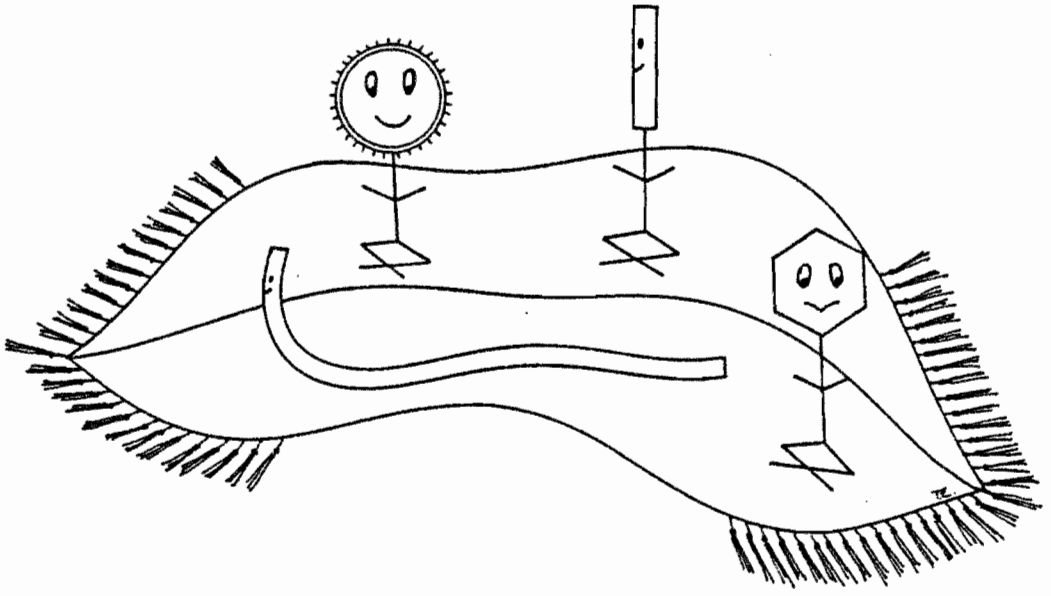
PROCEEDINGS OF
THE WORKSHOP ON EPIDEMIOLOGY
OF PLANT VIRUS DISEASES



ORLANDO, FLORIDA

August 6-8, 1986

PROGRAM

WEDNESDAY (6 August)

SESSION I — Effect of Resistance on Virus Epidemiology

8:00- 8:45 Registration, Examine posters for Session I

8:45- 9:05 Opening - Local Arrangements- Steve Garnsey (Florida)
Introduction of Speakers - O. W. Barnett (South Carolina)

9:05- 9:25 Speaker - A. T. Jones (Scotland)
Vector Resistance as a Means of Virus Control, Prospects and Problems

9:25-10:15 Discussion - Jim Moyer (North Carolina) moderator

SESSION II — Epidemiology of Thrip, Pollen and Seedborne Viruses

10:15-10:55 Examine Posters for Session II and Coffee

10:55-11:15 Speaker - G. I. Mink (Washington)
Pollenborne Viruses

11:15-12:00 Discussion - L. Lange (Denmark) moderator

12:00- 1:30 LUNCH

SESSION III — Control of Virus Spread

1:30- 2:10 Examine Posters for Session III

2:10- 2:30 Speaker - R. W. Gibson (England)
Chemical Control of Aphidborne Viruses

2:30-2:50 Special Topic - J.B. Kring (Florida)
Aphid Repellents and Attractants

2:50-3:30 Discussion - T. P. Pirone (Kentucky) moderator

SESSION IV — Quantitative Epidemiology

3:30- 4:10 Examine Posters for Session IV and Coffee

4:10- 4:30 Speaker - P. M. Burrows (South Carolina) Nearly Unbiased Estimation of Nonlinear Prevalence Functions

4:30- 5:10 Discussion - W.G. Ruesink (Illinois) moderator

7:00-10:20 Evening Social, Oasis A&B

THURSDAY (August 7)

SESSION V — Epidemiology of Leafhopper and Mirid Transmitted Viruses and Virus-Like Disease Agents

8:00- 8:45 Examine Posters for Session V

8:45- 9:05 Speaker - H. Hibino (Philippines)
Epidemiology of Rice Tungro

9:05- 9:25 Special Topic - J. W. Randles (Australia) Myndus taffini and Foliar Decay Disease of Coconut in Vanuatu

9:25-10:05 Discussion - P. E. Thomas (Washington) moderator

SESSION VI — Epidemiology of Viruses Nonpersistently Transmitted by Aphids

10:05-10:45 Examine Posters for Session VI and Coffee

10:45-11:05 Speaker - Ben Raccah (Israel)
Role of Flying and Colonizing Aphid Species in Spread of Nonpersistent Viruses in Annual Crops

11:05-11:45 Discussion - M. A. Conti (Italy) moderator

11:45- 1:00 LUNCH

1:00- 7:00 Tour of Demonstrations at Leesburg Agricultural Research and Education Center

Pierce's Disease of Grapes,

Cucurbit Virus Research,

Florida Research Programs

FRIDAY (August 8)

SESSION VII — Epidemiology of Whitefly-Transmitted Viruses

8:00- 8:45 Examine Posters for Session VII

8:45- 9:05 Speaker - C. Fauquet (Ivory Coast)
A Summary of the Epidemiology of African Cassava Mosaic Virus

9:05- 9:45 Discussion - S. Cohen (Israel) moderator

9:45-10:05 Special Topic - Keith Hendrie (Illinois) Meteorology and Plant Virus Epidemiology

10:05-10:20 Minidiscussion

SESSION VIII — Non-Insect Transmission of Plant Viruses

10:20-11:00 Examine Posters for Session VIII and Coffee

11:00-11:20 Speaker - E. E. Banttari (Minnesota)
Unusual Sources and Methods of Dispersal of Plant Viruses

11:20-12:00 Discussion - M. B. von Wechmar (South Africa)

12:00- 1:30 LUNCH

SESSION IX — Epidemiology of Viruses Persistently Transmitted by Aphids

1:30- 2:10 Examine Posters for Session IX

2:10-2:30 Speaker - G.R. Johnstone (Australia)
Epidemiology of Viruses Transmitted Persistently by Aphids

2:30- 3:10 Discussion - E. S. Sylvester (California) moderator

SESSION X — Diagnosis of Virus Diseases

3:10- 3:50 Examine Posters for Session X and coffee

3:50-4:10 Speaker - B. D. Harrison (Scotland)
Impact of Biotechnical Techniques on Plant Virus Epidemiology

4:10- 4:50 Discussion - J. E. Duffus (California) moderator

SUMMATION AND BUSINESS MEETING

4:50- 5:30 J. M. Thresh (England) Principles of Plant Virus Epidemiology

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 and Carowa, Australia 25-27 August 1983. The Committee has been chaired by Michael Thresh since its conception.

Committee members from 1983 to 1986 have been:

J. M. Thresh, chair, East Malling Research Station, UK

C. Fauquet, ORSTOM, Ivory Coast

R. Kisimoto, Mie University, Japan

R. Gamez, Universidad de Costa Rica, Costa Rica

O. W. Barnett, Clemson University, USA

The local organizing committee for this workshop included:

O. W. Barnett, Clemson University, SC, Program Chairman

Warren Adlerz, University of Florida Agricultural Research Center, Leesburg, Local Arrangements Chairman

Steve Garnsy, Horticulture Research Laboratory, Orlando

Don Hopkins, University of Florida Agricultural Research Center, Leesburg

Jim Tsai, University of Florida Agricultural Research Center, Ft. Lauderdale

Jim Duffus, USDA-Agricultural Research Station, Salinas, CA

Mike Irwin, University of Illinois, Urbana

The following sponsored this workshop:

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Department of Plant Pathology and Physiology, Clemson University, Clemson, SC

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International Society of Plant Pathology

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.

Minor editorial changes were made by the Program Chairman. Appreciation is expressed to Kathryn P. Harrell and Maria T. Zimmerman for their reproduction and editorial expertise.

IN MEMORY OF

DR. WARREN C. ADLERZ

1928-1986

Dr. Adlerz, an entomologist, conducted research on virus-vector relationships, virus ecology, and virus disease epidemiology. He was especially interested and knowledgeable concerning virus diseases of vegetable crops, particularly cucurbits. Warren was a member of the Entomological Society of America and the American Phytopathological Society, and was an active participant in the two previous meetings organized by the Plant Virus Disease Epidemiology Committee of the ISPP. Until his sudden death in July, he was Local Arrangements Chairman for this workshop. Without his dedication, organizational skills, and attention to detail this meeting would not have been possible.

