa, Mexico	J. K. Brown & D. Rochester
Cassava Latent Virus (CLV)	Africa	J. Stanley
Squash Leaf Curl Virus (SLCV)	California, USA	S. G. Lazarowitz
Tomato Golden Mosaic Virus (TGMV)	South America	D. M. Bisaro

TABLE 2. Virus isolates and host plants used in dot blot hybridization assays.

Virus Isolates	Host Plant	Geographic Origin	Reference(s)
Bean Calico Mosaic Virus (BCMoV)	Bean	Sonora, MEX	Brown et al. (1988)
Bean Golden Mosaic Virus (BGMV)	Bean	Puerto Rico	Goodman et al. (1977)
Chino del Tomato Virus (CdTV-S)	Tomato	Sinaloa, MEX	Brown et al. (1988)
Chino del Tomato Virus (CdTV-T)	Tomato	Tamaulipas, MEX.	Brown & Nelson (1989)
Cotton Leaf Crumple Virus (CLCV)	Cotton	Arizona, USA	Brown & Nelson (1984)
Cassava Latent Virus (CLV)	Tobacco	West Africa	Stanley (1985)
Pepper Mild Tigre Virus (PMTV)	Pepper	Tamaulipas, MEX.	Brown et al. (1989)
Unchar. Pepper Virus-W	Pepper	Texas, USA	Brown & Nelson (1989)
Squash Leaf Curl Virus-D (SLCV-D)	Squash	California, USA	(Duffus 1987;Lazarowitz 1987)
Squash Leaf Curl Virus/Watermelon Curly Mottle Virus (SLCV/WCMoV)	Pumpkin	Arizona, USA	Brown & Nelson (1986)
Tomato Golden Mosaic Virus (TGMV)	Tobacco	Brazil	Hamilton et al. (1981)
Tomato Yellow Leaf Curl Virus (TYCV-L)	Tomato	Lebanon	Makkouk (1978)
Tomato Yellow Leaf Curl Virus (TYLCV-I)	Tomato	Israel	Cohen & Nitzany (1966)
Watermelon Chlorotic Stunt Virus (WCSV)	Watermelon	South Yemen	Jones (1988)

TABLE 3. Reaction of sulfonated probes to fourteen whitefly-transmitted geminivirus isolates in a dot blot hybridization assay (+ = faint rxn, ++ = moderate rxn, +++ = strong rxn).

PLANT SAMPLE			SULFONATED PROBE					
			BGMV-A	CdTV-A	CLV-A	SLCV-A	TGMV-A	5 Probe Cocktail
Virus Isolates	1	BGMV	+++	++	++	++	++	+++
	2	BCMoV	++	+	+	++	+	++
	3	CdTV-S	+	+++	++	+	++	+++
	4	CdTV-T	+	+++	++	++	++	+++
	5	CLCV	+	+	+	+	+	+
	6	CLV	+	+	+++	+	+	+++
	7	PMTV	+	+	+	+	+	+
	8	Pepper Virus-W	+	+	+	+	+	+
	9	SLCV-D	++	++	+	+++	++	+++
	10	SLCV/WCMoV	++	++	+	+++	++	+++
	11	TGMV	++	+	++	+	+++	+++
	12	TYLCV-L	+	+	+	+	+	+
	13	TYLCV-I	+	+	+	+	+	+
Healthy Controls	14	WCSV	+	+	+	+	+	+
	15	Bean	-	-	-	-	-	-
	16	Tomato	-	-	-	-	-	-
	17	Cotton	-	-	-	-	-	-
	18	Pepper	-	-	-	-	-	-
	19	Pumpkin	-	-	-	-	-	-
	20	Watermelon	-	-	-	-	-	-

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MULTIVARIATE ANALYSIS OF ANTIGENIC VARIATION AMONG GEMINIVIRUS ISOLATES ASSOCIATED WITH CASSAVA MOSAIC DISEASE

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INTRODUCTION

Extensive work on the geographical variation among geminivirus isolates associated with cassava mosaic disease in Africa and India has been conducted for several years in the Virology Division of the Scottish Crop Research Institute and results of these studies have been summarized elsewhere (Aiton and Harrison, 1988; Aiton et al., 1988; Harrison et al., 1987). Virus isolates from 10 countries in Africa and the Indian subcontinent were tested against a panel of monoclonal antibodies (MAbs) prepared against a west Kenyan isolate of African cassava mosaic virus (ACMV; Thomas et al., 1986). On the basis of the occurrence of 17 epitopes, the isolates fell into three groups. Group A isolates came from Ivory Coast, Nigeria, Angola, South Africa and western Kenya; Group B isolates came from coastal Kenya, Malagasy, Malawi and Tanzania, and Group C isolates were from India and Sri Lanka. Within each group there was some variation: group A isolates shared at least 14 epitopes with the west Kenyan isolate, group B isolates shared 4 to 9 epitopes with it and group C isolates shared only 2 or 3. Moreover, isolates in the same group tended to share the same epitopes (Harrison and Robinson, 1988).

The numerical data obtained by ELISA in the work described above involved the reactions of several dozen virus isolates with a panel of 17 MAbs. Thus although an analysis and diagrammatic representation of the whole set of data is desirable, this is difficult in practice. A diagram summarizing the data was produced by Harrison and Robinson (1988) but this took only the qualitative information into account, each reaction between a virus isolate and a MAb being scored either positive or negative. We describe here a different system for representing the data, based on multivariate analyses of the quantitative values obtained by ELISA. First, Hierarchical Cluster Analysis (HCA) was done to put the grouping of virus isolates on a mathematical basis. Principal Component Analysis (PCA) was then conducted to establish, in space with a limited number of dimensions, the positions of individual isolates, the occurrence of clusters (groups) of isolates, and the variation within and between these groups. In addition, the grouping of different MAbs, on the basis of their pattern of reactions against the isolates, was determined and

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[1]On secondment from ORSTOM (Institut Francais de Recherche Scientifique pour le Developpement en Cooperation)

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illustrated. Finally, Stepwise Discriminant Analysis (SDA) was done to establish which MAbs comprise the minimum number needed to distinguish between the groups of isolates.

MATERIAL AND METHODS

A relatively large panel of MAbs was available, but not all the isolates were tested with the same selection of MAbs during the six-year study. Therefore two versions of the analyses were performed to minimize the amount of missing data:

(a) A smaller number of MAbs (12) and a larger number of isolates (32 in group A, 17 B and 12 C) were used. These MAbs are those known as SCR 14, 16, 17, 18, 19, 20, 21, 22, 23, 25, 26 and 29.

(b) A larger number of MAbs (14) and a smaller number of isolates (26 A, 17 B and 12 C) were used. The MAbs included those listed above, together with SCR 28 and 32.

For both data sets an ELISA reading was taken after incubation with substrate for 1 hr at room temperature and also after subsequent incubation overnight at 4°C. The mathematical procedures used for HCA, PCA and SDA are essentially as described by Gnanadesikan (1977).

RESULTS

The three groups of isolates obtained by HCA correspond to the groups proposed previously, i.e. the West African group (A), the East African group (B) and the Indian group (C). Increasing the number of MAbs (and decreasing correspondingly the number of isolates analysed) did not change the grouping appreciably. Analyses of overnight readings allowed a better distinction between the groups than similar analyses of 1-hour readings and therefore the subsequent analyses were done on the overnight readings.

For PCA, the results were used from 61 isolates tested against a panel of 12 MAbs. The first principal component was a weighted average for all the MAbs (with low weights for SCR 18 and 20) and accounted for 61.2% of the variance. The second component had large positive coefficients for SCR 17, 23 and 26 and large negative coefficients for SCR 16, 19, 21, 22, 25 and 29. This accounted for 26.6% of the variance. The third component had large negative coefficients for SCR 18 and 20 and smaller positive coefficients for the other MAbs. This accounted for 5.1% of the variance. Therefore, PCA allows us to represent in two dimensions nearly 88% of the variance of the results contained in a 61 x 12 table. Indeed by including a third axis about 93% of the information can be represented.

Plots of the first two principal components clearly separated the three groups of isolates. However, the groups differ in the amount of variation they contain, with group B being the most variable. The variation can be attributed to several factors. It can be caused by differences in reaction intensity, such as those caused by differences between isolates in virus concentration. This kind of variation would appear preferentially along axis 1 (which represents an average of the reactions given by all the MAbs). Variation can also be attributed to the failure of some MAbs to react because the isolate lacks the corresponding epitopes. This would appear preferentially on the axis on which these MAbs have their greatest weight. Finally, variation can be

linked to differences in the relative intensity of reaction of some MAbs, such as those caused by minor changes in individual epitopes. All three kinds of variation were apparent in these studies, although most of it is caused by differences in reaction intensity and by the absence/presence of some epitopes. However, the considerable variation found among isolates in group B mainly reflects the absence of different combinations of epitopes in different isolates within this group rather than differences in concentration. Group C appears to be much more compact in plots of the first two components, but some minor variation is clearly expressed along axis 3 and is caused by variation in reactions with MAbs SCR 18 and 20 which have a strong influence on the position of points along this axis. Group A is the most compact cluster in the three dimensions, despite including the most isolates and the results of experiments conducted over a period of six years. The small variation within group A is attributed mainly to differences in reaction intensity (axis 1) and to differences in relative intensity of reactions with a few MAbs, such as SCR 19 and 22 (axis 2).

Further analysis of the correlation matrices indicates that the reactions of MAbs SCR 16, 19, 21, 22, 25 and 29 were highly correlated with each other. Similarly, the reactions of MAbs SCR 17, 23 and 26 were highly correlated. The reactions of SCR 18 and of SCR 20 were not significantly correlated with those of any other MAb. The reactions of SCR 14 were correlated significantly, although not strongly, with those of every other MAb except SCR 18 and 20. Principal Component Analysis allows a clear representation of the relationships between the reaction patterns of the MAbs, on the basis of the readings obtained, in plots of the two first principal components.

The application of Stepwise Discriminant Analysis to the results given by 55 isolates in reactions with 14 MAbs allowed us to identify the best combination of MAbs necessary to distinguish the different groups of isolates. Reactions with MAbs SCR 17, 20, 21 and 23 classified correctly 53 of the isolates. Indeed, increasing the number of MAbs did not significantly improve the classification. Only two isolates were misclassified and these exceptions can be explained by technical problems.

DISCUSSION

Multivariate analyses proved to be useful to group the virus isolates (HCA), to display the variation between and within groups of isolates (PCA), to illustrate the relationships between the patterns of reactivity of different MAbs (PCA) and to select the MAbs needed to discriminate between groups of isolates (SDA). These analyses confirm and refine previous interpretations of the data (Harrison and Robinson, 1988). They provide a clear picture of the relationships which exist and are a tool powerful enough to cope with further analyses, in which the number of virus isolates or viruses is increased. These sorts of analysis could also be helpful for assessing antigenic variation in countries or areas where the availability of MAbs is restricted and where ELISA plate-reading facilities are not available. For example, SDA would help to select the MAbs needed to study the range of variation among strains or related viruses. In addition, a grading system based on visual assessment of the strengths of ELISA reactions could be worked out which would reveal much of the antigenic variation that exists.

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SOME ASPECTS OF THE EPIDEMIOLOGY OF OKRA LEAF CURL IN COTE D'IVOIRE

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INTRODUCTION

Okra leaf curl virus (OLCV) is a serious disease that limits the culture of numerous okra varieties (*Abelmoschus* spp.) in Côte d'Ivoire. The characteristic symptoms include curling of the leaves, vein thickening and a decrease of leaf area. It can lead to a severe stunting and for some varieties to the death of the plant. In the field, the disease is transmitted by the whitefly *Bemisia tabaci* Genn; experimentally, it can be transmitted by grafting. Virus particles of the geminate type have been observed associated with the disease (Fauquet & Thouvenel, 1987). In 1986 and 1987, experiments were conducted to study some aspects of the epidemiology of the disease in Côte d'Ivoire, its transmission, prevalence in the country and development in space.

TRANSMISSION OF THE DISEASE

In preliminary experiments, it was established that the disease could not be transmitted through the seeds or by mechanical inoculation : Fifty seeds from infected okra plants were grown in insect-proof glass-houses. The experiment was repeated three times. No symptom of OLCV was noticed in any of the plants. Extracts of infected okra were inoculated to okra, cassava and *Nicotiana benthamiana*. In total, 75 plants of each species were tested. However, no symptoms were observed in any of the inoculated plants. These experiments suggest that mechanical inoculation to these plants is unlikely and confirm that there is no transmission through the seed. Consequently, field spread only depends on whitefly transmission.

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The percentage of viruliferous whiteflies in the field was assessed by transferring groups of 20 whiteflies originating from okra-infected fields onto healthy okra plants (cv. Clemson spineless) in the glass-house. In total, 75 okra were tested and the number of plants showing symptoms was recorded. The percentage of viruliferous whiteflies was deduced from the formula of Gibbs and Gower (1960) and was found to be c. 4%. Transmission from okra to cassava, from okra to *Nicotiana benthamiana* and from cassava to okra were also tested but no sucessful transmission was observed. No pupae were found on the tested plants, suggesting that whiteflies did not adapt sucessfully when transferred to these species.

PREVALENCE OF THE DISEASE

Previous observations have shown that Okra leaf curl occurred in numerous regions of Côte d'Ivoire. In 1987, experiments were conducted to assess in more details the prevalence of the disease in the country. Along a north-south transect in Côte d'Ivoire, four sites were selected and two varieties, one known to be susceptible (Clemson spineless), the other known to be resistant (ORS 520) were planted in a 20 x 20 m plot in early April. Sites were Sinematiali (north of Cote d'Ivoire in the savanna area; one rainy season from June to October; 1200 mm), Marabadiassa (centre of Côte d'Ivoire; savanna and forest, one rainy season from April to October, 1500 mm), Go-hermankono (South of Côte d'Ivoire, forest region, double rainy seasons, March to July and September to November, 2000 mm) and Adiopodoumé (same region as Go-hermankono but further south and along the coast). Disease incidence was assessed and the number of whiteflies was estimated weekly.