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SESSION VII - EPIDEMIOLOGY OF WHITEFLY-TRANSMITTED VIRUSES

SESSION VIII - NON-INSECT TRANSMISSION OF PLANT VIRUSES

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VECTOR RESISTANCE AS A MEANS OF VIRUS CONTROL: PROSPECTS AND PROBLEMS

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A wide range of variation in resistance to the vectors of viruses exists in plants and only now is this beginning to be explored to any significant extent as a means of virus control. This is not surprising because the study of interactions between virus, vector and host plant are complex and progress in such studies generally requires close collaboration between virologists, entomologists/mycologists/nematologists, and plant breeders. In the past this collaboration has not always been encouraged, in part because of the heavy reliance on chemicals to control pests and nonviral pathogens, and in part because of the narrow discipline-orientated view of many scientists.

In field studies, vector resistance has been associated with a decreased incidence of virus infection in more than 20 different virus/vector combinations; reports of decreased virus transmission or acquisition in glasshouse and laboratory studies on vector-resistant plants include several more such combinations (reviewed by Jones, 1987). In several instances, virus spread in commercial crops has been well controlled by vector resistance where chemical control of vector-mediated virus spread is either ineffective, difficult or too costly. In other instances, although virus control through vector resistance is only partially effective, the delays produced in virus epidemics and/or decreased secondary spread offer possible economic benefits. In addition, an increasing number of reports indicate the potential of even low levels of vector resistance (Jones, 1979; Lecoq et al., 1979, 1980; Moyer et all, 1985; Gunasinghe & Irwin, 1986) and further studies on the use of such plant varieties in integrated control programs involving chemical, cultural and biological methods may increase the effectiveness of virus control (Lecoq & Pitrat, 1983). In many crops therefore, and often in existing commercially acceptable material, there is variation of several kinds waiting to be exploited. However, not all forms of vector resistance are equally effective in preventing virus spread and some forms may actually increase virus incidence (Baerecke, 1958; Kennedy, 1976).

The extent to which virus spread is prevented depends on many interacting factors, such as the host range and vector relations of the virus, the mobility and breadth of host range of the vector, the type, effectiveness and durability of resistance to the vector and to the virus, and environmental factors. The specific mechanism(s) involved in resistance to vectors call for particular attention because relatively recent studies indicate that many preconceived ideas on the value of vector resistance as a means of virus control will need to be revised. Thus, near immunity to vectors, commonly regarded as the only effective mechanism for the control of nonpersistent viruses (Knight, Keep & Briggs, 1959; Kennedy, 1976; Gibson & Plumb, 1977), if not all viruses, is not necessarily a prerequisite for good virus control.

For convenience, four types of resistance that interfere in different ways with normal plant/vector relations and that can influence virus infection can be distinguished. In addition to the primary effect of decreasing virus acquisition and inoculation, the expression of any of these types of resistance can also lead to premature vector dispersal, thereby influencing secondary spread.

Interference with host finding by vectors. Although in some instances vectors reach hosts simply by a random encounter, the well documented responses of insects to color and other physical and chemical stimuli indicate that active mechanisms are involved in host recognition. Changes in the type or strength of these signals may completely eliminate alighting responses or feeding thereby preventing acquisition or inoculation of virus. For example, host finding can be inhibited by changes in leaf color, and by changes in the form of the crop canopy (Muller, 1956; Baker, 1960; Davis & Shifriss, 1983; Amin, 1985; Lowe et al., 1985).

Interference with the initial settling of vectors. Having located a host, chemical and physical stimuli from the plant are involved in the identification and establishment of suitable feeding sites. If the resistance is sufficiently strong to prevent probing altogether, no acquisition or inoculation of virus will occur, but if the vector is required to probe the plant before it is deterred from feeding inoculation of nonpersistent viruses is likely to occur. However, a delay in making the first probe, if sufficiently long, may exceed the time for which nonpersistent viruses are retained by vectors, so lessening the probability of virus transmission. If the vector is sufficiently deterred from feeding after several brief exploratory probes, it may leave the crop. Where this happens, the spread of persistent and semi-persistent viruses might be expected to decrease, because no transmission is likely to occur in these brief probes and because secondary spread would be minimized. Plants have a range of physical (e.g. hairs, glandular hairs, thick cuticle) and chemical (e.g. surface waxes and volatile compounds) attributes that can interfere with this initial settling phase before feeding begins (Rizvi & Raman, 1983; Lapointe & Tingey, 1984; Gunasinghe & Irwin, 1986).

Interference with sustained feeding behavior of vectors. When resistance in plants is due solely to antibiosis, the fact that vectors are more likely to remain on plants for lengthy periods would seemingly restrict the usefulness of this form of resistance in preventing virus spread. The most likely benefit might be a decrease in secondary spread through decreasing vector populations and possibly vector activity, but this would probably only affect the spread of persistently transmitted viruses. However, recent detailed studies on insect feeding behavior have shown that, in several instances, antibiosis seems to operate by preventing ingestion or phloem-finding, and these effects seem to influence the likelihood of acquisition and/or inoculation, both of persistent and of semi-persistent viruses (Neilson & Don, 1974; Oya & Sato, 1981; Auclair & Baldos, 1982; Auclair et al., 1982).

Specific interference with vector transmission of virus. In some specific instances, resistance of plants to inoculation of nonpersistent viruses by aphids cannot be explained by any of the previous mechanisms of resistance. In some, but not all of these instances, the phenomenon

is vector specific but not virus specific (Sylvester & Simons, 1951; Simons & Moss, 1963; Lecoq et al., 1979, 1980; Romanow et al., 1985).

The value of preventing virus spread in these different ways is, in addition, increased by the probability that cultivars would not need applications of chemicals to control the vector as a pest - a major consideration in subsistence farming in the tropics and sub-tropics. Even partial resistance to pests has been shown to produce attractive savings in the use of pesticides. Such decreased applications of chemicals are compatible with biological control.

However, the use of vector resistant material may not be practical in every situation. Thus, with viruses that are spread by several vector species or with crops that are infected with several viruses having different vectors, breeding for effective resistance to all these vectors may be impractical unless the resistance mechanism is nonspecific, e.g. plant hairs. Nevertheless, resistance to several vector species has been incorporated into single cultivars of some crop plants, e.g. wheat, rice.

The appearance of new vector biotypes as a result of growing resistant cultivars is a potential problem and has been the cause of major problems in some crops, e.g. rice (Sogawa, 1982). However, there are also many examples where resistance to vectors has proved to be durable for many years. In general, biotypes occur most commonly in monophagous vector species and where resistance is determined by single genes, and seem less common in polyphagous vectors and where resistance is controlled by several genes and is nonspecific, e.g. plant hairs.

As far as the plant breeder is concerned, many problems exist in the detection and exploitation of some of the mechanisms of resistance that promise to be useful. In particular, screening methods are needed for some of the less obvious mechanisms of vector resistance, and field scale testing methods are needed for evaluating the benefits of some forms of resistance that are not apparent in small scale tests. In addition, further studies are needed on integrated control methods using material containing the less effective forms of vector resistance.

New techniques may offer solutions to some of these problems. For example, new biochemical techniques for the rapid detection of specific secondary plant metabolites, which are closely correlated with pest resistance in some plants, could be useful for selecting resistant plants and hybrids. Furthermore, at the basic level, they may aid research on the precise mechanism(s) underlying resistance. Advanced genetical techniques can enable chromosome pieces to be transferred between species and even genera, and this has enabled vector mite resistance to be introduced in wheat (Martin et al., 1976, 1983, 1984) and blackcurrant (Knight et al., 1974). In addition, genetic engineering methods now offer, at least in principle, powerful and precise methods for introducing genetic material into plants. However, to capitalize on these techniques, and to exploit the potential while avoiding some of the limitations of vector resistance as a means of virus control, a greater level of collaboration between workers in different disciplines will be required than has been evident heretofore.

Table 1. Association of vector resistance with a decrease in virus incidence in some crop plants.

Vector	Crop	Virus	Reference
Fungus			
Polymyxa graminis	wheat	soil-borne mosaic	Palmer & Brakke, 1975
		spindle streak mosaic	Jackson et al., 1976
Nematode			
Xiphinema index	grapevine	fanleaf	Bouquet, 1981
Eriophyid mite			
Aceria cajani	pigeon pea	sterility mosaic	Muniyappa & Nangia, 1982
Aceria tulipae	wheat	streak mosaic	Martin et al., 1976, 1984
Cecidophyopsis ribis	blackcurrant	reversion	Knight et al., 1974
Thrip			
Frankliniella schultzei	groundnut	tomato spotted wilt	Amin, 1985
Leafhopper			
Circulifer tenellus	tomato	beet curly top	Thomas & Martin, 1971
Nephotettix virescens	rice	tungro	Heinrichs & Rapusas, 1983
Planthopper			
Nilaparvata lugens	rice	grassy stunt	Heinrichs, 1979
		ragged stunt	Parejarearn et al., 1984
Sogatodes oryzicola	rice	hoja blanca	Jennings & Pineda, 1970
Aphids			
Aphis craccivora	groundnut	rosette	Evans, 1954
Myzus persicae	potato	leaf roll	Rizvi & Raman, 1983
Amphorophora idaei	raspberry	BRNV, leaf mottle	Jones, 1979
A. agathonica		leaf spot	
Aphis gossypii	muskmelon	CMV, WMMV	Lecoq et al., 1979
Macrosiphum pisi	red clover	RCVMV, BYMV	Wilcoxson & Peterson, 1960
Aphis citricola	soybean	mosaic	Gunasinghe & Irwin, 1986
Myzus persicae			
Rhopalosiphum mardis			

BRNV = black raspberry necrosis virus; CMV = cucumber mosaic virus; WMMV = watermelon mosaic virus; RCVMV = red clover vein mosaic virus; BYMV = bean yellow mosaic virus.