OLCV and whitefly vectors seem to be widespread in Côte d'Ivoire although marked differences did occur between the four sites. Disease incidence in the susceptible variety reached 91% in Sinematiali, 69% in Marabadiassa, 5% in Go-hermankono and 36% at Adiopodoumé. Not only the final disease incidence but also the course of infection varied between sites, the epidemics in the north sites began later than in the south. Dynamics of whitefly populations also varied and higher populations were observed in the north than in the south. Other factors are likely to influence the epidemiology of OCLV as differences in disease incidence cannot be fully explained by corresponding differences

in whitefly populations: In particular, average number of whiteflies per plant was similar in Adiopodoumé and in Go-hermankono although disease incidence was five times greater in the former than in the latter. Thus, further information is needed in order to understand better the epidemics, especially about the nature and the prevalence of the virus reservoirs.

DISTRIBUTION OF THE DISEASE IN SPACE

Disease incidence and whitefly distribution were recorded in a 0.5 ha okra field planted in late March 1986. Infection was not homogenous within the field and strong border effects were observed. In particular, higher disease incidence was noticed on the south-west border, which is regularly exposed to the prevailing south-west wind. Disease incidence gradually diminished towards the centre of the field forming a decreasing gradient of infection. This gradient was apparent 30 days after planting and persisted during the rest of the experiment, although it tended to become less obvious with time. Whitefly distribution was not homogenous either and showed similar patterns characterised by higher populations on the south-west wind-exposed border. Modifications of the wind characteristics at the edge of the field may explain the greater concentration of whiteflies and the subsequent higher disease incidence on the wind-exposed borders (N'Guettia et al., 1986).

DISCUSSION

Spatial distribution of okra leaf curl shares some similarities with that of African cassava mosaic virus (ACMV) with gradients of infection from the wind-ward border to the center of the field (Fargette, 1985). Temporal patterns of spread also showed some similarities with ACMV with period of high disease incidence at the begining of the rainy season and only very low levels in the short dry season (N'Guessan, unpublished results). These common characteristics of the epidemiology are likely to be explained by features of the biology of their commun vector, *Bemisia tabaci*

Difficulty of mechanical inoculation and low percentage of transmission by the whitefly vector may be characteristics of the geminiviruses and

further work on the relationship between virus, host plant and vector is needeed. Further information is also required on the nature and the prevalence of the virus reservoir for a better understanding of the epidemiology. However, the pathogen agent should be identified and specific and sensitive means of detection should be developed before progress can be made in this direction.

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AUTRES MODES DE DISSEMINATION / OTHER MEANS OF SPREAD

CICADELLES / HOPPERS

RTV Epidemiology in Andhra, India.

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John identified RTV in India in 1968. Since then it has been reported frequently all over the country. Two severe epidemics occurred in the recent eighties in Andhra Pradesh of South India.

Occurrence of RTV around Tirupati has been suspected for long but not studied. Nellore, 120 K.M. north east of Tirupati, is a known place of regular occurrence of RTV but not studied. Seven villages around Tirupati within 7 K.M. radius and the fields at the Agricultural Research Station, Nellore, were surveyed for RTV and its vector, Nephotettix virescens.

MATERIALS AND METHODS :

10 x 10 M plots were selected in the different villages and visited weekly. Both nurseries and transplanted fields were surveyed. Six varieties, IR-20, IR-34, IET 1444, Emergent, Molagolukulu and Surekha around Tirupati and four varieties IR-20 RII, NLR 5000-57-3 RII, -51-4-3 RII, -69-2-RII at Nellore were studied for the vector population and disease incidence.

Vector population was determined by 50-60 sweeps with a 60-mesh nylon net over each plot in each visit and averaged. The disease incidence was determined by orange-red discoloration of the leaves and stunting of the plants in each plot. Three successive crops in a year were surveyed. Adjacent fields were always in different stages of crop development as each farmer raised his crop according to his convenience.

Air and leaf temperature and relative humidity for each plot were noted in each visit.

Dr. Anjaneyulu, Virologist at Central Rice Research Institute, Cuttack, provided the antiserum for RTBV and RTSV to determine the virus isolate occurring at each location. Latex flocculation test was used.

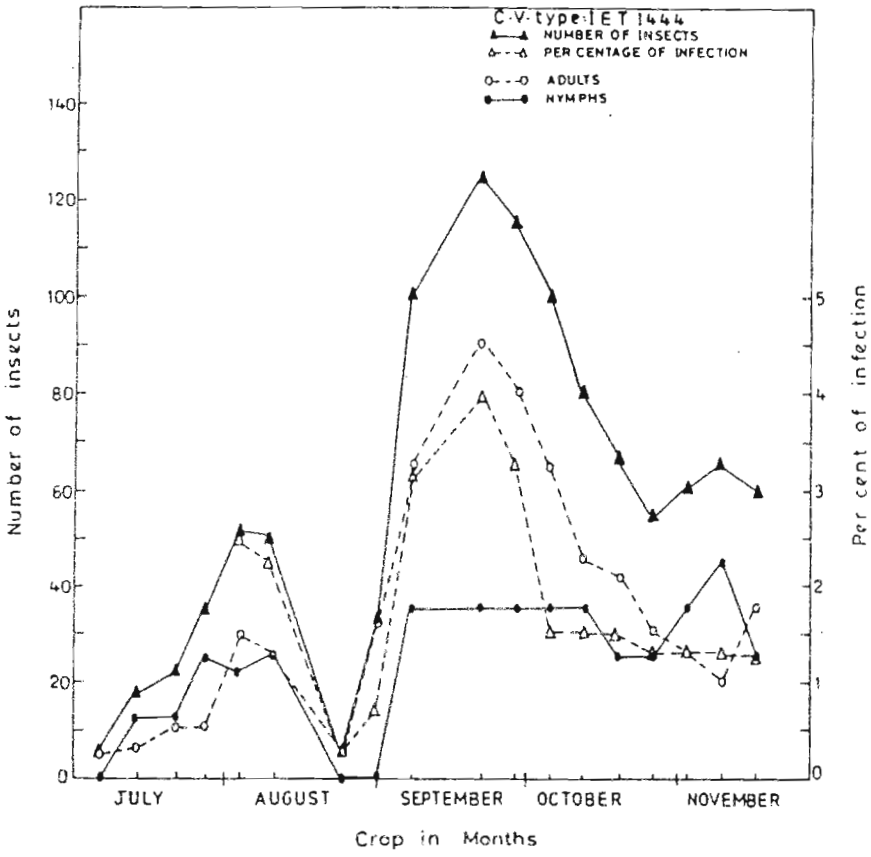

Fig.1: Vector population and tungro per cent

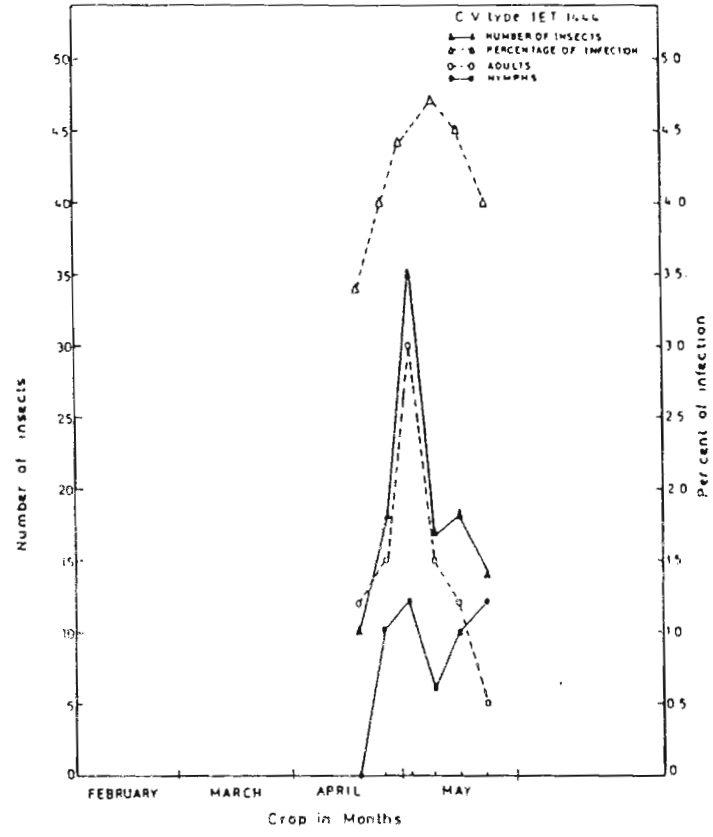

Fig. 2: Vector population and tungro per cent

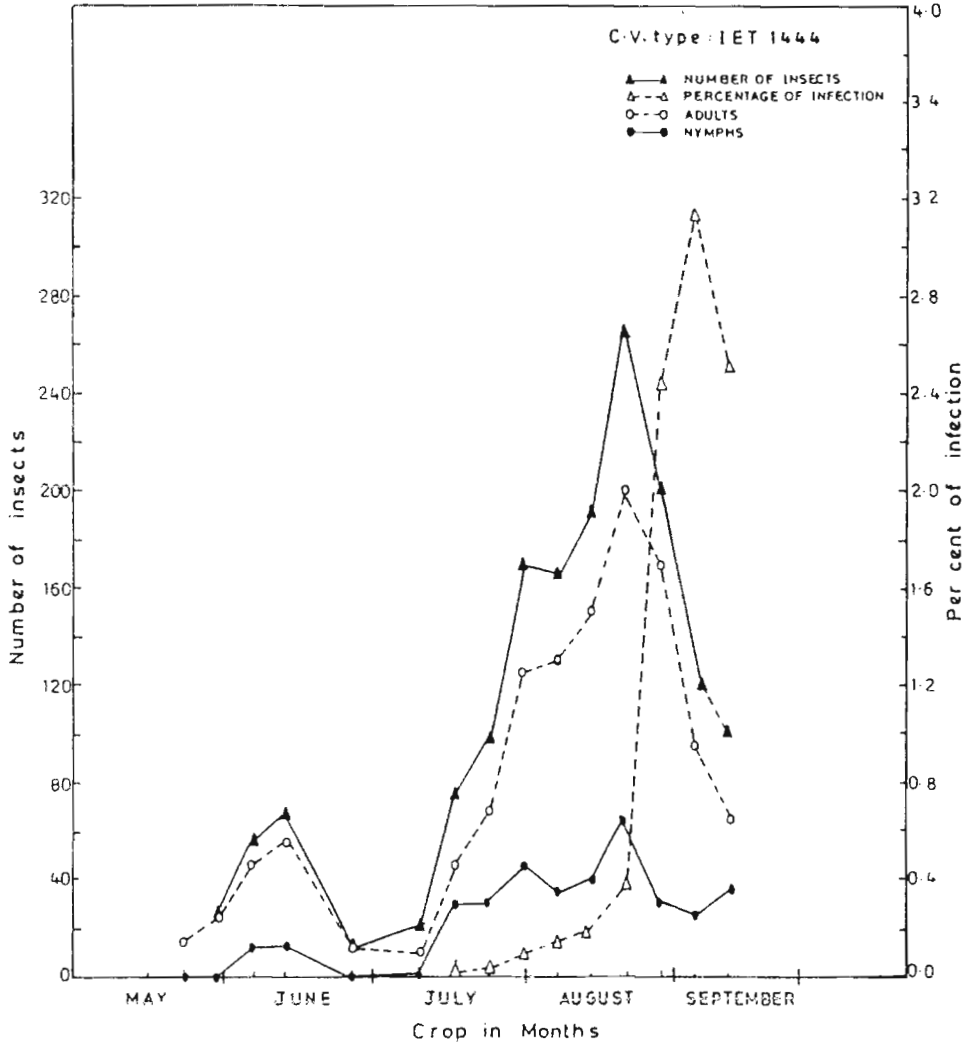

Fig.3 : Vector population and tungro per cent

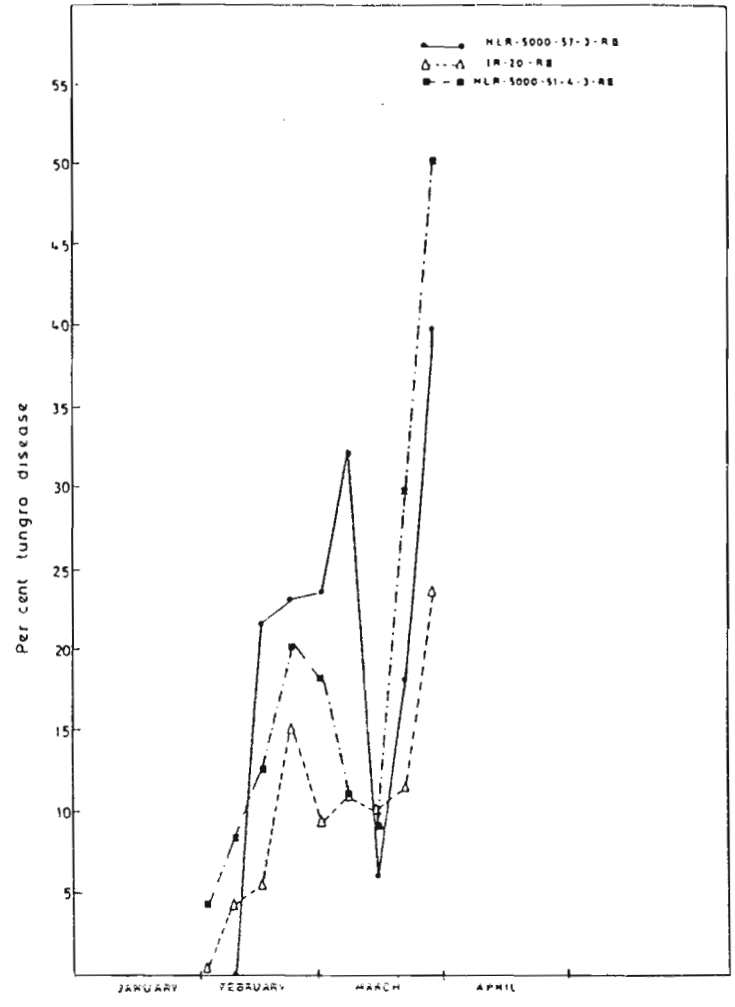

Fig.4 : Tungro per cent - Nellore

RESULTS :

Around Tirupati the insect population varied from 5 to 273/plot and disease incidence upto 4 % (Fig. 1-3) and at Nellore it was 4-20 insects/plot and disease upto 49.8 % (Fig. 4). Insecticide application was regular at Nellore station fields.