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VECTOR RESISTANCE AS A MEANS OF VIRUS CONTROL

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Host resistance to virus vectors is recognized as a potential means of control of virus diseases and has been sought when direct forms of resistance have not been available. The objective of this strategy to minimize losses due to virus diseases is to reduce the incidence of infected plants rather than to directly reduce the effect of the virus on the host. The suitability of host resistance to a virus vector as a strategy for control of the virus is dependent upon our ability to identify a form of host resistance which also interferes with the processes of transmission of the virus by the vector to the host. Frequently, however, resistance to the vector has been detected serendipitously while searching for resistance to the virus. Thus, this type of resistance may have been described as "resistance to infection or inoculation" or it may have been one of several potential factors resulting in "field resistance." It is unfortunate that this area of virus-vector relationships has not developed sufficiently to allow host resistance factors to be well understood or even well classified in terms of their influence on plant virus epidemics.

Complete resistance (= immunity) to virus vectors would result in functional escapes from infection; however, resistance to virus vectors is seldom complete. The task thus becomes one of altering the host-virus-vector relationship so as to reduce significantly the probability of virus transmission and subsequent incidence of virus infected plants. There are many interacting vector-related factors which contribute to the spread of plant viruses and thus will influence the success of any vector resistance intended to control virus spread. Although virus acquisition, retention and inoculation efficiencies by the vector are important components of the virus-vector relationship, they are only three of many biological factors which potentially can regulate the rate of progression of plant-virus epidemics. In a review focused specifically on insect vectors, Kennedy (1) discussed the possible implications of the type of resistance and the level of the resistance on the spread of persistently and nonpersistently transmitted viruses. In this discussion, he pointed out that the interrelationship of ecological factors, such as the relative importance of primary and secondary spread of the virus, the type of resistance and the virus-vector relationship can each cause significant deviations in the influence of host resistance to the vector on the resulting epidemic. Given the diversity of host-virus-vector relationships and the complexity of interactions that can alter virus disease epidemics extreme caution should be exercised when attempting to extrapolate from one type of epidemic to another with regard to the efficacy of a given form of host resistance to the vector.

It almost goes without saying that "significant progress in the use of vector resistance for control of virus diseases will require a better understanding of the complex interdependency among the factors

responsible for virus disease epidemics." It is considerably less apparent where to begin to acquire this understanding. The elucidation of these interdependencies require the cooperation of specialists in each of the disciplines involved: e.g., virology, entomology, epidemiology, genetics, and statistics. A suggested approach is to first determine if a consensus of opinion can be reached concerning the current status of our understanding of vector resistance and its potential use for control of plant virus diseases. Do we know enough to predict when and where the deployment of vector resistance for the control of virus diseases is most likely to succeed or perhaps even more importantly doomed to failure?

Examination of this question might be most efficiently addressed by identifying conditions or relationships between any two components of the virus epidemic which would suggest an (un)acceptable probability of success. In examining the vector-host interaction, one might initially hypothesize that as the dependency of the vector on the virus-host as a food source increased there would be a corresponding increase in the effectiveness of vector resistance on virus disease control. First, to accept this hypothesis a necessary requisite for transmission would be the establishment of a feeding relationship between the vector and host. However, resistance to a specific aphid species has been associated with resistance to nonpersistent virus transmission which does not require a feeding relationship. Although the basis of the resistance to the aphid was shown to be associated with recognition of the phloem tissue, recognition phenomena responsible for the suppressed transmission probably were also interrupted in the epidermis. This hypothesis might also be true if the form of resistance were not based on tolerance but on other forms of resistance such as antibiosis. However, the level of resistance would have to be sufficiently high to reduce the vector population below the threshold needed for significant virus spread. It is also theoretically possible that if an additional effect of resistance were to increase vector activity, as is the case with some insect resistance, any gain due to decrease in number could be compensated for by increased activity.

One might also hypothesize that as the specificity of the relationship between the virus and its vector increases, so will the utility of vector resistance in controlling the virus. As with the previous hypothesis, there are many interrelated considerations. For example, if the virus were spread by only one species, or only one potential vector species were present, then the task would be simplified. It would then be feasible to incorporate a single form of resistance.

A third hypothesis might be that vector resistance would be more effective when there is only one virus host (= crop) in the immediate area. If true, this hypothesis would suggest that vector resistance may be more effective when a significant proportion of the increase in incidence of virus-infected plants can be attributed to secondary spread and that primary spread is small and not quantitatively related to the final disease incidence other than to provide the initial source of inoculum. Although when it is necessary for a feeding relationship to be established for virus transmission to occur, the appropriate form of vector resistance may also reduce primary spread of the virus.

In conclusion, it is recognized that vector resistance is an indirect strategy for controlling virus diseases. Thus, it may be most effective when there are few or no alternative modes of virus spread other than by the vector to which the resistance is directed. The objective then is to be able to recognize when the proper ecological circumstance exists, to identify an appropriate form of resistance to the vector, and to incorporate this resistance into agronomically or horticulturally acceptable cultivars.

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ROLE OF WEED HOSTS AND INSECT VECTORS ON THE INCIDENCE OF VIRUS DISEASES OF PEPPER IN CALIFORNIA

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Virus diseases cause substantial losses in pepper production in California. In search for sources of inoculum of viruses that infect pepper, samples of symptomatic and non-symptomatic plants in 11 plant species belonging to six plant families were collected from Ventura County during 1985. The indirect enzyme-linked immunosorbent assay (ELISA) was used to test for the presence of the following viruses: Potato Virus Y (PVY), Tobacco Etch Virus (TEV), Pepper Mottle Virus (PeMV), Cucumber Mosaic Virus (CMV), Alfalfa Mosaic Virus (AMV), Potato Virus X (PVX) and Tobacco Mosaic Virus (TMV). With the exception of PeMV all the above viruses are among those that were most frequently encountered in California in the past. The presence of PeMV in California has only recently been confirmed (1). Natural sources of PeMV were of special interest because its incidence in pepper was found to be relatively high.

Datura metaloides A. DC. (Jimson weed) and Brassica geniculata Desf. were found to harbor PVY, TEV, PeMV and AMV. All seven viruses were detected in Nicotiana glauca Graham (Tree tobacco) and in Heterotheca grandiflora Nutt. (Telegraph weed); whereas Solanum douglassii Dunal (Douglas nightshade) was found to be infected with PeMV only. Three viruses, namely CMV, AMV and PVX were detected in Artemisia douglasiana Bess. (Sage brush) while only TEV and AMV were detected in Phacelia ramosissima Dougl. ex Lehm. (perennial heliotrope). Conyza canadensis (L.) Cronq. (Horseweed), Sonchus oleraceae L. (Sowthistle), Amaranthus albus L. (Tumbling pigweed) and Chenopodium album L. (Lamb's quarters) tested negative to all seven viruses. A. albus and C. album have, however, been reported as hosts for certain pepper viruses. It should be noted that the latter four hosts are all annual plants and perhaps were sampled too early in the season before becoming infected with any viruses. All the other weed hosts listed which are serving as reservoirs for one or more viruses are perennial plants with the exception of Heterotheca grandiflora which is a biennial. The data do clearly indicate that mixed virus infections are more frequently encountered in the field than are single virus infections.

Insect vectors are probably the primary means by which pepper viruses are spread in the field. In an earlier study of PVY and TEV in pepper, other workers reported five aphid species of the eight species they tested were vectors for both viruses (3). We tested nine aphid species for their ability to act as vectors for PeMV, and found that six of the species were indeed capable of vectoring the virus. These were: Myzus persicae Sulz. (green peach aphid), Aphis gossypii Glover (melon/cotton aphid), Aphis craccivora Koch (cowpea aphid), Acyrthosiphon pisum Harris (pea aphid), A. kondoi Shinji (blue alfalfa

aphid) and Brachycaudus rumexicolens Patch. The relative efficiency of transmission of PeMV by pea aphid, green peach aphid and blue alfalfa aphid (three prevalent vectors in Southern California) was tested. The pea aphid was the most efficient followed by green peach aphid and blue alfalfa aphid. The three aphid species that did not transmit PeMV were: Sitobion avenae Fabrici (English grain aphid), Lipahis brysimi Kalterbach (turnip aphid) and Eucarazzia elegans Ferrari.