In variety Emergent, tho' the apparent percent infection was 1-3 %, the yield loss was 72 %. In some cases the disease and vector were noted in a week after transplantation, while in some others disease was noted 3-4 weeks after vector was noted in the transplanted field. Vector occurred all thro' the year.

Depending on when the crop was transplanted, the peaks of the vector population varied. There were invariably two peaks of the vector with an interval of 20-60 days depending on the time of the year. The peaks of the adults and nymphs invariably coincided. The peaks occurred in March, April, May, June, August, September and November depending on the cropping period.

CONCLUSION :

It is suggested that the vector is present all thro' the year and the RTV is now endemic. Epidemics develop whenever highly favourable conditions occur. Effective control can only be by integrated management including regulation of the cropping period.

SOME EPIDEMIOLOGICAL ASPECTS OF TOMATO LEAFROLL

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INTRODUCTION

Tomato leafroll is a disease that in some years affected greatly the tomato crops in a restricted area, in the South of Portugal (Sequeira & Borges, 1985).
The symptoms displayed by the infected plants in the field, consisted of leaf rolling, mainly of the younger leaves which became brittle and showed sulphur yellow discolouration. These symptoms are similar to the ones described for some strains of beet curly top virus (BCTV) infecting tomato (Bennett, 1971).
The yields were severely reduced when the infection occurred in the nurseries and higher rates of infection corresponded to years with drier springs.
Considering the great economic importance of the disease, which had not been previously recorded in Portugal, studies were conducted in order to determine its etiology and clarify some epidemiological aspects concerning particularly transmission and possible sources of the causal agent.

RESULTS

The first experiments on tomato leafroll showed that its causal agent is graft--transmissible (Sequeira & Borges, 1985, 1987). It was noticed, however, that the symptoms displayed by the infected plants, in greenhouse conditions, were somewhat different from the ones observed in the field plants. The leaf tissue of the plants in the greenhouse was less brittle and yellow discolouration was almost absent. Rather than rolling, the leaflets showed corkscrew-like distortion.
Purification experiments using infected tomato leaves enabled suspensions of tomato leafroll virus (TLRV) to be obtained in which geminate particles, typical of the Geminivirus group, were seen under the electron microscope.
Serological studies, using antiserum prepared to TLRV and antiserum to BCTV, kindly supplied by Dr. J. Duffus, indicated that both viruses are serologically related. The two antisera reacted identically against TLRV antigen, when the homologous antiserum was 4 times more diluted than the heterologous antiserum.
Having obtained antiserum to TLRV, it was possible to detect sources of virus by ELISA (Enzyme-linked immunosorbent assay). It was found that virus antigen was present in several plants grown in vegetable gardens, in the area where the nurseries were set up and possibly in some weeds. Such plants could therefore be sources of virus and, in favourable years, the vectors could easily infect the plants in the nurseries.
Bearing in mind that TLRV is serologically related to BCTV, which is a leafhopper -transmitted geminivirus, the populations of leafhoppers were observed in the area where TLRV was detected and some leafhoppers were collected for transmission experiments. The more abundant leafhoppers were *Empoasca fabae* but other species were also collected mainly *Exitianus taeniaticeps*, *Psammotettix alienus* as well

as *Agallia* sp. and *Peragallia* sp.
Empoasca fabae leafhoppers were particularly abundant on pepper plants,in the vegetable gardens near the nurseries.Those pepper plants,which had some leafroll symptoms,were tested by ELISA and were found to contain TLRV antigen.Experiments using leafhoppers did not provide conclusive results because it was not possible to keep the leafhoppers for long periods under laboratory conditions.Most of them died a few hours after being caged.The leafhoppers were groung in a mortar with some buffer and the suspensions tested by ELISA. The results suggested that some TLRV antigen might be present in the leafhoppers but did not prove that they are vectors. Therefore, the vector (s) of TLRV still remains to be determined.
The possibility of TLRV being seed-transmitted was also investigated.The results of experiments with seed collected from infected fruits indicated that fresh seed could transmit the virus at low percentages. Probably TLRV is not transmitted by dry seed.
Once the presence of virus sources near the tomato nurseries was confirmed, the growers were advised to set up the nurseries in a different place, far from the vegetable gardens. This measure, by itself, was sufficient to limit the disease. In fact, tomato leafroll has not been detected since then (1985),even in fields where it had occurred before.

CONCLUSIONS

As a result of the studies conducted, a few aspects concerning the epidemiology of tomato leafroll have been clarified.
Strong similarities have been found between the causal agent of the disease and beet curly top virus. In addition to host range and symptomatology, it was found that both geminiviruses are serologically related. Although the vector of TLRV has not been determined, the similarities to BCTV suggest that it is probably a leafhopper.
Several plants mainly tomato,pepper and potato, were found to be hosts for TLRV. Such plants may account for the survival of the virus from one year to the other, particularly in the vegetable gardens where those plants are grown all the year round.Fortunately the main sources of virus seemed to be restricted to the area of those vegetable gardens and the virus could not remain in the tomato fields because all the plants were burnt after the harvest.On the other hand the spread of the virus is difficult because leafhoppersare highly sensitive to pesticides that growers regularly apply. Presumably, because of such restritive conditions, tomato leafroll, which can be a very serious disease, never reached alarming proportions. The outbreaks of the disease were a consequence of plant infection in the nurseries, and depended very much on the weather conditions. In the years with drier springs, more favourable to vector multiplication and activity, the incidence of the disease was higher. Setting up the nurseries far from virus sources, inside a pine tree forest, proved to be a simple but efficient way of avoiding further outbreaks of tomato leafroll.

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THRIPS

POTATO STEM-NECROSIS EPIDEMIC DUE TO TOMATO SPOTTED WILT VIRUS IN INDIA

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Since 1982, an outbreak of an unusual potato disease causing stem/foliar necrosis (rings/spots/streaks) has been observed in parts of North-western/ Central plains of India. The disease was characterized by extensive stem and petiole necrosis, leaf deformations, chlorosis and stunting normally observed in mid-December/early-January, i.e. 6-8 weeks after planting. The yield losses were estimated to vary from 11.6 to 34.4% depending on the variety and age of crop. The disease incidence was usually very high (up to 90%) in cv. Kufri Chandramukhi planted by October-end but below 5% in fields planted in early-November. The disease agent was not tuber-borne but mechanically transmitted to a number of hosts. On the basis of symptomatology, transmission, serology, host range, particle morphology and instability in crude sap, the disease was found to be due to tomato spotted wilt virus (TSWV). The physical properties of the virus were DEP between 1:100 - 1:200; TIP 40-46°C, and LIV between 2-5 h at 18-22°C. It was readily detected by ISEM and also confirmed by das-ELISA using host-absorbed TSWV-specific IgG. Typical TSWV particles of 70-90 nm dia were observed in leaf macerates from glutaraldehyde-fixed diseased but not healthy plant materials. In ultrathin sections of freshly necrosed leaf cells, clusters of TSWV particles were frequently observed in cytoplasm enclosed in vesicles of endoplasmic cisternae. Insecticidal sprays at fortnightly intervals did reduce the disease incidence/index to some extent. Secondary spread of the disease within the crop was non-significant. Apparently, the large populations of incoming thrips, mainly from cotton, possibly **Frankliniella schultzei** or **F.occidentalis/Scirothrips dorsalis** cause spontaneous primary infections on young stems/foliage on the feeding sites.

PRELIMINARY EVIDENCE FOR AN UNUSUAL MODE OF TRANSMISSION IN THE ECOLOGY OF PELARGONIUM ZONATE SPOT VIRUS (PZSV).

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Zonate spot is a disease of tomato caused by pelargonium zonate spot virus (PZSV), a virus with quasi-isometric particles which encapsidate a bipartite genomic RNA. So far the virus is taxonomically ungrouped although it resembles ilarviruses in some properties and some aspects of its ecology are still obscure, including the natural way of transmission. Previous studies showed that PZSV is not transmitted by aphids (Quacquarelli and Gallitelli, 1979; M. Conti unpublished results).

In Southern Italy, the virus was isolated from tomato in 1982 (Gallitelli, 1982; Gallitelli *et al.*, 1983) and since then it became prevalent in tomato crops for canning (Vovlas *et al.*, 1986).

Infected plants show peculiar symptoms on the foliage and fruits consisting of severe malformations, yellow rings, line pattern and necrosis. Very often, the whole plant dies.Field surveys have shown that incidence of the disease is usually below 1% but may reach 30% in rows of plants growing in the vicinity of roads and irrigation canals. Among indigenous weeds usually found in these environments, PZSV has been isolated only from *Diplotaxis erucoides* which is infected symptomlessly(Vovlas *et al.*, 1989).

This study was undertaken to asses the importance of the alternative host (*D. erucoides*), seed transmission and vectors in the spread and survival of the virus.

Starting from a road heavily infested by *D. erucoides*, tomato samples (Italpeel hybrid) were collected in the first 10 rows of the crop at weekly intervals 7 to 40 days after transplanting. *Nicotiana glutinosa* was used as assay host.

The first tomato infections were detected 30 days after transplanting in plants growing in the first two rows of the field. A slow progression of the disease to other rows was then observed and it was stopped by the first insecticide spraying given by the farmer. After this, infected plants were detected sporadically in the field. Using standard mechanical inoculation procedures, pollen collected from naturally infected tomato plants proved to be contaminated by the virus.

D. erucoides plants growing on the farm road were also assayed and the insect population thriving on them determined. The flowers of the weed were found heavily infested by a predominant thrips species (Melanothrips ? fuscus Sulzer). When the insects were collected and observed with a scanning electron microscope their body was found to carry pollen grains. Adult insects were collected from naturally infected D. erucoides and carefully transferred in groups of ten, to tomato seedlings. Observations with the scanning electron microscope of the surface of tomato leaves artificially infested with M. fuscus revealed breakage of leaf hairs and release of pollen grains. Tomato seedlings were kept under glasshouse at 20-22°C. Characteristic yellow zonate spots appeared 25 days later in 1 out of the 10 infested seedlings were transferred and the infecting virus serologically identified as PZSV. In comparative experiments, a suspension of intact pollen in phoshate buffered saline (PBS) was gently inoculated onto tomato seedlings without abrasive . In this case, 8 out of 10 plants became infected within 10 days after inoculation.

So far, the possibility that thrips could transmit PZSV by feeding on infected leaves or pollen has not been tested.

PZSV is not transmitted by tomato seeds in detectable level (Vovlas and Gallitelli, unpublished results) but it is seedborne in D. erucoides (M.A. Castellano and V. Savino, personal communication). To give an estimate of percentage of seed transmission in this weed, seeds were collected from a naturally infected D. erucoides and sowed. Seedlings were then tested by mechanical inoculation and 9 out of 40 (i.e. 5%) proved to be infected by PZSV.

The results of this study are preliminary but nevertheless they cast some light on the following aspects of the ecology of PZSV:

1) In Apulia (southern Italy), PZSV is endemic in D. erucoides (M.A. Castellano and V. Savino, personal communication) which, so far, is also the only known (alternative) host of the virus and grows frequently in proximity of tomato crops. In the crop, the first rows can be infected by PZSV within 30 days after transplanting..

2) The virus is transmitted through the seed of D. erucoides (about 5%) and has been recovered by mechanical inoculation of intact pollen, suggeststing that the virus may contaminate the surface of pollen grains.

3) D. erucoides is an usual food-host of the thrips M. fuscus which infests regularly its flowers, thus becoming loaded with pollen grains. Direct evidence has been obtained that the plant pollen is carried on the surface of the insect body.

4) As D. erucoides plants age, the pollen-infested thrips are stimulated to move and they migrate to the surrounding tomato crops. As shown by direct visual observations, it is probably at this stage that PZSV is inoculated to tomato plants, possibly through wounds caused by crawling.

5) Insecticide sprays routinely done within 20-25 days after transplanting tomato kill, all the thrips thus preventing secondary virus spread.

The unusual mode of transmission which is proposed for PZSV has recently been demonstrated for tobacco streak ILARvirus (Sdoodee and Teakle, 1987), a virus with which PZSV shares some properties.

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COLEOPTERES / BEETLES

COMPARATIVE ROLE OF PERENNIAL WEEDS, *URENA LOBATA* L. AND *SIDA ACUTA* L. AND TWO BEETLE PESTS OF OKRA IN EPIDEMIOLOGY OF OKRA MOSAIC VIRUS DISEASE IN NIGERIA

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Okra mosaic disease (OMD) caused by okra mosaic virus (OkMV) is the most important virus disease of okra (*Abelmoschus esculentus* (L.) Moench.) in West Africa where it is endemic. Incidence usually reaches 100% before harvest, resulting in estimated yield losses of 20-40%, depending on cultivars and time of infection. The virus is transmitted by two beetle pests of okra, *Podagrica sjostedti* Jacq. and *P. uniforma* Jacq., but not through seeds hence the hypothesis that OkMV is perpetuated in weed hosts. In addition to okra, OkMV has been isolated from several weed hosts, including two perennials *Urena lobata* (UL) and *Sida acuta* (SA). Studies were, therefore, conducted to determine relative importance of UL and SA and the two beetle vectors of OkMV in epidemiology of OMD in Nsukka, Nigeria.