Resistant cultivars are the best method of controlling pepper virus diseases. Since no cultivars resistant to PeMV were available in California, those developed in other states were tested against a California isolate of PeMV to evaluate their possible use in controlling the disease in California. For the test these cultivars were inoculated with the virus using viruliferous aphids (green peach aphid). Delray Bell (2), Tambel-2 (4) (bell peppers) and Tam mild chile-2 (chili-type pepper) (Dr. B. Villalon, personal communication) were the resistant cultivars used in the study. Susceptible cultivars, Yolo wonder B (bell type) and Anaheim chili were also inoculated for comparison. Based on symptom expression a 0-11% rate of transmission of PeMV resulted when resistant cultivars were infested with 10-30 viruliferous aphids per plant whereas a 70-100% rate of transmission resulted when these cultivars were infested with 100 viruliferous aphids per plant. A 33-83% rate of transmission resulted when susceptible cultivars were infested with 10-30 viruliferous aphids per plant while infestation of susceptible cultivars with 100 viruliferous aphids per plant resulted in a 100% transmission rate. No virus symptoms were observed in the control plants which were exposed to 100 nonviruliferous aphids per plant. Virus titers in both susceptible and resistant pepper cultivars were determined by indirect ELISA.

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OCCURRENCE OF PEPPER VIRUSES IN VENEZUELA AND FIELD EVALUATION OF VIRUS-RESISTANT CULTIVARS

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Virus diseases pose a serious constraint to pepper growing worldwide due to the considerable losses that they cause. These diseases are widespread in Venezuela, where they constitute the main limiting factor for pepper production by lowering yields and fruit quality noticeably. To determine the identity, distribution, and frequency of occurrence of the viruses present in that country, a survey was conducted in several pepper growing areas. Virus symptoms consisting of mosaic, veinbanding, distortion of fruits and leaves, and stunting, were observed in all pepper fields visited. Often 100% of the plants showed symptoms towards the end of the pepper growing cycle. Virus was recovered from 174 samples collected at 23 fields in 11 of Venezuela's 20 states.

Tobaco etch virus (TEV) was the virus most frequently detected. It was present in 81 samples (47% of all samples diagnosed) collected in all 23 fields visited. Pepper mild mosaic virus (PMMV), an apparently new potyvirus first detected during this survey (3,5) was detected in 68 samples (39%) collected at 18 fields located in eight states. TEV and PMMV were frequently found together in mixed infections. Doubly infected pepper plants showed typical TEV symptoms, which by being more conspicuous mask the milder PMMV symptoms. Cucumber mosaic virus (CMV) was detected in 16 samples (9%) from seven fields in three states. Potato virus Y (PVY) was present in only four samples (2.3%) from four fields located in two states. The least frequent virus found was tobacco mosaic virus (TMV) present in three samples (1.7%) collected at three fields in three states. Identification of these viruses was based on the reactions of manually inoculated diagnostic hosts, although the identities of some isolates of TEV and CMV were confirmed serologically.

The ubiquity of TEV and PMMV, and their usually very high incidence in commercial and experimental plantings, indicate that they play a major role in the pepper viral problem of Venezuela. The other three viruses detected appear to be less important, because of their limited distribution and very low incidence in affected fields.

Of the five viruses infecting peppers in Venezuela, the four most frequently detected are all spread nonpersistently by aphids. Since the use of genetic resistance is the most feasible means of controlling this type of virus, especially under tropical conditions, field trials were conducted to explore this possibility by growing pepper cultivars with resistance to at least the more common viruses present. Results of the first trials, reported elsewhere (4), demonstrated a clear response to the use of a cultivar with multiple virus resistance, Florida VR-2 (1), to reduce virus-induced losses significantly.

To confirm these findings and evaluate other virus-resistant cvs, new field trials herein reported were conducted. Three experimental plantings in the same number of years were established at Saman de Guere, Aragua State, in a randomized block design with four replications. In addition to traditional pepper cvs grown in the country, and a new high yielding hybrid (XPH-828 Asgrow), three cvs with multiple virus resistance from the breeding program of Dr. A. A. Cook at the University of Florida were included in these trials. Dr. Cook's cultivars were: Florida VR-2 resistant to PVY, TEV, and TMV; Delray Bell (2) resistant to pepper mottle virus (PeMV), PVY, and TEV; and 'VRDB,' resulting from a cross between the two former cvs, which had resistance to PVY, PeMV, TEV, and TMV (Dr. A. A. Cook, personal communication).

In the first trial, Delray Bell outyielded all other cvs under evaluation, producing 34 Kg of marketable fruits, of which 69% were of the large export type over 6 cm in diameter. Florida VR-2 was the second best yielding cv with a production of 31 Kg, 67% of which were of the export type. Yields and percentage of export type fruit of the cvs that followed in decreasing order were: Early Cal Wonder, 22 Kg (33%); Yolo Wonder, 19 Kg (38%); Keystone RG N° 3, 17 Kg (25%); and Cal Wonder 300, 15 Kg (30%).

In the second trial (Table 1), yields of the cvs with multiple virus resistance Florida VR-2 and Delray Bell, were more than twice those of the other two cvs under test, Keystone RG N° 3 and R. Florida Giant. Incidence of virus symptoms was very low in plants of Delray Bell at the end of the growing cycle, and very high for the other three cvs included in the trial. Delray Bell symptoms were caused by CMV, while those exhibited by plants of Leystone RG N° 3 and R. Florida Giant were mostly induced by TEV and PMMV, often in mixed infections. PMMV infection was responsible for the high percentage of Florida VR-2 plants with virus symptoms; however, this cv appears to be tolerant to this virus, as evidenced by its high yield.

In the third trial, two cvs with multiple virus resistance, 'VRDB' and Delray Bell, greatly outyielded the other three cvs included in the test. Additionally, all plants of these two cvs remained symptomless up to the end of the trial, 10 wk after planting. On the other hand, 100% of the plants of the other three cvs exhibited virus symptoms at this time. For the five cvs evaluated the number of marketable fruits produced and their weight were: 'VRDB' - 82 fruits, 8.825 Kg; Delray Bell - 52 fruits, 5.075 Kg; 'XPH-828' - 15 fruits, 1.475 Kg; Keystone RG N° 3 - 7 fruits, 0.750 Kg; and Florida VR-2 - 6 fruits, 0.6 Kg. Yield of 'VRDB' was 12 times greater than that of Keystone RG N° 3, the more widely grown pepper cv in the country. Contrary to previous experiences, in this case Florida VR-2 yielded very poorly and was highly infected, not only with PMMV, but also with TEV, to which it had shown resistance. This behavior was attributed to the seed used this time from a commercial source, which did not conform to the characteristics of the cv.

Results of all the trials confirm the effectiveness of growing virus-resistant cvs to reduce losses caused by the viruses that infect this crop. Good performance of the cvs with multiple virus resistance

depended in part on their resistance to TEV. They also showed resistance or tolerance to PMMV. In addition, they possess favorable horticultural characteristics, especially Florida VR-2 and 'VRDB.' All showed excellent adaptation and expressed their production potential under the conditions of Venezuela, since experimental yields obtained in this country and in the United States are comparable in some of the trials. Based on the results of our trials, growing of these cvs has been recommended to avoid or reduce virus induced losses in Venezuela. Florida VR-2 is already being grown to some extent for this purpose.

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Table 1. Yields and percentage of plants showing virus symptoms of four pepper cvs under evaluation at Saman de Guere, Aragua State.

Cultivars	Marketable fruits		Plants with virus symptoms (%) weeks after planting		
	N°	Weight (Kg)	6	8	12
Delray Bell	273	28.930	0	0	1.3
Florida VR-2	247	27.500	0	1.3	85
Keystone RG N° 3	131	13.050	0	13.6	100
R. Florida Giant	116	11.390	0	20	96

QUANTITATIVE EFFECTS OF VIRUS-SUPPRESSION AND APHID RESISTANCE ON THE SPATIAL PATTERN OF PLANTS INFECTED WITH WATERMELON MOSAIC VIRUS 2

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The spatial pattern of virus-infected plants is a fundamental characteristic of the epidemiology of a virus disease. Sequential observations of the spatial pattern of virus infected plants throughout an epidemic have revealed trends indicative of long or short distance primary spread or secondary spread.

A random pattern of infective plants is often attributed to primary spread; however, primary spread also may result in clusters of infected plants. These clusters may occur if the vectors are physically and behaviorally able to inoculate multiple plants or if the virus source is adjacent to the newly available host. The spatial characteristics of the clusters developing from each of these two mechanisms would be significantly different. In the case of inoculation of multiple plants the clusters would tend to be small, compact and randomly located in the field. In the second situation clusters of plants may occur at the edges of the field adjacent to the virus source.

Secondary spread of a virus generally results in clusters of infected plants, but the characteristics of the clusters (e.g., size, shape, and rate of expansion) differ depending on the vector and the mode of transmission. Highly mobile vectors, such as winged insects are not confined to movement between adjacent plants; therefore, clusters may be loosely defined and not easily identified by several of the techniques for spatial pattern analysis. Vectors such as crawling insects, nematodes and fungi are limited in their range of movement and a virus will be spread usually between adjacent plants. This limited spread will result in the formation of closely associated clusters or runs of infected plants. In either case secondary spread from an initial focus will result in an increase in cluster size over time, whereas, a cluster formed as a result of primary spread would not be expected to increase in size over time.