Surveys conducted between July 1 and 30, 1987, in ten widely separated okra-growing areas to determine relative occurrence of UL and SA, beetle vector populations, and incidence of OkMV infection on the weeds showed that in three of the locations UL was slightly more abundant than SA. Over 90% of UL present had high infestation of *Podagrica* spp. especially *P. sjostedti*, and incidence of OMD ranged from 80-100%. In the remaining seven locations, SA count was more than UL, but infestation by Podagrica spp. and incidence of OMD and SA were less than 1%, respectively.

Observations on contiguous experimental plots of UL, SA, or okra (OK) at University farm, for beetle populations in relation to incidence of OkMV infection showed that *P. sjostedti* was the predominant beetle vector on all three plant species from April to September (with a peak in August) when it was greatly outnumbered (with a peak in October) by *P. uniforma*. Counts of the two beetle species were generally low during the dry months, from December to March, moderate from April to July and highest from August to November. Both beetle species exhibited a statistically significant preference for UL followed by OK and SA in that order. Incidence of OkMV infection on UL, OK, and SA, 86 days after planting (DAP) was 100%, 35% and 1%, respectively, and 100%, 100%, and 10%, respectively, 150 DAP. Incidence of OkMV infection was positively related to populations of beetle vectors on UL, SA, or OK. Field survival rates for UL and SA, 270 DAP were 96% and 41%, respectively, and 61% and 36%, respectively, 360 DAP. Surviving infected UL and SA produced new symptomatic leaves few weeks after the onset of rains the following year. All OK died between 150 and 200 DAP.

Field experiments conducted to determine the effect of six planting dates (bi-monthly plantings starting from February 1, 1988) of five okra cultivars on beetle vector populations and incidence of OMD showed that 60 DAP, February 1 and April 1 plantings had very low beetle populations and no OMD. The highest incidence of OMD (20%) occurred in June 1 planting, but the incidence decreased

progressively with the least incidence (1%) in December 1 planting. Increases in incidence of OMD were positively related to large increases in populations of P. sjostedti but not those of P. uniforma. Okra cultivar West African Local had the highest OkMV incidence (10%) followed by cultivars OP 80 (9.4%), Awgu Early (9.0%), TAE 38-Lady's Finger (3.1%) and NHAc-47 (1.5%). Disease incidence was related positively to total beetle populations in cultivars Awgu Early, OP 80 and West African Local, and negatively to total beetle populations in cultivars TAE 38-Lady's Finger and NHAc-47. Cultivars West African Local and Awgu Early were the most susceptible to mechanical inoculation followed by OP 80, NHAc-47, and TAE 38-Lady's Finger in that order.

Studies on retention period of OkMV in both beetle species and relative ability of UL and SA to serve as virus acquisition source showed that both beetle vectors retained OKMV for a maximum of two days and that UL was a better source of the virus than SA. Under greenhouse conditions, both beetle species transmitted OkMV from UL or SA to okra, from okra to okra, and from okra to UL or SA.

These results show that in Nsukka, Nigeria: (1) UL is more important than SA as primary source of OkMV in nature; (2) Podagrica sjostedti is primarily responsible for transfer of OkMV from UL to OK at the start of planting season; (3) both beetle species are apparently important in secondary spread of OkMV and in return of the virus from OK to UL or SA or both at the end of okra growing period; (4) genes for resistance or tolerance to OMD probably exist in okra cultivars, and (5) there is need to investigate possible control of OMD through roguing of UL near okra fields in combination with early planting of okra (January to April) if irrigation is available.

ACARIENS/MITES

Transmission of Pea seed borne mosaic virus on peas by the mite *Tetranychus urticae* Koch in the glasshouse

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During studies on seed transmission of Pea seed borne mosaic virus in pea in the glasshouse it was observed that higher number of plants were found infected at a later growth stage than the known low seed transmission rate of the seed lot. The stem and lower leaf surfaces of plants were heavily infested by the spider mites and the upper leaf surfaces exhibited numerous white specks typical of mite infestation. This strongly suggested the possibility of secondary transmission of PSbMV from infected to healthy pea plants. Experimental evidence of the mite transmission of PSbMV was obtained on strictly controlling the other possibilities of secondary dissemination of the virus. The mite species vectoring PSbMV was identified as *Tetranychus urticae* Koch. This constitutes the first report of PSbMV transmission by a mite vector in theglasshouse.

DIVERS / MISCELLANEOUS

VIRUSES CAUSING EPIDEMICS IN TOMATOES IN EGYPT

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SUMMARY

Thorough investigations are continued in Egypt for understanding the epidemiology of viruses infecting tomatoes. Previous results were fragmentary and focused only on tomato yellow leaf curl (TYLC) or tobacco mosaic virus (TMV) together with tomato mosaic virus strains (ToMV). Crop yield reached a crisis for the first time in Egypt in 1989. However, the proceeding two years were indicative of the cumulative problem country-wide. Some of the major factors behind the severe epidemic infections and yield losses of tomatoes in Egypt (80-90%) were concluded as follows:

1- Imported foreign seeds and sometimes seed production from the F_1 hybrids of the imported seeds (a traditional habit of the Egyptian farmers).

2- Numerous sources of foreign seeds: (France, Holland, United States of America, United Kingdom, Denmark and Israel). The last source gained wide market in the last three years.

3- Inadequate or absence of quarantine which, in many instances, never done for many imported seed stocks.

4- Insufficient or ineffective pest control of the major insect vectors: aphids and whiteflies.

5- Lack of hygene since root debris play the second major role together with handling of the plants.

6- Existence of weeds and other field crops werving as propagative hosts or reservoirs all year round.

7- Gaining new viruses and mixed infections.

INTRODUCTION

Tomato plants are grown all year round for local consumption and exportation. During the last 3 years yield decreased from 20 to 3 ton/hectare. Viral diseases infecting tomato were negligible and their epedimics were mainly restricted to tomato yellow leaf curl virus (TYLCV) as recorded by Nour-Eldin et al., 1969. Since then, TYLCV was known as the first causative agent behind the great losses (80%) of tomato yield especially in summer and fall. Mazyad et al., 1986, presented the role of the environmental variations in the epidemics of TVLCV, However, other viruses causing natural infections were unsurveyed except for the practical studies of Mazyad, 1966 and Allam et al., 1971.

During a survey conducted by the author in Ismaillya in August 1986, tomato fields were severely infected.

Since then and up till now, nation wide surveying of viral infections in tomato fields in undertaken. Deterioration in the [illegible] (Hussein under publication) and epidemiology of tomato viral diseases in Egypt.

METHODS

Surveying Viral infections:

Tomato fields were selected in 8 Governorates: Giza, El-Fayoum Beni Suef, Kaluobyia, Sharkyia, Monoufyia, Dakahlyia. Natural infections were recorded, estimated and sampled at different times between January-May, 1988-1989 1989.

Host range and pathogenicity of mechanically infected plants:

Mechanical inoculations, susceptability and symptomatology were performed on 23 plant species including, tobacco, tomato, beans, cucurbits and others. (Hussein, Under publication).

Serological Identification:

Antisera against cucumber mosaic virus CMV; ToMV; tomato bushy stunt virus, TBSV; tomato yellow leaf curl, TYLCV; tomato spotted wilt virus, TSWV; potato leaf curl virus, PLCV; potato vikus X, PVX; potato virus Y , PVY and alfalfa mosaic virus, AMV were obtained from ATCC, U.S.A. Hollan, France, New Zealand and Australia and some were raised in our laboratory. Double immuno-diffusion (Hussein & Sharaf 1987) was performed.

Electronmicroscopy:

Squeezed juice of infected leaves was negatively stained with 2% phosphotungstate and examined using Jeol 51 electron microscope.

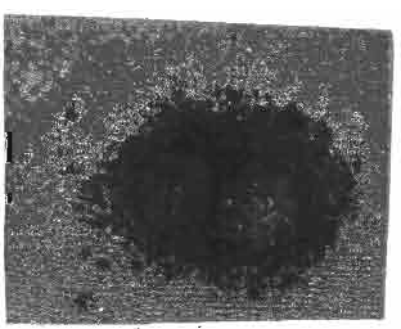
RESULTS AND CONCLUSIONS

Viral infections: Samples were taken 3 times per planting season and cumulative means of the data are shown in Table 1. High incident of infections was recorded in Ismaeilyia, Kaluobyia, Sharkyia and Giza.

Data from Table 2 showed that TYLCV ranks first, ToMV second and CMV third while other viruses were reported for the first time in Egypt. However, unknown viruses, and perhaps mixed viral infections, represent high incidences.

Figure-1 shows a twin particle of a gemini virus (TYLCV) from tomato sample collected from Belbis (Sharkyia).
Mean of the crop yiled was 10 and 3 tons per hectare for 1988 and 1989 respectively.
In some fields losses were 80-90%
The factors ;behind this crisis were:

1- The imported seeds (approximately of 206 cultivars and lines).
2- Highest infections were from seeds imported from U.S.A.; Holland, and Israel. Seeds from Denmark, France, Britain, and Japan were less susceptible to viral infections. Most infections were wither masked or latent.
3- Early infections affected the yield higher than later infections irrespective for the total percent of infected plants.



Electron micrograph of TYLCV

4- Numerous sources of foreign seeds including F_1 and F_2 of the hybrids.
5- Lack quarantine, laboratory testing and improper investigation of imported seeds and propagative stocks.
6- In most devastating severe infections contaminating hands were the first cause of epidemics in fields and green houses).
7- Neighboured infected fields especially in aphid- and whitefly-berne viruses.
8- Mixed infections, new viruses and unidentified viruses played a major role in severity and rate of viral epidemics.

Table 1: Incidence of viral infections in fall and spring plantings of 1988 and 1989.

Location	1988		1989	
	Fall %	Spring %	Fall %	Spring %
Giza	70	60	90	90
El-Fayoum	70	--	90	--
Beni Suef	50	70	80	80
Kalubyia	70	80	90	90
Monofyia	30	40	50	60
Sharkyia	80	80	80	80
Ismaeilyia	80	80	90	90
Dakahlyia	40	50	60	60

Table 2: Percentage of viruses causing epidemis as identified by antisera. Data are means of the two seasons within. 1988 and 1989.

Virus / Location	CMV	AMV	ToMV	TBSV	PVX	PVY	TYLCV	PLCV	UNKNOW
Giza	20	2	30	2	2	2	20	5	17
El-Fayoum	15	-	25	1	4	3	30	-	22
Beni Suef	17	-	11	-	-	-	35	15	22
Kalubyia	20	5	20	-	10	5	10	5	25
Monofyia	5	2	10	-	10	8	30	5	20
Sharkyia	25	15	25	-	1	4	20	-	15
Ismaielyia	20	20	10	2	5	5	20	3	15
Dakahlyia	10	3	20	5	15	10	23	2	12

Previous studies were focused on resistance and susceptibility of tomato cultivars (El-Hammady et al., 1976; Hassan et al., 1982; Nassar et al., 1982 and Mazyad et al., 1985. Few investigation were concerned with epidemiology (Mazyad et al., 1986).

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The health status of uncertified locally produced legume seeds from Egypt

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INTRODUCTION

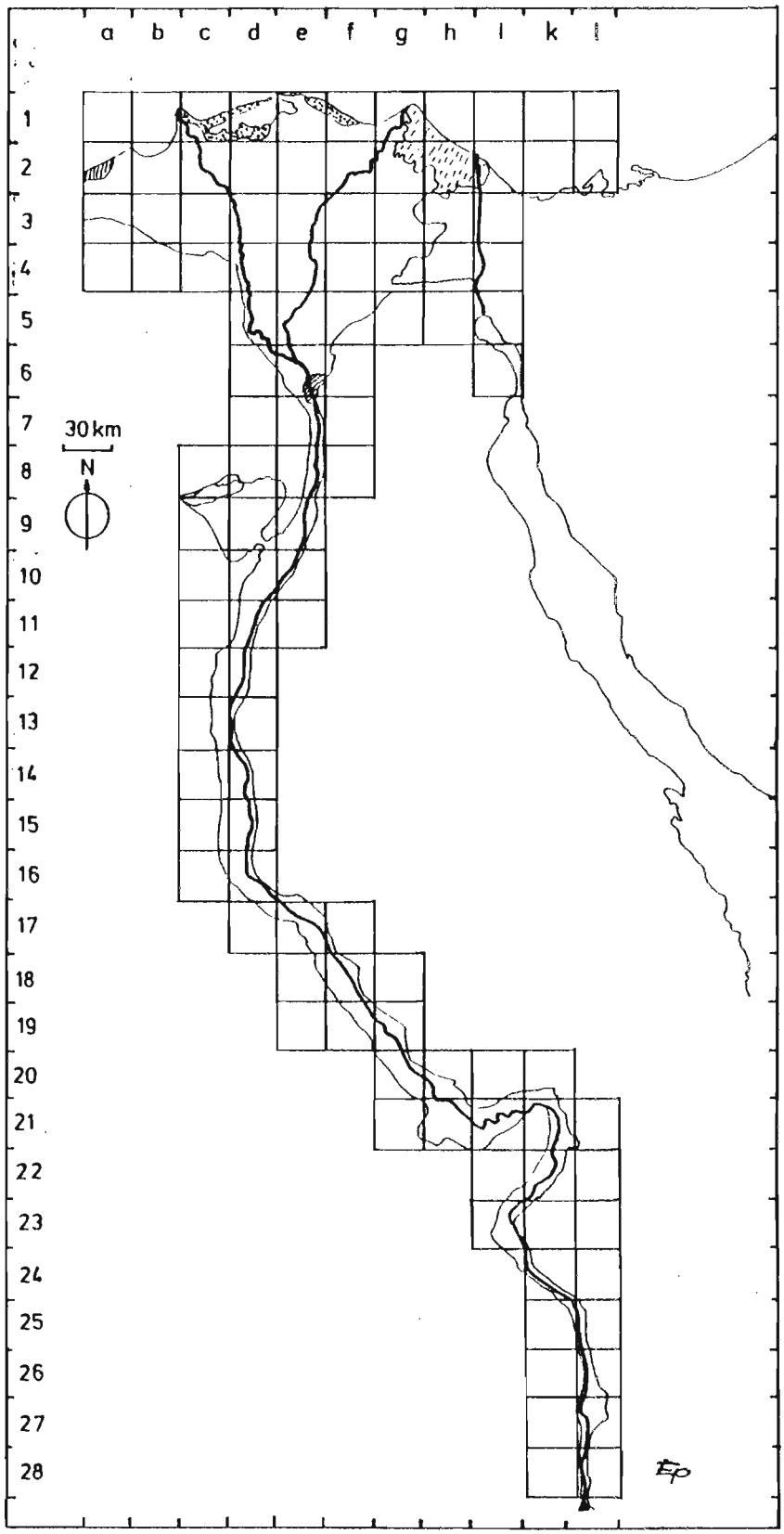
Seedborne virus diseases are a challenge to plant pathologists, because proper certification procedures allow to reduce the inoculum brought into the fields drastically, but interfere mostly with the traditional ways of seed production and distribution in many countries. To evaluate the amount of virus-contaminated seeds in Egyptian legumes, the agricultural area was subdivided by a grid into 30 x 30 Km squares (Fig.1)(Eppler & Kheder, 1988) and seed lots were bought on as many local markets as possible according to this grid. The seed-lots were brought to Germany and kept at about 8 C before plants were raised either under greenhouse conditions or in the field under insect-proof cages (in minimum 10 plants per lot). The plants were carefully checked for symptoms and then tested for virus-presence using indicator-plants and serological techniques which where either the traditional ELISA as described by Clark & Adams (1977) or the indirect ELISA-method described by Legrand (1986). The results presented were obtained using the latter two techniques. The antisera used originated either from the own laboratory or were provided by colleagues (G.Adam, O.W.Barnett; R.Koenig; L.Lange; G.I.Mink). The following viruses were tested for their presence: Bean yellow mosaic virus/pea mosaic virus (BYMV)(broad bean, cowpea and pea); Echtes Ackerbohnenmosaik Virus (EAM)(broad bean); broad bean stain virus (BBStV)(broad bean); bean common mosaic virus (BCMV)(french bean); pea streak virus (PStV)(pea); pea enation mosaic virus (PEMV)(pea); pea seed-borne mosaic virus (pea, lentil, chickpea); cowpea mild mottle virus (CopMMV)(cowpea, soybean); cowpea mosaic virus (CopMV)(cowpea, pea); grapevine fanleaf virus (GVFLV)(soybean); arabis mosaic virus (ArMV)(soybean); tobacco ringspot virus (TobRSV)(soybean); soybean mosaic virus (SoyMV)(soybean); cucumber mosaic virus (CuMV)(Frenchbean,cowpea). No experiments were made to localize the virus in the seeds and thus to differentiate true seed transmission, which is characterized by invasion of the embryo by the virus from virus contaminations of the seed providing inoculum to infect the germinating seedling and which might have been eliminated by a seed treatment, for example by washing.

RESULTS

Broad bean (*Vicia faba*)

Broad beans could be collected at 40 sites and 430 samples were tested. BYMV was present in 1.8% of the samples, EAMV in 4.8% and BBStV in 6.7% and the viruses were found at 7, 17 and 18 sites respectively (Eppler & Kheder, 1988). The four varieties Giza 2, Giza 3, Giza 402 and Romy did not show significant differences, the infection for BYMV ranged from 0% (Romy, Giza 3) to 2.3% (Giza 2), for EAM from 0% (Romy) to 6.3% (Giza 3) and for BBStV from 3.3% (Giza 402) to 8.8% (Giza 3).

Fig. 1: Sampling grid for legume seed lots in Egypt



French Bean (*Phaseolus vulgaris*)

French bean samples originated from 18 sites and belonged to three cultivars: Giza 3, Mont Calm and Balady. BCMV was found in samples from 12, CuMV in samples from 10 sites and the average values of infestation were 23 and 7% respectively.

Chickpea (*Cicer arietinum*)

The chickpea samples tested were out of lots collected at 12 sites and two cultivars could be identified (Giza 1, Shamy) while the others belonged to local types. PSbMV was detected in 6 seed lots and in average 5 % of the samples were shown to be infected. None of the different cultivars was free of PSbMV and the infestation rates did not differ significantly (Kheder & Eppler 1988b).

Cowpea (*Vigna sinensis*)
In case of cowpea seed 33 seed lots were collected at 30 sites and the seedlings tested for the presence of CopMMV, BYMV, CopMV and CuMV. Only the latter two were found to be present at levels of 33% and 4 % respectively. Four different cultivars were identified, Karem 7, Fatriaat, Black eye and a third of the samples belonged to local lines of the cultivar Balady which was infected with CopMV to a degree of 45% in average and up to 90% in individual seed lots. For the cultivar Fatriaat the situation was comparable with an average value of 40% while cv. Karem 7 and Black eye gave values of only 15% and 20% infection. Compared to these values the infestation with CuMV was only minor, reaching from 0% (Black eye), 1.1% (Fatriaat), 3.6% (Balady) to 10% (Karem 7).

Lentils (*Lens culinaris*)

Lentils were available at 12 sites and 160 samples were tested. Neither PEMV, BYMV and CuMV nor ToBSV and CopMV were found to be present, but PSbMV was found in 8% of the samples and at 8 sites. Only few seed-lots could be attributed to a particular variety, but both identified, Giza 9 and Giza 370 got the virus. In addition a TOBAMO-like virus was found in a sample originating from Middle-Egypt (Eppler *et al.* 1988).

Peas (*Pisum sativum*)

Pea seeds were collected at 12 sites belonging to four different cultivars, Mitcor, Little Marvel, Progress No.9 and the local cv. Balady. Pea mosaic virus (BYMV) and CopMV were not found in the plants raised but PStV was detected at a rate of 1.7%, PEMV at 4% and PSbMV was found in 24% of the samples tested. PSbMV was present in all cultivars with infection rates reaching from 5% (Balady) to 32% (Mitcor) (Kheder & Eppler 1988a).

Soybean (*Glycine max*)

Eleven soybean seed lots were collected at 11 sites and tested for the presence of SoyMV, TobRSV, CopMMV, GFLV and ArMV. Only ArMV and SoyMV were found to be present at rates of 3.6% and 14% respectively. SoyMV was found in samples from 6 seed lots, ArMV in one sample each out of 4 seed lots. The seeds could not be attributed to cultivars.

Tab. 1: The geographic distribution of seedborne viruses in legumes in Egypt

Legume	Virus	Present at (grids according to Fig.1)
Broad bean *Vicia faba*	BBStV	2b, 2c, 2e, 2f, 2g, 3c, 3e, 4e, 4h, 5d, 5e, 5f, 6e, 9d, 12d, 18e, 22k;
	EAMV	2c, 2f, 2g, 3c, 3e, 3g, 4d, 4e, 4f, 4g, 4h, 9d, 17e, 18e, 19g, 21i, 22k;
	BYMV	2b, 2g, 3c, 4e, 4h, 18e, 25l;
French bean *Phaseolus vulgaris*	BCMV	2b, 2d, 3b, 3c, 3d, 5d, 7e, 8e, 19g, 20g, 24i, 25k;
	CuMV	2b, 2d, 3b, 3c, 3d, 4g, 7e, 19g, 20g, 25k;
Chickpea *Cicer arietinum*	PSbMV	3b, 4g, 16e, 17e, 20g, 24i;
Cowpea *Vigna sinensis*	CopMV	2a, 2b, 2c, 2d, 2e, 2f, 3b, 3c, 3d, 3e, 4e, 4g, 4h, 5d, 5e, 5f, 7e, 8e, 9d, 9e, 10d, 12d, 15d, 16d, 17e, 19g, 24k, 25l;
Lentil *Lens culinaris*	PSbMV	2b, 4f, 4g, 17e, 18e, 21i, 23i, 28k;
Pea *Pisum sativum*	PSbMV	2b, 4f, 4h, 9d, 12d, 15d, 19g, 20g, 21i
	PEMV	2b, 12d, 13d, 15d, 28k;
	PStV	9d, 15d;
Soybean *Glycine max*	ArMV	2c, 4f, 6e, 13d;
	SoyMV	2c, 6e, 9e, 12d, 13d, 17e;

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CASSAVA FROGSKIN DISEASE

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Cassava frogskin disease (FSD) is a disease of unknown etiology which is graft transmitted and is suspected to be of viral origin. On most clones of cassava, the infected plants have distinctive symptoms in which the roots remain thin and fibrous with lesions forming on the peel. While many clones of cassava do not show symptoms on the leaves, there are clones which consistently show a mosaic on the leaves. The cassava clone, Secundina, shows mosaic symptoms both in the field and in the greenhouse and was used as the indicator plant for the field and whitefly transmission experiments. The disease can spread rapidly in the field with infection rates as high as 70% after one year. The rate of transmission varies according to location and appears to be related to the whitefly populations. A disease which produces mosaic symptoms on Secundina was transmitted by the whitefly Bemisia tuberculata collected from a field with a high incidence of FSD. The transmission of this mosaic disease has been confirmed using Bemisia tuberculata reared in cages on healthy cassava plants. Ten whiteflies per plant were given a 24 hour acquisition time on young cassava plants with mosaic symptoms and transferred to healthy plants for seven days. Approximately 10 percent of the plants developed symptoms within one month.

GENETIC VARIATION OF TOBAMOVIRUSES UNDER EPIDEMIC AND ENDEMIC SITUATIONS.

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Pepper Mild Mottle Tobamovirus (PMMV) causes severe epidemics in protected crops of peppers in SE Spain since the spread of cultivars carrying the L1-gene of resistance to other tobamoviruses. Field isolates were collected from 1980 till 1987 and their genomic heterogeneity and variation was studied by comparing their RNase T1 oligonucleotide fingerprints, a method widely applied to the study of variation of animal viruses, but not till now to that of plant viruses. The populations studied show a high genetic stability: divergence among types is very limited, and clear evolutionary lines cannot be established. The introduction in 1986 of cultivars with the L3-gene of resistance to PMMV does not induce changes in this pattern.

The variability of the U5-strain of tobacco mosaic virus, infecting endemically the wild solanaceous Nicotiana glauca Grah. in the same area, follows a very similar pattern. A major difference between PMMV and U5-TMV is the distribution of genotype frequencies: as has often been described for phytopathogenic fungi, a few genotypes are prevalent in epidemics on pepper, while a higher genetic diversity is found in the endemic populations on N. glauca.

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TRANSMISSION OF DIANTHOVIRUSES. C. Hiruki, Department of Plant Science, University of Alberta, Edmonton, Alberta, T6G 2P5, Canada.

The dianthovirus group consists of three viruses: the type member carnation ringspot virus (CRSV), red clover necrotic mosaic virus (RCNMV), and sweet clover necrotic mosaic virus (SCNMV). They infect a wide variety of plants and are readily propagated in Phaseolus vulgaris and Nicotiana clevelandii. The member viruses are transmitted through soil, and are released into the soil from infected plants, serving as a source of subsequent infection. The chytridiomycete fungus Olpidium radicale, and nematodes such as Xiphinema diversicaudatum, Longidorus elongatus and L. macrosoma have been implicated as the vectors of certain dianthoviruses. Insects have been suspected as natural aerial vectors but supportive evidence is lacking. In this paper, a critical appraisal of recent experimental data pertaining to the transmission of dianthoviruses will be presented.

VIRUS CONTENT OF SEED POTATO STOCKS PRODUCED IN A UNIQUE SEED POTATO PRODUCTION SYSTEM

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Potato seed production in the Mediterranean climate of Western Australia is unlike that found anywhere else in the world. The State is totally isolated by virtue of the Nullabor desert separating it from the rest of Australia, and has developed a self contained seed production scheme. The present system has virtually remained unchanged since the early 1920's with small family holdings providing all the top quality seed available for the State. A single cultivar Delaware, has been grown for 80 years almost to the exclusion of other varieties and still makes up 85-95% of the total crop. The seed potato industry is based at Albany in the southern coastal region, where sea breezes and a comparatively cool climate help maintain low aphid populations. The seed crops are grown in summer in the many small isolated swamps found along the coast in the Albany region. These swamps flood over the winter period, helping to kill volunteer tubers and affording some measure of control for weeds and soil pathogens. Cropping on these swamplands is continuous without any rotations, being totally reliant on the flooding capacity to control carryover problems. The Albany seed growers preserve an old fashioned system which does not employ the commonly accepted "flush through" methods for seed potato production where fresh virus tested seed stocks are continuously introduced to a seed scheme. Instead the seed is recycled by retaining a portion of the seed harvest and using it to plant in the swamps the following summer. There is no virus testing of any kind in this scheme, virus control being based solely on a seed certification system of visual inspections and roguing of severely symptomed plants and any volunteers escaping the flooding with a tolerance level of 2%.

To determine the consequences of 60 years of using this unique, unsophisticated seed production system on the content of viruses within seed stocks, a detailed survey of Delaware seed from the swamps of 14 growers was undertaken. 100 tuber samples from each were tested by ELISA for potato viruses X, S, Y, and leafroll. The majority of crops surveyed showed 95-100% infection with PVX and PVS. PLRV however was detected in only one sample at a low level. No PVY was detected in any samples. A further survey of winter plantings in the Perth metropolitan region, grown from Albany certified seed, was also conducted, and here a similar picture emerged with high levels of PVX and PVS, and no PLRV or PVY being detected.

Despite the lack of rotation and fresh input of clean seed, the simple system of inspection anf roguing adopted by these small growers is effective in controlling the aphid-borne viruses. The complete lack of PVY in the State may also be due in part to the favored practice of cutting large tubers for seed rather than planting smaller seed. In contrast the recycling of the same seed stocks over many years has resulted in almost complete infection with the contact transmitted viruses PVX and PVS, the inspection and roguing system being ineffective in controlling these. However despite the very high levels of PVX and PVS infections reasonable yields are still obtained, and the Albany scheme remains economically viable for Western Australian conditions.