The dynamics of the spatial pattern of infected plants throughout an epidemic can provide a great deal of information on the biology of the pathosystem. However, it is difficult to quantify the spatial pattern of virus infected plants for comparison over time and space. Recently, we introduced a technique to quantitatively analyze spatial patterns of virus infected plants within a lattice (1). This technqiue is applicable to crops planted on a lattice and can be used to compare spatial patterns over time or space. The two-dimensional distance class analysis provides a quantitative description of the spatial pattern of all infected plants in relation to one another throughout the epidemic and can be used to better evaluate the type of spread, the proximity and

perhaps direction of the virus source, and provide information on the behavior and mobility of the vector.

We have used this analysis to quantitatively analyze 14 watermelon mosaic virus 2 (WMV 2) epidemics occurring in the three muskmelon (Cucumis melo) genotypes. Top Mark, a commercial cultivar, is susceptible to both WMV 2 and Aphis gossypii, the only aphid species regularly colonizing C. melo in North Carolina; the accession 91213 which possesses antibiosis/antixenosis mediated resistance to A. gossypii and a form of resistance to WMV 2 which suppressed the level of virus multiplication (3); and Aphid Resistant Top Mark (AR-Top Mark) which possesses the antibiosis/antixenosis resistance to A. gossypii found in 91213, but lacks the resistance of 91213 to virus multiplication. Romanow et al. (4) have quantified the effects of both resistance components on acquisition and inoculation of WMV 2 by A. gossypii and Myzus persicae. The suppressive virus resistance reduced the acquisition efficiency of WMV 2 by aphids from 91213 relative to that from virus-susceptible genotypes. The aphid resistance was specific for A. gossypii and reduced the efficiency with which A. gossypii, but not M. persicae inoculated plants with WMV 2. Field studies conducted on spring and summer plantings indicated the final incidence of disease caused by WMV 2 was significantly reduced in the AR-Top Mark and 91213 genotypes relative to the Top Mark genotype during the spring when secondary spread was important. In the summer, when primary spread was important, however, neither resistance component was effective in reducing the final disease incidence (2). The spread of WMV 2 in both plantings was caused by winged aphids (no colonization of any plant occurred) of A. gossypii and other non-colonizing species. The proportion of A. gossypii trapped during the summer relative to the other aphid species was significantly lower than in the spring.

Infected plants were clustered in separate plots of all three genotypes during the spring planting, but the characteristics of the clusters were different for each genotype. Infected Top Mark plants were closely associated within clusters of up to 20 plants. The clusters increased in size over time indicating secondary spread was important. The infected AR-Top Mark plants were arranged as doublets along or across rows when disease incidence was less than 30%. As disease incidence increased, loosely defined clusters or runs of infected plants were defined and a majority of the infected plants were located at the edges of the plots. A similar edge effect was apparent in the 91213 plots, but larger clusters (up to 10 plants) were evident early in the epidemic and spread along rows near the edge of the plots.

In the summer plantings disease incidence increased to nearly 100% within 5 wk for all genotypes. We could not analyze the spatial pattern of the WMV 2-infected Top Mark plants due to incomplete data. The amount of disease was similar in the 91213 and AR-Top Mark plots and the infected plants were distributed randomly. Small clusters of infected plants were evident in the AR-Top Mark plots when disease incidence was near 50%. The aphid resistance was not important during the summer because the resisted aphid, A. gossypii, was a minor component of the entire aphid population. Thus, we would not expect the epidemiology of WMV 2 to differ in Top Mark or AR-Top Mark. The random pattern of

infected plants in the 91213 plots throughout the epidemic suggests primary spread from sources outside the test area. Since the suppressive virus resistance affects only acquisition of the virus and not inoculation, the suppressive virus resistance would be overcome under conditions of primary spread, and the epidemiology of WMV 2 in the 91213 plots should be similar to that in the other two genotypes.

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COMPARATIVE EPIDEMIOLOGY OF THREE CUCURBIT VIRUSES (CMV, WMV2 AND ZYMV) IN SUSCEPTIBLE AND PARTIALLY RESISTANT MELON CULTIVARS IN FRANCE

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Three aphid-borne viruses are now commonly observed in muskmelon (Cucumis melo L.) crops in France: cucumber mosaic virus (CMV), watermelon mosaic virus 2 (WMV2) and zucchini yellow mosaic virus (ZYMV). Since CMV was first reported as more prevalent, a breeding program for resistance to this virus has been developed and resistant lines were evaluated in the field by visual assessment of plants showing mosaic symptoms (2,4). However, in recent years the increase in frequency of both WMV2 and ZYMV pointed out the need for a better characterization of the virus spread patterns in both susceptible and resistant cultivars. This was possible by the development of serological methods enabling a rapid and easy identification of viruses infecting individual plants.

MATERIAL AND METHODS

Two melon lines were used: "Védrantais," a susceptible cultivar of the "Charentais" type widely grown in France, and "Virgos," a breeding line issued from the fifth backcross of PI 161375 to "Charentais" type cultivars. "Virgos" possesses two genetically distinct resistance mechanisms:

The first type prevents infection by CMV "common" strains (i.e. nearly 2/3 of the CMV isolates encountered in natural conditions) and is under an oligogenic and recessive genetic control. It does not prevent infection by CMV "Song" strains although some level of tolerance is noticed, including lower virus multiplication and poor efficiency as a virus source for the aphids.

The second type prevents transmission of CMV, WMV2, and ZYMV by Aphis gossypii, an important vector in the field. This resistance is monogenic and dominant. It is ineffective against virus transmission by other aphid species (including Myzus persicae, A. fabae...) which are also efficient virus vectors (1).

Plots of approximately 600 m^2 were planted with 225 plants of each line in 1983, 1984 and 1985. All plants were observed individually for mosaic symptoms every 2 or 3 days. At weekly intervals 30 samples were collected from the plants developing mosaic symptoms in each plot and their virus content was characterized using the SDS immunodiffusion technique (3) and antisera against CMV, WMV2 and ZYMV. The percentage of plants infected by each virus within the plots was deduced from the percentage of plants infected by each virus within the sample and the total number of plants with mosaic.

Virus progression curves were analyzed using Van der Plank's logistic model, and the parameters of the equations were estimated by regression analysis (5).

RESULTS AND DISCUSSION

In the susceptible cultivar, epidemics of CMV and WMV2 occurred every year, soon after planting. In contrast, ZYMV occurred only in 1983 and 1985 and its occurrence was later in the growing season. The epidemic curves have the same "S" shape with a steep slope; generally all plants became infected by a virus 2-3 wk after 5% of the plants were found infected. The similarity in the virus progression is particularly clear in 1983 where no significant difference was observed in the progression rate parameters for WMV2, CMV and ZYMV.

In the resistant line "Virgos," CMV was first detected much later than in the susceptible cultivar (mean delay of 24 days) and the virus epidemics developed more slowly. Progression rates were significantly lower than those observed for the susceptible.

In contrast, WMV2 and ZYMV epidemics were very similar in both lines and no significant differences were observed in progression rates. Only a short delay was noticed in the WMV2 spread in "Virgos" every year (mean of 4 days). This is probably the effect of the resistance to WMV2 transmission by A. gossypii. Its limited efficiency is likely due to the important aphid populations, among which a high number of species other than A. gossypii were observed in our conditions.

The similarity of the CMV and WMV2 epidemics during the 3 years allowed the calculation of "mean" virus development curves for each line, providing a good estimation of the field efficiency of the resistances (Fig. 1).

This study demonstrates the high level of protection conferred to the crop by the composite resistance to CMV. However, it reveals the importance of WMV2 and ZYMV, and points out: 1) the need for the search of new sources of resistances towards these viruses, and 2) the inaccuracy of visual assessments and the need for the use of serological methods in breeding programs for virus resistance.

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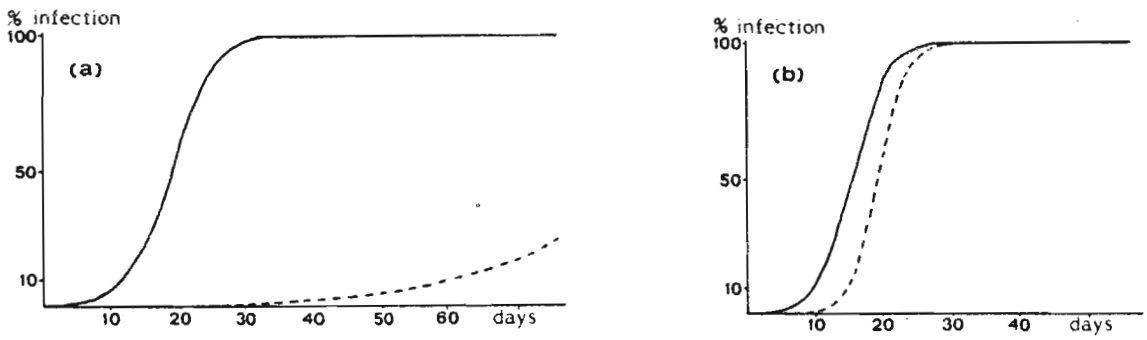

Fig. 1. "Mean" CMV (a) or WMV2 (b) development curves in plots of (——) susceptible or (---) partially resistant melon lines (estimated from 1983, 1984 and 1985 data).