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Effect of turnip mosaic virus and arabis mosaic virus on horse-radish yield

Horse-radish in Poland, is an important plant grown on nearly 2000 ha. Horse-radish is infected almost 100 % by turnip mosaic virus /TuMV/ by arabis mosaic virus /AMV/ about 20 % . Very aften both viruses infect the some plant. Information in the world about the influences of this virus on horse-radish yield are rare but in Poland there is none. Therefore, we conducted field experiments in 1987 and 1988.

The purpose of the first experiments was to investigate the effects of both viruses inoculated directly before transplanting cutting, 6 and 12 weeks later on the on, yield of horse-radish.

The experiments run in 4 replications using the split-plot system. In 1987, plants directly from meristem were used but 1988 cuttings were used which I obtained from healthy plant meristems./Data shown in table 1/.

TuMV caused a smaller number of plants to be obtained from cutting, about 25 % less, especially if infection come directly before transplanting. Infection TuMV before this date caused about 40 % total yield reduction. The yield from 1 plants of the total yield was lower too. This date of inoculation decreased yield in the root of second class, and number of cuttings. A slighty weaken effect on total yield, yield of roots second class the inoculation had 6 weeks after planting cuttings. Infection 12 week after planting has no effect on horse-radish yield.

The effect of TuMV and AMV on horse-radish yield cultivar Alpo /Meristem [illegible]/

The virus	Date in relation to the planting of cuttings	No of plants /63 cuttings were planted		Total yields in kg from plots /0,68 x 8,40/		Yield accor class from plots /average/		[illegible]	[illegible]
		1987	1988	1987	1988	1987	[illegible]	[illegible]	[illegible]
TuMV	no infected	61,8	42,5	27,0	22,1	2,69	[illegible]	[illegible]	[illegible]
	0	42,8	31,5	16,3	15,4	1,35	[illegible]	[illegible]	[illegible]
	6	59,8	41,5	21,0	19,5	0,9[illegible]	[illegible]	[illegible]	[illegible]
	12	61,5	35,5	28,1	21,4	1,79	[illegible]	[illegible]	[illegible]
Average		56,5	37,7	23,1	19,7	1,69	[illegible]	349,7	[illegible]
		1987	1988	1987	1988	1987	[illegible]	1987	[illegible]
AMV	no infected	61,5	38,0	28,7	22,3	3,39	[illegible]	[illegible]	[illegible]
	0	46,5	48,0	18,8	21,0	2,73	[illegible]	[illegible]	[illegible]
	6	62,5	40,5	23,8	19,0	2,44	[illegible]	[illegible]	[illegible]
	12	60,5	38,8	28,6	20,2	2,66	[illegible]	[illegible]	[illegible]
		57,7	41,3	25,0	20,6	2,80	[illegible]	[illegible]	[illegible]

AMV has less of an effect on the number of plants from planted cuttings but total yield decreased if infection come directly before transplanting, as yield of root second class, and number of new cuttings. Infection 6 weeks after planting has less of an effect on horse-radish yield, and 12 week after planting there was no effect on yield.

Generally, TuMV has a stronger effect on horse-radish yield than AMV /table 1/.

In the second field experiment, run using the rondom block system in 4 replications using root cuttings infected with TuMV, AMV, and TuMV, AMV together. Results of this experiments, suport the thesis that TuMV has a slighty larger effect on yield than AMV. Mixed infection of TuMV and AMV caused similar yield reduction as the weaken virus /table 2/.

Horse-radish mosaic virus /TuMV/ which has a lower concentration of virus in the roots, caused smaller yield reduction compared to the higher virus concentration in the roots /table 3/.

3 cultivars of horse-radish were compared; Alpo, Piotrkowski and Krenox /Alpo cuttings were obtained using the meristem technic/ Piotrkowski is slighty less susceptible to TuMV.

This data suggests that tolerance is possible to TuMV, based on the fact that Piotrkowski is a less susceptible cultivar and that cuttings with lower virus concentrations react better to virus infection.

The effect of TuMV, AMV and mixed infection on horse-radish yield

The virus	Total yield in kg from plots		Total yields from 1 plots		Yield 1 class from plots		No of cutting from plot	
	1987	1988	1987	1988	1987	1988	1987	1988
TuMV	22,8	7,2	0,47	0,29	2,89	1,35	27	17
AMV	21,2	7,7	0,45	0,26	2,62	0,90	18	20
TuMV + AMV	21,0	8,8	0,78	0,29	3,75	1,45	196	21

The effect of a small slight and heavy infections by TuMV

	Total yield in kg from plots		Total yields from 1 plots		Yield 1 class from plots		No of cutting from plots	
	1987	1988	1987	1988	1987	1988	1987	1988
slight	25,1	6,5	0,54	0,30	3,77	1,30	378	67
heavy	22,8	7,2	0,47	0,29	2,89	1,35	175	179

VIROIDES / VIROIDS

Viroids in Grapevines: Relationships, Factors Responsible for Widespread Dissemination, and Implications for Vine Growth and Performance

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INTRODUCTION

VIROIDS were first identified in the 1970's as the causal agents of plant disease. These subviral molecules can be readily transmitted to receptive plant species without producing any apparent host plant reaction which might be considered as an expression of "disease." It is possible, therefore, that the biological activity of this unique class of transmissible, small nuclear RNA molecules may be expressed by altering, within an acceptable range, a growth or developmental response of a "normal" plant. Supporting this proposition, viroids are distinct from viruses in their mode of replication, association with plant cells, and potentially disease expression.

The initial report of the existence of viroids in grapevines (Flores et al., 1985; Sano et al., 1985) was followed by the even more startling revelation that one or more of the three grapevine viroids (GV) described were virtually ubiquitous in commercial and Foundation plantings in California (Semancik et al., 1987). Even though the suggestion has been made that a viroid may be the causal agent of the yellow speckle disease (Flores et al., 1985; Koltunow and Rezaian, 1988), experimental verification for this relationship has not been accomplished and no association of the grapevine viroids with any disease syndrome has been established. Only GV-3 has been demonstrated to be a disease-causing agent in the alternate host cucumber where a reaction including leaf rugosity, internode shortening, and darkening of leaf color is noted.

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Viroid-free vines can be produced by culturing very small (0.1-0.15mm) shoot tips (Duran-Vila et al., 1988) and the grapevine viroids can be independently transmitted to these viroid-free vines by mechanical inoculation of purified nucleic acid extracts (Szychowski et al., 1988).

With this background, the study of grapevine viroids presents most intriguing questions concerned with the ubiquitous spread and the role of the viroids as, not only potential agents of disease, but even more interestingly, as nuclear RNA molecules which may influence those growth characters which define clonal variation and usage parameters, such as wine quality.

RESULTS AND DISCUSSION

Three grapevine viroids **(GV)**, designated as **GV-1**, **GV-2**, and **GV-3**, of about 371, 365, and 300 nucleotides, respectively, were detected by relative electrophoretic migration in 5% polyacrylamide gels under "native" and denaturing conditions in the presence of 8 M urea (Rivera-Bustamante et al., 1986) followed by silver staining.

Random-primed cDNA probes were made to electrophoretically purified grapevine viroid preparations. When hybridized against the three grapevine viroids electrotransfered onto nylon membrane directly from denaturing gel, only the homologous reactions were positive. This was true for viroids extracted from varieties of European origin grown in California for extended periods as well as for viroids from tissue recently collected in Europe. This indicated unique nucleotide sequences among the three grapevine viroids. A viroid of about the size of GV-3 and demonstrated to be related to the hop stunt viroid has been reported to occur in Japan (Sano et al., 1985).

The viroids were found widely-disseminated in commercial vines as well as Foundation plantings in California. Essentially, all grapevines tested carried at least one viroid, most contained two, and some, harbored all three. Patterns of distributions of the grapevine viroids could be found among wine and table grape varieties as well as in commercial rootstocks and rootstock germplasm sources (Table 1).

The three most recurrent viroid distribution patterns were detected principally in different classes of vines. Namely, the pattern, GV-1, -2, -3, predominated in table grape varieties, the pattern, GV-3, in rootstock selections, and the most common pattern, GV-1, -3, was characteristic of wine grape varieties. To test the proposition that perhaps the selective occurrence of the grapevine viroids has been influenced by factors such as usage and production practices, viroid-free vines have been established in field plots to evaluate the possible affect of the grapevine viroids on vine growth properties and performance.

Table 1. FREQUENCY IN THE PATTERNS OF DISTRIBUTION OF GRAPEVINE VIROIDS IN GRAPEVINES GROWN IN CALIFORNIA AND EUROPE

			Numbers of varieties analyzed		
GV-1	GV-2	GV-3	Wine	Rootstock	Table
+	-	-	1	0	0
+	-	+	50	16	9
+	+	+	5	1	7
-	+	+	0	2	0
-	-	+	6	8	1
-	-	-	0	1	0
* -	+	-			
* +	+	-			
		TOTALS	62	28	17

*Possible viroid combinations which have not been detected.

Evidence for extensive field spread through standard cultural practices or vector transmission have not been verified. The absence of a uniform distribution of the three grapevine viroids throughout an ancient crop dependant upon vegetative propagation and severe annual pruning, suggests a low level of field spread. Furthermore, vectors have not been implicated as a major factor in the spread of any viroid induced disease.

Therefore, the ubiquitous spread of the grapevine viroids has probably occurred through propagative transmission of homologous plant materials as well as from the mixing of heterologous scion/rootstocks combinations. Cultural practices characteristic of vine usage may also influence the spread of the grapevine viroids. Since table grapes and rootstocks are largely self-rooted, a conservative viroid pattern should be retained. The grafted scion/rootstock combinations common to wine grapes should produce the most uniform pattern of distribution. Both of these theoretical patterns would be complicated by the frequency in occurrence of field spread which at present is unknown.

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ANALYSIS OF RADIOACTIVE PROBES FOR THE DETECTION OF POTATO SPINDLE TUBER VIROID BY DOT-BLOT HYBRIDIZATION.

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Molecular hybridization of probes on plant sap spotted membranes is already a routine technique in many laboratories for the detection of viroids in plant extracts. We have analyzed the performance of different variants of this methodology by means of comparing the results obtained with probes synthesized in several ways for the detection of Potato Spindle Tuber Viroid (PSTV) in experimental hosts. Cloned PSTV cDNA sequences have been used as templates for the generation of radioactive probes, i) by nick translation of recombinant plasmids, ii) by labelling newly synthesized cDNA randomly primed, and iii) by run-off transcription of the PSTV cDNA and synthesis of radioactive PSTV RNA of both (+) and (-) senses. Results relative to the use of monomer versus dimer-lenght viroid probes will also be presented.

ds - RNA

Detection of double-stranded RNA in oilpalms as an indication for a virus infection

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Fatal yellowing or lethal spear rot (Turner 1981), a serious disease affecting oilpalms (Elaeis guineensis Jacq.), was observed in Brazil and other regions of South America during the last two decades.

At DENPASA (Dende do Para, S.A.) oilpalms Estate, Belém, Brazil, the progression of this disease became very rapid since 1985 causing the loss of about 10% of the plantation in 1988.

Neither the cause nor the vector of the disease are known until now. In 1988 Singh et al., detected RNAs with viroid-like behaviour on return-polyacrylamid-gels (Schumacher et al., 1986). These RNAs were found in leaves and roots of oilpalms with and without symptoms, but not in young healthy palms.

In our investigations leaves of each five diseased and symptomless oilpalms from the DENPASA plantation were analysed for the occurence of viroids by three different methods and led to the following results:

a) By 2 D-gel electrophoresis five to seven RNA-bands showed an extremely diminished electrophoretic mobility under denaturing conditions. Their migration was much slower than that of PSTV.
b) By return-gel electrophoresis these bands showed a behaviour - in accordance to the finding of Singh et al. - similar like PSTV. They were found in diseased and in older symptomless trees.
c) Northern blotting followed by hybridization with ^{32}P-labelled PSTV-transcripts did not show an indication for a homology between these RNAs and PSTV.

Because of their important behaviour in the 2D-gel electrophoresis samples were tested for the occurence of dsRNA. Using a sandwich-ELISA with dsRNA-specific monoclonal antibodies (Schönborn et al.) a positive signal appeared. After immunoblotting of the 2D-gel and the relating "return-gel" the RNA bands with viroid-like behaviour could be characterized as dsRNA. In addition, their double-stranded character could be confirmed by temperature gradient gel electrophoresis (Rosenbaum and Riesner, 1987).

It may be the subject of further investigations to prove if these dsRNAs are of viral origin or a response of the palm to an infection.

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A D D E N D U M

SCREENING HARD RED WINTER WHEAT CULTIVARS FOR RESISTANCE TO WHEAT STREAK MOSAIC VIRUS USING MONOCLONAL ANTIBODIES.

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Wheat streak mosaic virus (WSMV) is a member of the potyvirus group and is transmitted by the wheat curl mite, **Aceria tulipae** Keifer. The incidence of WSMV in the Great Plains states in the USA has increased in recent years. In 1988 losses were estimated at 5-10% with some areas reporting near total loss of the crop. No agronomically suitable hard red winter wheat cultivars have been developed with resistance to WSMV. Monoclonal antibodies (MABs) have been demonstrated to be useful in the identification of plant viruses and in the evaluation of plant material for resistance to plant viruses. Stable hybridoma cell lines secreting MABs to WSMV were produced by fusing spleen cells from BALB/c mice immunized with an isolate of WSMV from OK to mouse myeloma cell line P3x63Ag8.653. Hybridoma clones produced antibodies which reacted to WSMV in enzyme linked immunosorbent assay (ELISA) and Western blots. The purpose of this work was to develop and evaluate MABs for detection of WSMV and evaluate hard red winter wheat cultivars for reaction to inoculation with WSMV under field conditions.