DISEASE RESISTANCE AND SEED YIELD OF SOYBEAN INFECTED WITH COWPEA CHLOROTIC MOTTLE VIRUS

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Cowpea chlorotic mottle virus (soybean strain) (CCMV-S), a member of the bromovirus group, is one of several soybean viruses that have economic importance. The virus has been reported to reduce soybean seed yield and can cause minor alterations in the quality and quantity of oil and protein in seeds (2,3). The virus has a narrow host range, mainly legumes, and is transmitted mechanically and by beetles. In a previous study (1), different types of resistance, based on virus concentration and disease reaction, were found when over 500 soybean genotypes were evaluated.

Virus concentration and agronomic performance of six soybean genotypes with different levels of susceptibility and resistance to CCMV-S were studied under field conditions. In general symptoms of mosaic and stunt were milder in a 1984 experiment than in two experiments in 1985. Virus concentration also was lower in 1984, particularly in the resistant genotypes. No seed yield loss was detected in 1984; however, Davis (a susceptible genotype with high virus concentration, mosaic, and stunt) had a loss of 19% in one 1985 experiment, plant introduction (PI) 96983 had an average loss of 37% in the two 1985 experiments, and no losses occurred in four resistant genotypes. Low seed quality was observed in five genotypes in one or more experiments. Plant height was the agronomic character that was affected most frequently by the virus infection; reductions varied from 13-42% with all genotypes affected in at least one experiment. Lodging, seed weight, and maturity date were affected to a limited extent by CCMV-S.

Virus concentration was not always directly related to seed yield losses and plant height reductions. Only 3-15% as much virus was produced in PI 96983 as in Davis, yet seed yield losses and plant height reductions were consistently greater in the former. The CCMV-S/PI 96983 interaction is complex. We believe PI 96983 has a temperature sensitive gene which inhibits systemic virus movement at 24 C. At 30 C and in the greenhouse and field, virus movement is not inhibited and a strong disease reaction is expressed, despite the low virus concentration.

Four types of resistance were noted in these field studies. The susceptible cultivar Davis is tolerant to seed yield reduction under some growing conditions. (Tolerance is defined as a negligible disease response in a host with relatively high virus concentration levels and relatively unrestricted movement of the virus.) The moderate resistance (virus concentration inhibited 25-75%, mosaic, and mild stunt) in cultivars Coker 237 and Jackson also was adequate to protect against seed yield loss. PI 346304 and Bragg were similarly resistant to all

agronomic characteristics analyzed in the study, but the type of resistance differed in the two genotypes. Very mild or no symptoms occurred on PI 346304 which had virus concentration inhibited 80 to 99%. Bragg reacted with necrotic lesions on inoculated leaves, and extremely low quantities (less than 1 μg/g of leaf tissue) of virus in symptomless, uninoculated leaves. The resistance in PI 346304 may be more desirable than the resistance in Bragg because the former is resistant to all nine strains of CCMV that we have available whereas two strains can overcome the necrotization reaction in Bragg.

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EPIDEMIOLOGY OF MAIZE DWARF MOSAIC VIRUS IN THE NORTHERN UNITED STATES

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Maize dwarf mosaic virus (MDMV) infects maize, sorghum, and a number of grasses throughout the central United States. In the South the predominant strain is strain A which infects Johnson grass, a common perennial grassy weed. Johnson grass does not survive in the North because of severe witners. Strain B, which by definition does not infect Johnson grass, predominates in the North. It has been presumed that strain A predominates in the South because of its alternate host, the highly susceptible perennial Johnson grass. Strain B is believed to predominate in the North because it has different overwintering hosts that give it an advantage. We have examined these hypotheses.

First, we examined susceptibility to inoculation by viruliferous aphids, in a natural setting, on the level of infected plants. A population of genetically diverse Sudan grass which had been self pollinated was naturally infected with unidentified strains of MDMV. The progeny from infected plants were compared with the progeny from uninfected plants to test their susceptibility to inoculation. Seedlings from infected parents were significantly more susceptible to inoculation with both strains A and B of MDMV than were the progeny from uninfected plants. The difference between the progeny from infected and uninfected parents was more pronounced with strain B, but overall susceptibility to inoculation was much higher with strain A.

The Kansas extension service reported in an extension bulletin that maize is more susceptible to strain B and sorghum more susceptible to strain A. Strain A predominated in the South of Kansas while strain B was more common in the North. In our survey of grasses as well as field crops in Nebraska and northern Kansas we also found far more strain B than strain A. In a perennial grass nursery near Manhattan Kansas several cultivars of six commonly growh species showed virus symptoms. Of 121 plants tested by ELISA and mechanical inoculation to indicator plants for the presence of strains A and B, 10 plants had neither virus, 111 had strain B and 2 had strain A.

In our next studies conducted in the greenhouse with mechanical inoculations we examined the graminaceous host range of MDMV-A and B, the susceptibility to inoculation of these plants, and the relative virus titer as measured by quantitative ELISA and by back-assay to sorghum. Fifty-three grasses were tested. In broad generalities, more grasses were susceptible to strain A than to B. No grasses were infected by strain B but not by A. Most grasses were more susceptible to inoculation by strain A than to inoculation by strain B. Johnson grass which is not infected by strain B had a very high titer of strain

A, while most of the other grasses had a low titer of A but a higher titer of strain B.

Northern grasses are more susceptible to strain A, and strain A infects a higher proportion of the plants challenged. A possible explanation for the preponderance of strain B in the north (and strain A in the south) lies in the titer of the virus in perennial reservoir grasses. There may be a threshold level below which aphids cannot efficiently recover the virus for transmission. This hypothesis should be tested.

EPIDEMIOLOGY OF POLLEN-BORNE VIRUS DISEASES OF SWEET CHERRY IN THE PACIFIC NORTHWEST

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Several biological variants (biotypes) of two pollen-borne viruses, Prunus necrotic ringspot virus (NRSV) and prune dwarf virus (PDV), cause a variety of diseases in sweet cherry trees grown in the semi-desert areas of the Pacific Northwest. Diseases such as "blind wood" and "narrow leaf" caused by PDV do not affect fruit yield or quality and these are of no economic importance to growers. Likewise, chlorotic leaf spotting caused by some isolates of NRSV are of no economic concern. However, trees exhibiting cherry rugose mosaic disease (CRM) caused by other NRSV isolates produce unmarketable fruit.

Primary infection centers for CRM frequently appear in 10- to 15-year-old cherry orchards apparently from virus introduced through contaminated pollen carried on rental bees. Research with caged cherry trees demonstrated that rental bees entering Washington from California during cherry bloom season can deliver infectious pollen to flowers which subsequently produce fruit containing either NRSV or PDV-infected seed. However, in tests conducted over a 6-year period, we have not yet been able to demonstrate infection of the seed bearing trees via pollen.

Despite our inability to demonstrate tree-to-tree spread under experimental conditions, field spread of both viruses occurs in nearly all commercial orchards. The rates of field spread for both viruses appear similar over a 10-year period.

Although the incidence and distribution of both NRSV and PDV can be monitored by enzyme-linked immunosorbent assay (ELISA) during the winter months, distribution maps for NRSV seldom agree with the distribution maps for CRM diseased trees. Serological results over a 7-year period revealed the presence of a symptomless NRSV biotype in many orchards which, although serologically similar to the disease-causing biotype, is biologically distinct on woody and herbaceous plants. In some orchards, prior infection with the symptomless biotype appears to protect trees either from subsequent infection or from symptom expression by some CRM biotypes. Thus the symptomless biotype may be useful to reduce field spread in orchards where CRM disease is a problem.

So far, rapid detection techniques such as ELISA do not distinguish between the symptomless and disease-causing biotypes and therefore have been of no value in efforts to control spread of CRM by eradication.

EPIDEMIOLOGY AND CONTROL OF POLLEN AND SEEDBORNE VIRUSES

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Most of the plant diseases caused by viruses can be characterized epidemiologically by two factors: systemic infection of the host plant and efficient secondary spread by a vector. This means that they rank among the plant diseases of high epidemiological risk, especially serious in cases where the virus is also seed-borne and the vector is an ubiquitous insect. Further complications for their detection arise if the virus in question may be present latently in its host and if seed-borne transmission may occur in trace amounts.

There are two points where prophylactic control measures can be taken against seed- and pollen-borne virus diseases. The first is to safeguard the country or region against introduction of a new disease by proper plant quarantine inspection. The second is to incorporate virus testing in the quality control of seed multiplication programs. The new developments in virus testing methodology potentially facilitate the introduction of seed health testing for viruses on a truly routine basis in both plant quarantine and in seed production.

Quarantine for Seed-borne Plant Viruses. In quarantine checks of plant materials for diseases one must follow one of two strategies, exclusion of infected materials or eradication of the pathogen(s) present (Neergaard, 1980). In the case of plant viruses only the former is applicable as reliable eradicative treatments in general do not exist for this group of pathogens. The quarantine procedure for plant viruses is thus in principle based on inspection, examination and testing followed by release of material which has been found to be free of infection.

Many of the viral plant quarantine objects (as e.g., peanut stunt, cucumber mosaic and cowpea aphid-borne mosaic virus) are characterized by being transmitted through seed (often in traces only) and by occurring as latent infections. This places high demands on the sensitivity and efficiency of the testing procedure to be used and implies that inefficient quarantine inspection may lead to the introduction of virus diseased materials which under favorable conditions may spread extremely rapidly (e.g., the presence of a potential vector and susceptible host plants). This introduction is especially serious if it is a virus which is new to the area; but also where a new strain is introduced it may prove to be very serious, (e.g., if the crops grown locally prove to be much more susceptible to the new strain).

If the virus is first introduced and established you have created an additional obstacle for horti- or agriculture in the area; a problem which may be difficult to overcome as eradication of virus diseases is extremely difficult. The best way to control virus diseases is to keep

the pathogen out° A classical example of the introduction of a virus disease to a new area is the African cassava mosaic which is now a serious constraint on cassava cropping in many African countries.

The danger involved with the international spread of virus diseases is obvious. As a consequence, most countries have included a rather large number of plant viruses in their list of quarantine objects (Neergaard, 1980; Reddy et al., 1984). However, surprisingly few quarantine testing programs exist at present for the detection of plant viruses which allow enforcement of the quarantines. This situation implies two things: either potentially virus-infected materials are permitted entry without appropriate testing, or the quarantine responds with a strict embargo of plant materials coming from areas where the diseases are found. It is obviously extremely dangerous to let materials pass without proper testing, but the latter solution is also inadequate. One may here succeed in keeping out potentially dangerous materials, however, quarantine is then acting as a barrier to international trade and international exchange of breeding materials for the improvement of agriculture and for research. This is an unsatisfactory and unsound policy. Quarantine should act only as a filter to protect against the introduction of new diseases.

Virus Testing in Seed Production. Testing for viruses as an integrated part of the quality control in a seed multiplication program differs in certain respects from plant quarantine. First of all, tolerance levels must be established for the various generations of the seed multiplication program. The sound approach for this is to make trials with varying (known) levels of infection and to record the effect of the given seed-borne inoculum level under field conditions. Later the tolerance level and the detection method should be brought in agreement. The second factor which is different from plant quarantine is that we in seed testing for production can rely on testing of samples. An estimate of the infection percentage can then be calculated statistically from the testing result of many subsamples.

The third special aspect is a problem found inherent with the new highly sensitive techniques. The sensitivity may be so high that you risk to record false positives in cases where the seed-borne inoculum occurs in non-embryonic parts of the seed which would never lead to establishment of the disease in the growing plants. So far the best way around this point is to test only the embryonic parts of the seed.

Consequently, high demands are placed on seed testing stations and quarantine services and on the skill of the personnel who implement the procedures. To some extent these demands are fulfilled for the testing of fungal pathogens as efficient and reliable routine testing procedures have been developed over the last decades. However, for the viral pathogens test methodology lags behind. This is not only because fewer efforts have been made in this field, but also because virus testing is so much more difficult. From the above description of the epidemiological characteristics of insect-vectored, seed-borne viruses, which may occur in trace amounts and be present latently in their host, it is obvious that test methodology is critical. Some of the newest virological testing methods may within a short span of years fulfill the

demands for sensitivity and simplicity necessary to qualify for routine testing. Such progress in test methodology is the only way to bring about realistic test procedures for viruses; an achievement which is urgently needed both in quarantine and in production of healthy seed.

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TOMATO SPOTTED WILT VIRUS IN LOUISIANA: EPIDEMIOLOGICAL ASPECTS

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Tomato spotted wilt virus (TSWV) is a serious problem of some solanaceous crops in Louisiana. The virus was first identified in the state in 1972 (1), and since then has become increasingly more prevalent in several production areas. Severe losses have occurred in tomato, Lycopersicon esculentum L.; pepper Capsicum annuum L. and C. frutescens L.; and tobacco, Nicotiana tabacum L. Surveys have shown that TSWV incidence in tomato has averaged 10 to 30% in some production areas since the late 1970's, and occasionally has reached 60% in individual commercial tomato fields and 100% in home gardens.

Six thrips species have been reported to be vectors of TSWV. Thrips tabaci Lindeman, Frankliniella fusca Hinds, F. occidentalis Pergande, F. schultzei Trybom; Scirtothrips dorsalis Hood; and T. setosus Moulton. Thrips tabaci and F. fusca have been known to occur in Louisiana for many years and have been assumed to be the vectors of TSWV in this area. Identification of F. occidentalis in Louisiana for the first time in 1984 led to the hypothesis that this thrips species was responsible for the increased incidence of TSWV in solanaceous crops in the state over the past 6 to 8 years (4). In 1985, thrips were trapped with white pan traps placed in tomato and pepper fields at 17 locations throughout the state. These locations were selected to include areas of the state known to have either a high or low incidence of TSWV. Thrips species identified from all locations listed in order from the most to the least abundant were: F. tritici, T. tabaci, F. fusca, Sericothrips spp., Microcephalothrips spp., and F. occidentalis. Only F. tritici was found in traps at all locations over the entire cropping season and it was 19 times more abundant than all other species combined. Of the thrips species trapped, only F. fusca, F. occidentalis, and T. tabaci are reported to be vectors of TSWV. The occurrence or abundance of certain thrips species could not be clearly associated with locations in which there was a high incidence of TSWV. In the 1985 study, F. occidentalis was trapped only at the Caddo parish location, and the TSWV incidence was less than 2% in that tomato field. The low percentage of TSWV in the field in which they were trapped and the absence of F. occidentalis at all other locations lead us to conclude that the high TSWV incidence which has been observed is not related to the recent occurrence of this thrips species in the state. It is unclear at the present time which thrips species is the most important vector of TSWV in solanaceous crops in Louisiana.

Weeds growing in the vicinity of tomato, tobacco, and pepper crops have been assayed for TSWV to evaluate them as possible TSWV reservoirs (2). Indigenous plant species found to be naturally infected with TSWV

include: Amaranthus spinosus L., Euphorbia heterophylla L., Lactuca floridana (L.) Gaertner, Parthenium integrifolium L., Plantago rugelii Dcne., Ranunculus spp., Rudbeckia amplexicaulis Vahl, Solanum carolinense L., Sonchus asper (L.) Hill, Taraxacum officinale Wiggers, and Verbena brasiliensis Vellozo. Of these species, the winter annuals, L. floridana, Ranunculus spp., and S. asper, are thought to be the most likely weed hosts in which the virus overwinters in Louisiana. These weeds are abundant in Louisiana during April and May coincidental with establishment of spring solanaceous crops.

Field experiments were conducted to determine the effect of plastic film mulches on thrips populations and incidence of TSWV in commercial fields of tomato, pepper, and tobacco (3). Aluminum-surfaced plastic mulch, black plastic mulch, and a nonmulched control were compared in fields with histories of high tomato spotted wilt incidence. Thrips influx into plots was estimated by trapping on yellow sticky boards and disease incidence was determined by periodic counts of plants showing symptoms of TSWV infection. Several thrips species were identified from the traps including two known TSWV vectors, F. fusca and T. tabaci. Aluminum-surfaced mulch reduced the numbers of trapped thrips by 68% and the incidence of TSWV by 64% in tomato when compared with the nonmulched control treatment. In bell pepper, thrips numbers and TSWV incidence were reduced by 60% and 78%, respectively. The number of thrips trapped in tobacco was reduced with the aluminum-surfaced mulch by 33% and TSWV incidence by 63%. Number of thrips trapped and TSWV incidence in the black plastic mulch treatment plots were intermediate to nonmulched and aluminum-surfaced mulch treatments.

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SEED TRANSMISSION OF ZUCCHINI YELLOW MOSAIC VIRUS (ZYMV) IN *CUCURBITA PEPO*

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An epiphytotic of a virus disease occurred in cucurbits in New Jersey during the summer of 1985. Zucchini yellow mosaic virus [ZYMV (4)] was the major virus isolated from infected plants. Fruits shown by enzyme-linked immunosorbent assay (ELISA) to be infected with ZYMV were collected from a commercial field of Black Beauty squash (*Cucurbita pepo*). Seeds were extracted, surface disinfected, and later planted for determination of ZYMV seed transmission using indirect ELISA (1). Three to six wk after planting, seedlings were tested in groups of 10 using composite samples from the first true leaf of each plant. If group results were positive or plants exhibited suspicious symptoms then individual tests were conducted using the second and third leaves of plants.