The reaction of eight hard winter wheat cultivars to WSMV was determined by using symptom expression and a double antibody sandwich ELISA. Plants were inoculated using a DeVilbiss air gun at 60-75 psi in either the fall or spring. Inoculum was prepared from WSMV infected wheat seedlings ground in 0.1 M potassium phosphate (100 g tissue/ 1.5 L). Approximately 25 mL of the inoculum was applied to each 0.31 m row. Results from 2 years indicate that inoculation in the fall results in significant reductions in yield but not thousand kernel weight. Spring inoculation resulted in significant yield reductions from 4 of 6 cultivars in the first year, but no symptoms of WSMV infection developed in any of the 8 cultivars following the spring inoculation in the second year. Plants inoculated in the spring of the second year were nearly 2 weeks more mature at the date of inoculation which may have resulted in reduced infection. The cultivar 'Rall' showed reduced symptoms of WSMV infection and a lower titer of virus when measured by ELISA. This cultivar may be a useful parent in a program of breeding for resistance to WSMV.

BARLEY YELLOW DWARF IN PORTUGAL
II. INCIDENCE OF BYVD ISOLATES AND APHID-FLIGHTS IN THE SOUTH REGION.

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INTRODUCTION

The South of Portugal has been considered the cerealiferous region of Portugal. From time to time several epidemic occurrences in this area have been attributed to different causes including infection by barley yellow dwarf virus (BYDV). The symptoms observed covered from leaf discoloration in barley and wheat to purple in oats.

Aphid surveys showed the abundance of BYDV vectors (3). A PAV-like isolate, Plumb's "Type B", was detected in 1987 by Enzyme-linked immunosorbent assay (ELISA) in several cereals (2).

Identification of BYDV isolates and the distribution of cereal aphids in wheat fields of Alentejo are presented for the 1986-87 growing season.

METHODS

Aphid-survey. To obtain information about the aphid species predominant in wheat fields with BYDV symptoms, from February to June 1987 aphids were identified and counted. Aphids were collected every fortnight on two wheat fields, on plants from two parallel rows, one and ten meters distant from the edge. In each row five samples of twenty consecutive tillers, twenty meters apart, were collected and the aphids counted on a total of two hundred tillers.

ELISA. Direct ELISA was done at Rothamsted Experimental Station with two polyclonal IgG, Ky (a polyclonal antiserum that detects PAV + RPV strains, T. Pirone) and RES-F (MAV-like, R. Plumb). Indirect ELISA used the same specific polyclonal IgG and was done with monoclonal antibodies (ADAS, Harpenden Lab.) specific for PAV (Mac 91)-, RPV (Mac 92)-, and MAV (Maff 2)-isolates. Indirect ELISA done at Univ. Trás-os-Montes e Alto Douro used polyclonal IgG specific for PAV-, MAV-, and RPV-isolates (R. Lister) for coating and the same specific monoclonal antibodies as second antibody. Samples were tested in four replicate wells for Direct ELISA and duplicate wells for Indirect ELISA. Positive values were taken as greater than three times the healthy mean.

RESULTS AND DISCUSSION

Distribution of cereal aphids in wheat fields in Alentejo. The main BYDV vectors are present in Alentejo (Fig. 1). During the 1986-87 growing season *Rhopalosiphum padi* was the predominant aphid and was particularly abundant by February-March, a period of intense growth of the cereals sown at the previous Fall.

Sitobion sp. appeared only by April but were the predominant species by May. *Metopolophium dirhodum* was present from the end of March up to the beginning of May but in very small quantities. *R. maidis* and *Schizaphis graminum* were rare.

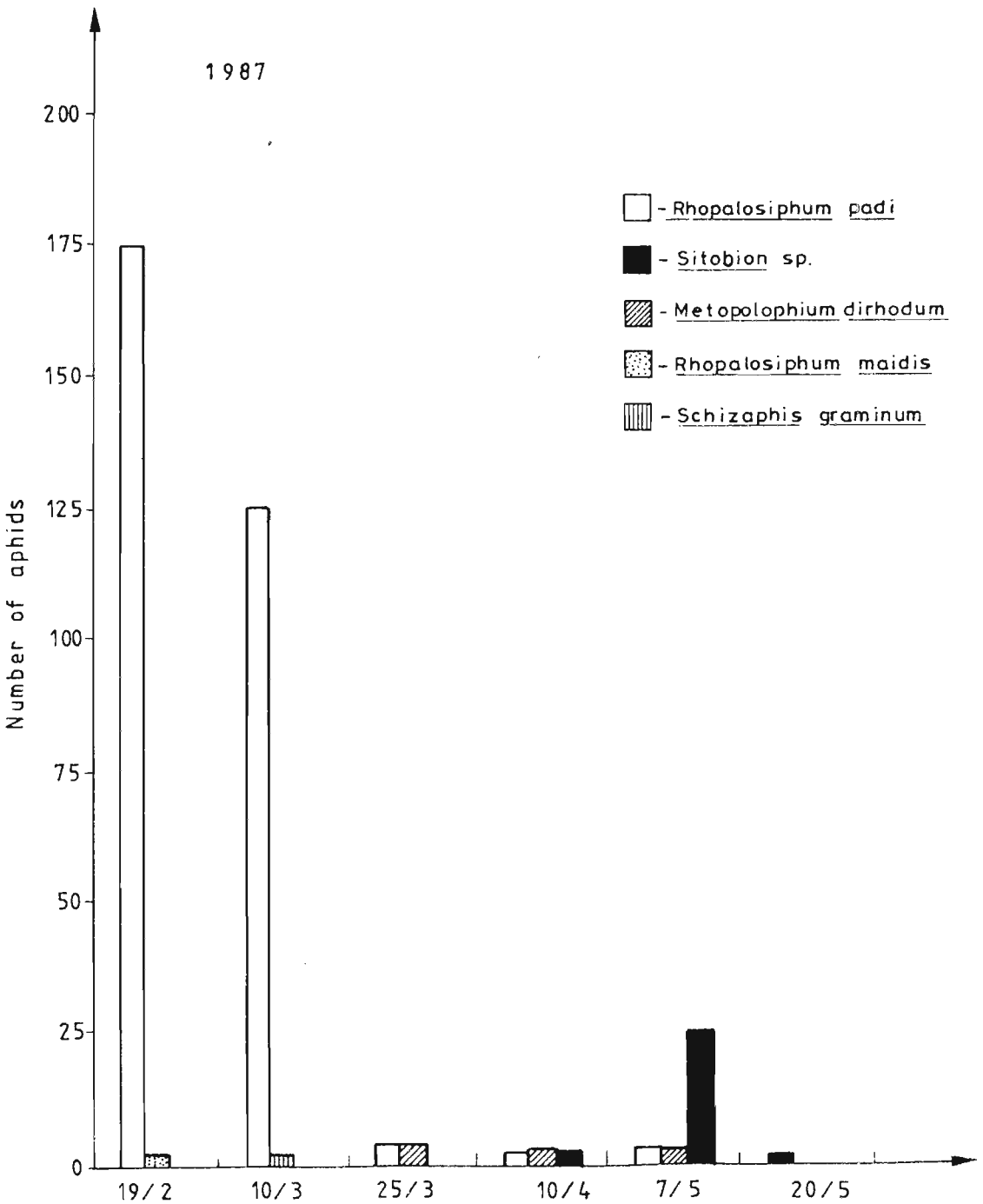

Figure 1. Distribution of the main BYDV vectors in wheat fields in Alentejo for the 1986-87 growing season.

BYDV isolates in cereal fields as indexed by ELISA. Indirect ELISA with specific monoclonal antibodies gives a more complete information about BYDV infection as compared with Direct ELISA with polyclonal antisera (Table 1). Most of the plants showing typical BYDV symptoms were indexed as positive by ELISA. Of the 15 wheat samples harvested by April 1987 in Alentejo, 12 were infected. Nine samples were of PAV alone, one of RPV alone and two mixture of PAV + RPV. The MAV-isolate was not detected. These results are in agreement with the predominance of *R. padi* by February-March 1987 (Fig. 1).

Samples collected by April 1989 in Oeiras (near Lisboa) showed the presence of the MAV-isolate in mixture with PAV and RPV in several oat cultivars (Table 2).

TABLE 1. CHARACTERIZATION OF BYDV ISOLATES IN WHEAT, COLLECTED IN APRIL 1987, AT DIFFERENT LOCATIONS IN ALENTEJO

WHEAT SAMPLES	DIRECT ELISA		INDIRECT ELISA			SEROTYPE
	Polyclonal antisera		Monoclonal antibodies			
	Ky	RES-F	Mac91	Mac92	Maff2	
#1	0.037*	0.051	0.317	0.007	0.008	PAV
#2	0.126	0.049	0.131	0.098	0.011	PAV+RPV
#3	0.035	0.049	0.296	0.003	0.003	PAV
#4	0.188	0.049	0.043	0.115	0.006	PAV+RPV
#5	0.029	0.045	0.199	0.007	0.005	PAV
#6	0.033	0.062	0.036	0.006	0.003	PAV
#7	0.034	0.064	0.002	0.001	0.003	
#8	0.014	0.045	0.002	0.000	0.003	
#9	0.024	0.061	0.030	0.006	0.004	PAV
#10	0.029	0.055	0.031	0.003	0.004	PAV
#11	0.106	0.051	0.021	0.031	0.011	RPV
#12	0.028	0.050	0.046	0.003	0.012	PAV
#13	0.010	0.055	0.001	0.007	0.003	
#14	0.025	0.043	0.049	0.008	0.004	PAV
#15	0.017	0.041	0.129	0.006	0.010	PAV
Positive controls						
B (PAV+RPV-like)	0.715	0.125	1.353	1.062	0.003	
F (MAV-like)	0.011	0.344	0.004	0.004	0.613	
R-568(RPV-like)			0.022	1.313	0.014	
Negative controls						
Buffer	0.021	0.047	0.008	0.004	0.005	
Dry healthy mean	0.035	0.075	0.010	0.005	0.006	
S.D. (n=8 D.ELISA and +42 I.ELISA)	0.011	0.016	0.007	0.004	0.003	
3x Healthy mean	0.105	0.225	0.030	0.015	0.018	

* Values are means of 4 replicate wells for Direct ELISA and duplicate wells for Indirect ELISA.
Positive values were taken as greater than 3 times the healthy mean.
ELISA done at Rothamsted Exp. Sta.

TABLE 2. CHARACTERIZATION OF BYDV ISOLATES IN OATS, COLLECTED IN APRIL 1989, IN OEIRAS.

OAT CULTIVARS	NUMBER OF SAMPLES	ELISA POSITIVE FOR*: PAV+MAV+RPV	PAV+RPV
Maris Osprey	6	2	4
Pergúla	6	0	6
Streckenthiner II	6	1	5
Endress Weisshoper	6	0	6
"dos Arcos"	6	0	6
Maris Tabord	6	0	6

* Leaves and roots of dry samples were tested in duplicate by Indirect ELISA. One-gram samples were powdered in liquid nitrogen and homogeneized in 10 ml 0.01 M phosphate buffered saline, pH 7.4, with 0.05 % Tween 20 and 2% PVP (MW 40.000). Indirect ELISA using rabbit polyclonal Ig from P.PAV, RPV(2) and MAV antisera (R. Lister, Purdue Univ., USA) for coating and rat monoclonal antibodies Mac 91, Mac 92 and Maff 2 (from ADAS Harpenden Lab., England) as second antibody.

Taking together with the BYDV isolates detected in the North of Portugal (Trás-os-Montes) (1) these preliminary surveys indicate that besides annual variation, the incidence of MAV may increase from the South (Alentejo) to the North of the country (Trás-os-Montes).

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Transmission and Epidemiology of Maize White Line Mosaic Virus. T. A. Zitter and L. Zhang, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Maize white line mosaic virus (MWLMV, 35 nm diam.) and associated virus-like particles (17 nm diam.) were first reported in the United States in sweet and dent corn fields located near Ithaca, NY in 1979. The disease occurred in the state during the following 7 seasons but symptomatic plants were not found in either '87 or '88. The soilborne nature of the disease was demonstrated in field and greenhouse experiments, but no vector has been identified. Although not transmitted mechanically using conventional techniques, wheat and corn seedlings were infected when purified virus was added to wounded embryos. No differences in susceptibility to the virus was found among 11 sweet corn varieties with three different genes (sh 2, su, se) encoding sugary phenotypes. The virus-like particles associated with MWLMV could not infect plants without MWLMV and hence are a satellite virus of MWLMV (SV-MWLMV).

INTRODUCTION

Maize white line mosaic virus (MWLMV) was first reported in the United States in 1979 in sweet and dent corn fields located near Ithaca, NY.

The virus has since been identified in seven additional states. Diseased corn plants show bright mosaic symptoms consisting of white to yellow-green rectangular to linear patches and stripes on leaves. The diseased plants are severely stunted and bear few ears.

The soilborne nature of the disease was demonstrated in field and greenhouse experiments, but no vector has been identified. MWLMV cannot be transmitted to other corn plants by conventional techniques or by insects. This feature makes it very difficult to study this disease.

Our work shows that MWLMV can be transmitted by adding purified virus to wounded corn embryos. MWLMV-infected corn plants will grow from these inoculated seeds.

MATERIAL AND METHODS

Eleven sweet corn varieties with three sugary gene types (su, sh2 and se) were selected and inoculated by the following wounding technique in order to identify their susceptibility to MWLMV. The varieties 'Sunbeam' and 'Silver Queen' were used in preliminary experiments.

To identify the host range of MWLMV among cereal crops, rice, wheat, sorghum, millet, oat and barley seeds were also inoculated with MWLMV by this technique.

Virus was purified according to the procedure of G.A. de Zoeten and then diluted to 5mg/ml with 0.02 M phosphate buffer (pH 7.0) before it was used as the inoculum. Corn seeds were soaked in water for 4 hours at room temperature and were dried by paper towels. One drop (about 2 ul) of the virus preparation was placed on the embryo side of each seed. One or two cuts were made through the drop of the virus preparation into the corn embryo so that the virus could enter through the wound. The wounded and inoculated seeds were then planted in autoclaved greenhouse soil and kept in the greenhouse under natural day light. Wounded seeds inoculated with buffer served as controls.

RESULTS

Wounded and inoculated corn plants usually showed typical MWLMV disease symptoms on leaves as soon as they emerged from the soil. The infection rate varied among experiments and depended upon how the technique was performed and the percent of seed that germinated.

The pre-soaking time for corn seeds was tested for 0, 0.5, 1, 2, 4, 8, 12, and 24 hours before wounding and inoculation. The highest infection rate was obtained with 4 hours pre-soaking (Table 1).

Once seeds were inoculated, they could be held for as long as 64 days at room temperature with the seed still remaining infectious (Table 2).

Eleven varieties of sweet corn with three different sugary gene types (su, sh2 and se) showed no obvious difference in their susceptibility to MWLMV (Table 3). Corn seedlings infected by wounding and showing typical MWLMV symptoms appear in Figure 1.

Other corn viruses such as maize dwarf mosaic virus and maize subtle mosaic virus could also infect corn seeds using this technique.

This wounding technique could also be applied to corn that already germinated. Results showed that MWLMV when applied to the embryo area could infect corn seedlings even if they had germinated for 5 days and were about 5 cm in length (Table 4).

Among wounded cereal crop seeds, corn and wheat were infected by MWLMV. These results were confirmed by both ELISA and cDNA hybridization techniques. MWLMV-infected wheat plants showing typical mosaic symptoms are shown in Figure 2.

CONCLUSIONS

Our work shows that MWLMV can be transmitted by wounding and inoculating its host plant seed embryos. Virus can remain infectious in dry seed for an extended period of time.

Using this technique, we found that beside corn, wheat was also susceptible and confirms an earlier report of natural field infection of wheat.

Sweet corn varieties differ in their susceptibility to MWLMV under natural field infection, depending somewhat if they are early maturing varieties or produce weak seedlings especially among certain sugary phenotypes. No apparent difference in susceptibility to MWLMV by wound inoculation was found among 11 sweet corn varieties with three different genes (sh2, su and se) encoding sugary phenotypes.

The wounding technique has allowed us to pursue other studies on MWLMV. We are able to maintain a continuous supply of MWLMV, which has enabled us to demonstrated the satellite nature of the virus-like particles associated with MWLMV (SV-MWLMV). We have also established a testing system, by which some possible vectors of MWLMV are being tested.

Table 1. Different pre-soaking time on MWLMV infection of 'Sunbeam' by wounding technique

PRE-SOAKING (HOURS)	SEEDS TESTED	GERMINATION NO.	%	DISEASED NO.	%
0	50	11	22	1	9
0.5	50	11	22	1	9
1	50	14	28	2	14
2	50	18	36	3	17
4	50	18	36	5	28
8	50	25	50	2	8
12	50	8	16	0	0
24	50	14	28	0	0

Table 2. Length of time that 'Sunbeam' corn seeds remained infectious after inoculation with MWLMV and held at room temperature

TIME LENGTH (DAYS)	SEEDS TESTED	GERMINATION NO.	%	DISEASED NO.	%
0	50	19	38	2	11
1	50	14	28	5	36
2	50	19	38	4	21
4	50	14	28	9	64
8	50	21	42	17	81
16	50	36	72	14	39
32	50	27	54	12	45
48	50	30	60	6	20
64	50	23	46	11	48

Table 3. The infection of 11 sweet corn varieties (50 seeds each) with three sugary gene types (su, sh2 and se) inoculated with MWLMV by wounding technique

VARIETY	GENE TYPE	GERMINATED		DISEASED	
		NO.	%	NO.	%
Silver Queen	su	46	92	16	35
Sweet Sue	su	41	82	5	12
Sunbeam	su	36	72	14	19
Zenith	sh2	43	86	2	5
Landmark	sh2	32	64	5	16
Seneca Sentry	se	34	68	7	21
Snow Bell	se	23	46	3	13
Spring Calico	se+su	36	72	8	22
Pennfresh ADX	-	24	48	2	8
Hawaiian Super Sweet #9	sh2	30	60	3	10
Sprite	su	30	60	8	27
Sunbeam (not inoculated)	su	37	74	0	0

Table 4. Inoculation of corn seedlings by wounding technique

TIME LENGTH (DAYS)	SEEDLING LENGTH	SEEDS TESTED	GERMINATED		DISEASED	
			NO.	%	NO.	%
0	0 mm	40	26	65	11	42
1	2 mm	36	35	97	4	11
2	2 cm	36	30	83	0	0
3	5 cm	36	32	89	1	3
4	10 cm	36	22	61	0	0

TRANSMISSION OF SWEET CLOVER NECROTIC MOSAIC VIRUS

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INTRODUCTION

Sweet clover necrotic mosaic virus (SCNMV), a member of the dianthovirus group, possesses a bipartite genome and the following properties (5): virions contain positive sense single-stranded genomic RNAs of approximate molecular weights (Mr) 1.35 x 10^6 (RNA-1) and 0.55 x 10^6 (RNA-2) and a coat protein of Mr 38 x 10^3. The particles measure 33 nm in diameter and sediment at 126 S as a single component. It has a moderate host range, but causes characteristic necrosis, both local and systemic, often associated with severe stunting, which is a potential cause of reduction in crop yield. Research on modes of transmission is scarce. The virus is released into the soil from infected plants and transmitted to neighbouring plants (4). Insects have been suspected as natural aerial vectors (4) but supportive evidence is lacking. This paper reports recent experimental data analysing the possible role of the western flower thrips Frankliniella occidentalis which is found in constant association with pollen grains in the florets of sweet clover plants both in the field and in the glasshouse.

MATERIALS AND METHODS

Virus and virus purification. The SCNMV type strain 38 was originally isolated from sweet clover (Melilotus officinalis) in 1979 and has been maintained in the same host. For purification the virus was multiplied in Phaseolus vulgaris 'Red Kidney' and purified according to the method previously reported (2).

Growth conditions. All plants, unless stated otherwise, were grown in 12-cm-diameter pots containing an autoclaved mixture of loam, sand, and peat (1:1:1, v/v/v) in a glasshouse at 25 $\pm$ 2°C.

Inoculation and virus assay. Crude juice was obtained by grinding infected leaves with a mortar and pestle in the presence of 0.025 M phosphate buffer (Na_2HPO_4 - NaH_2PO_4), PH 7.0 at a fixed ratio. The extract was rubbed on leaves dusted with Carborundum. For local lesion assay, young seedlings of Chenopodium quinoa were used at growth stages of 4 to 8 uniform leaves.

Pollen and anther preparation. Pollen grains and anthers were collected from selected flowers of infected and virus-free sweet clover (M. alba) plants maintained in 24-cm-diameter pots, by tripping the flower under a stereomicroscope.

ELISA. Direct double sandwich enzyme-linked immunosorbent assay was used for SCNMV detection as described previously (7).

Thrips. F. occidentalis, the western flower thrips, originally obtained from M. alba was reared on SCNMV-infected or on virus-free sweet clover in separate plant growth cabinets or separate glasshouse compartments. Adults and first and second instar nymphs were used either separately or as mixed populations.

RESULTS

Detection of SCNMV in extracts of pollen and of thrips. To test whether pollen grains released from anthers of sweet clover infected with SCNMV contain the virus, young healthy seedlings of C. quinoa were inoculated with aliquots of extracts from pollen samples or from thrips samples in three replicates. Other aliquots of both pollen and thrips samples were used for ELISA. Results of the experiments, summarized in Table 1, show that pollen samples from virus infected sweet clover plants contained high concentrations of infective SCNMV, whereas those from healthy plants did not. The concentration and infectivity of SCNMV from extracts of viruliferous thrips remained relatively low.

Table 1. Detection of SCNMV antigen and infectivity in extracts of pollen and of western flower thrips that emerged from SCNMV-infected sweet clover in the glasshouse.

Experiment	Pollen extract from[a]		Extract from[a]	
	Infected Sweet Clover	Healthy Sweet Clover	Viruliferous thrips	Nonviruliferous thrips
	>2.000	0.153	0.393	0.007
ELISA	>2.000	0.073	0.258	0.017
(A405)	>2.000	0.098	0.644	0.003
Local lesions[b]	55	0	1	0
on C. quinoa	29	0	8	0
	33	0	9	0

a, Pollen grains from 30 florets per pollen sample or 10 thrips nymphs per thrips sample were tritulated in 0.025 M phosphate buffer, pH 7.0, and then the final volume of 100 µl, made up with extraction buffer, was divided into two aliquots, one for ELISA and the other for local lesion assays.
b, The figures represent average numbers of local lesions on 3 leaves.

Upon examination, using scanning electron microscopy, of thrips which had emerged from SCNMV-infected plants, pollen grains were found firmly attached to the surfaces of the thrips (results not shown).

Detection of SCNMV in washings of pollen grains. Pollen collected from the flowers of sweet clover plants that had been experimentally inoculated and kept in the glasshouse for several months was washed three times. High concentrations of SCNMV were found in the first washings, significantly lower amounts in the second washings, and none in the third washings (Fig. 1). Similar results were obtained when the experiment was repeated with pollen samples collected from sweet clover plants that were infected naturally in the field (Fig. 2).

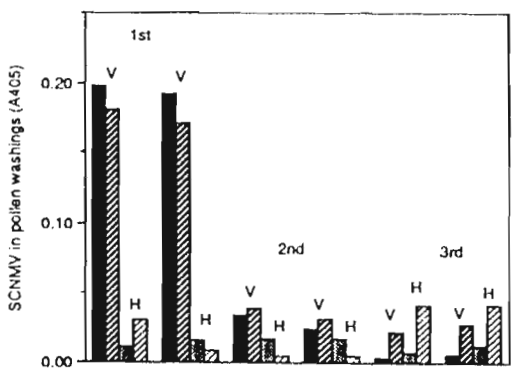

Figure 1. Detection of SCNMV by ELISA in pollen washings from sweet clover experimentally inoculated in the glasshouse.
Pollen grains from 100 virus-infected florets were placed on a Millipore filter (0.22µm) and washed using 1.2 ml extraction buffer (first washing) and then washing was repeated in the same way to obtain second and third washings. Pollen grains from 100 virus-free florets were subjected to the same treatment as controls. V, virus-containing pollen grains; H, virus-free pollen grains.

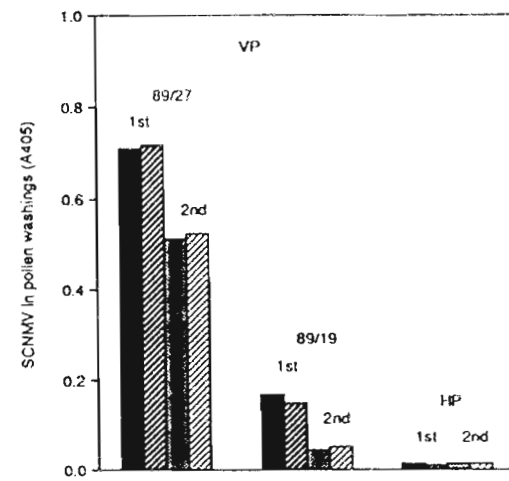

Figure 2. Detection of SCNMV in pollen washings from naturally infected sweet clover in the field.
Pollen grains from 20 florets of field samples 89/27 and 89/19 were washed using 0.8 ml extraction buffer at a time to obtain first or second washing as before. A comparable test was done with pollen grains from virus-free plants. VP, virus-containing pollen grains. HP, virus-free pollen grains.

F. occidentalis as the vector of SCNMV. Thrips recovered from naturally infested sweet clover (M. officinalis and M. alba) both in the field and in the glasshouse were identified as F. occidentalis. In a series of surveys, the thrips were found in constant association with florets of sweet clover.

Twelve experiments were carried out using 3 to 4 young seedlings per treatment: a) thrips only, b) virus-containing pollen only, c) thrips and virus-containing pollen, and d) no treatment (control). Each treatment was done in a sealed Petri-dish at room temperature for 24 hours, following which the transplanted seedlings were kept in the glasshouse for 4-6 weeks prior to being tested for SCNMV using the ELISA technique. None of the experiments showed transmission of SCNMV by the thrips.

In another series of experiments both a mature, healthy sweet clover plant and a plant that had been mechanically inoculated with SCNMV were fully infested with thrips and then they were caged with young seedlings of sweet clover test plants for a period of 7 weeks. Assays for SCNMV infection at the end of incubation showed that none of the test plants was infected with SCNMV. In an additional experiment using 8 sweet clover plants with floral buds which were ready for blooming, no SCNMV transmission was detected after a 4-week exposure to a mixed population of thrips from a SCNMV-infected sweet clover plant.

DISCUSSION

F. occidentalis is known as an efficient pollinater and several individuals often occur in each floret, and, in contrast to bees, they can penetrate a flower bud before it opens (6). Thus, they are exposed to pollen for a longer time. In this investigation, up to 80 florets were found on a single floral stalk of an average sized, potted sweet clover plant in the glasshouse (data not shown). Since numerous floral stalks develop on a single sweet clover plant, the population level of F. occidentalis is quite high. During our observation, many thrips were found to be carriers of pollen grains which in turn carry virus particles. Pollen samples collected from virus-infected plants both in the field and in the glasshouse contained high titres of SCNMV antigen that is highly infectious (Figs. 1 and 2). However, the concentration of SCNMV detected in the extract of viruliferous thrips was not high enough to suggest replication of the virus in the thrips (Table 1). Also, our results did not show any evidence of virus transmission by the thrips. Rather, our observations tended to show that sweet clover pollen grains are surface-contaminated with the virus in a similar manner to that reported previously (1,3), and transmission of SCNMV to sweet clover plants is probably caused mechanically (1) when a significant amount of viral inoculum is released from the pollen grains of virus-infected plants. Therefore, the mode of transmission does not resemble that of tobacco streak virus, in which Thrips tabaci transmits pollen-borne virus (8). Further investigation is necessary to determine whether SCNMV is transmitted by thrips from floret to floret.

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