A total of 246 of the 1298 plants (18.9%) tested were infected with ZYMV as determined by ELISA. A condensed summary of these results is shown in Table 1, in which the 14 fruits are divided into six groups based on similar rates of seed transmission. Percent transmission ranged from 0 to 81%, with the majority of transmission occurring in seeds of reduced size and spongy texture. Some of the plants infected by virus transmitted through seed developed small, inconspicuous necrotic spots from the cotyledonary leaves up to the second true leaf within 2-5 wk after planting.

Selected plants were assayed by ELISA at various leaf positions for virus distribution and dilution end point. ZYMV was detected in 9 of 9 cotyledonary leaves, 12 of 17 fourth true leaves, 8 of 17 fifth true leaves, and 2 of 17 sixth true leaves, but not in the seventh leaf of 17 or the eighth leaf of five plants tested. Not only was virus detected more frequently in lower leaves than in upper leaves of these plants, but the average absorbance (405 nm) decreased from the lower to the upper leaves. Reproductive tissues were also collected from these plants and virus was not detected by ELISA in whole flower buds of 14 plants or in sepals, corolla, stamens, and anthers of two plants. The highest reciprocal dilution of tissues collected 8-9 wk after planting from squash infected by seed transmission ranged from 80 to 1280 by ELISA but was greater than 2×10^4 in tissues of squash mechanically inoculated 2 wk previously with ZYMV.

ZYMV was transmitted from two plants infected by seed transmission about 8 wk after planting to 4 of 6 and to 6 of 6 Multipik squash plants, respectively, and was similarly transmitted from squash mechanically inoculated with ZYMV to 6 of 6 Multipik plants.

Although other workers have tested squash seeds for seed transmission of ZYMV (3,5) this is the first report that such transmission can occur. We believe that several factors elucidated in this study may help explain problems in detecting seedborne ZYMV: 1) the distribution of virus is the opposite of what is normally expected, i.e., seedborne ZYMV concentrations decrease from the lower, older leaves to the upper, younger leaves and is undetectable by ELISA in the youngest leaves; 2) the titer of virus detectable by ELISA or bioassay is at least 100 times lower than in leaves infected for 2 wk with mechanically inoculated ZYMV and requires a very sensitive ELISA system for detection; and 3) symptoms of seedborne ZYMV are very mild and inconspicuous and are restricted to the lower leaves where they are further obscured by senescence.

Since we have studied seed transmission in only one cultivar of squash we do not know the extent of this phenomenon or its significance. Investigations are currently underway to determine seed transmissibility in other cultivars. The aphid transmission studies show that these plants may serve as efficient sources of this virus and play a major role in the epidemiology of ZYMV by providing a reservoir for aphid transmission. We conclude that seed transmission occurs in at least one cultivar of squash and that this mechanism in conjunction with previous reports of aphid transmission efficience (2,4, Davis et al., unpublished) may explain the rapid geographic spread of ZYMV.

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Table 1. Transmission of zucchini yellow mosaic virus (ZYMV) through seedlings of Cucurbita pepo 'Black Beauty.'

Number of fruits[a]	Seedlings infected[b]	Seedlings tested	Seed transmission (%)
2	0	206	0.0
3	4	648	0.6
4	10	112	8.9
2	8	34	23.5
1	82	122	67.2
2	142	176	80.7
14	246	1298	18.9

[a]Fruits were tested by enzyme-linked immunosorbent assay (ELISA) and found to be infected with ZYMV.

[b]Based on group and individual ELISA assays.

THE EPIDEMIOLOGY OF ARABIS MOSAIC VIRUS IN HOPS IN GERMANY

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Symptomatology. Arabis mosaic virus (AMV), known to be associated with the diseases of hops "nettlehead," "severe split leaf blotch" and "bare bine," was detected for the first time in Germany in 1977. In the past, diseases such as "Kräuselkrankheit" and severe stunted forms of hop mosaic were sometimes mistakenly identified as "nettlehead." In a survey of symptoms in all the German hop-growing regions "nettlehead" was never found and the few plants detected which showed "split leaf blotch" were not consistently infected with AMV. It is not likely that "bare bine" would be observed in commercially grown hops in Germany as these are cut back early in the growing season just at the time that symptoms would normally appear. Furthermore, none of the other symptoms occasionally observed on hops and of, as yet, unknown origin (e.g. crinkle or stunting) could be correlated with AMV. Thus, AMV infection in German hops must be designated "latent."

Infestation in the hop-growing regions. Table 1 gives a resumé of the results obtained in the survey of virus distribution. Samples were taken at random and following a geographical grid. No hop-growing region was free of AMV; although no AMV was detected in the, then, few remaining hop gardens of RHW region, 7% of plants collected from field hedges of former hop gardens were infected. Holsthum, with low AMV incidence, is an area where hop-growing was re-introduced after the second world war after an interval of more than 60 yr. Here only 6% of plants were infected and, therefore, the few clones and varieties introduced could only have had a low AMV incidence. Striking results were obtained from the Spalt region where 51% of the 327 samples tested showed AMV infection, whereas in other regions less than 20% of the tested plants were infected.

The geographic distribution of AMV in the areas can be represented by the percentage of hop gardens with AMV. Whereas none of the existing tree gardens in RHW contained AMV-infected plants and only one of the seven hop gardens at Holsthum, the values in the major regions reached 37% in the Hallertau, 41% at Tettnang, 55% at Hersbruck and 80% in the Spalt region. The low incidence in the Jura region can be explained by the fact that a high proportion of the area has only recently been converted to hop gardens, using clones and varieties with a very low disease incidence. Baden and Pfalz, on the other hand, are the remains of traditional hop-growing regions with local selections having a high disease incidence.

Escaped hops were found to be 13% infected, wild hops 3.5%.

Transmission and vectors. The infection of some seedlings derived from non-infected mother plant provides evidence of pollen transmission.

Mechanical transmission and transmission by grafting could not be detected for the German strain of hop-AMV, but have been achieved for other strains. Vector transmission by the nematode Xiphinema diversicaudatum seems to be the only means of spread from plant to plant, although the nematodes found in Germany proved to be less efficient at transmitting the German strain of hop-AMV than were English nematodes of the same species. Using English AMV-H the German vector reached an efficiency of only 6% compared to 100% for the English vectors under identical experimental conditions.

The vectors were found in only three of the nine hop-growing regions. Only in the Spalt region was the vector frequent and present within hop gardens; elsewhere the nematodes were found only in hedgerows or woodland surrounding fields.

Conclusions. AMV is widely distributed in the German hop-growing regions, but the infections are latent and the infected plants do not display any disease symptoms. The AMV infection at Spalt is significantly higher than in any other region and the variety "Spalter" originating from there is more commonly infected than other varieties. This coincides with the presence of the nematode vectors in the hop gardens only in this region. In the other regions where the vector is not present the infected plants seem to be introduced and maintained by infected planting material, either originating from places where vectors were present or by making clonal selections from plants already infected. Further spread seems to be rare. Pollen transmission is of little danger to German hops as male hops must, by law, be grubbed in the hop-growing regions.

None of the varieties showed resistance to infection with AMV but all seem to be tolerant to the German hop strain of AMV, as no symptoms could be attributed to infection with this virus.

Table 1. Arabis mosaic virus occurrence in different regions of the Federal Republic of Germany.

Hop-growing region	% AMV		Vectors* present	
	gardens	samples	region	hop gardens
Baden	71	16	-	-
Hallertau	37	14	-	-
Hersbruck	55	16	+	-
Jura	19	11	+	-
Pflaz	100	17	-	-
Rheinpfalz (Holsthum)	14	6	-	-
Rottenburg-Herrenberg-Weil der Stadt (RHW)**	0	0	-	-
Spalt	80	51	+	+
Tettnang	41	16	-	-
Federal Republic of Germany	47	19		
Federal Republic of Germany excluding Spalt	41	15		
Escaped hops		13		
Wild hops		3.5		

*Xiphinema diversicaudatum

**Hop cultivation was terminated in 1978

VENEREAL TRANSMISSION OF CHERRY LEAF ROLL VIRUS

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The vertical transmission (from pollen to seed) of cherry leaf roll virus (CLRV) was investigated in three natural hosts but principally birch (Betula pendula Roth.). Electron microscopy of mature pollen grains in anthers developing on CLRV-infected birch trees revealed virus-like particles (VLPs) in close paracrystalline arrays in cells forming the anther walls, and in the vegetative and sperm cell cytoplasm of the grains. VLPs within tubules were also observed in anther cells and vegetative cells of pollen grains from CLRV-infected walnut (Juglans regia L.). Washings, from intact freshly collected birch pollen, were not infectious but contained VLPs (detected on grids previously coated with antiserum prepared against CLRV). CLRV-specific antigens (detected by enzyme-linked immunosorbent assay - ELISA) were more tenaciously held to pollen surfaces of cherry than to those of anaemophilous birch or walnut.

When CLRV-infected was compared to virus-free pollen in vitro, no statistically significant differences were observed in germination percentages or germ-tube elongation rates. In vitro infected pollen, germinated, but the extent of callose plug formation was greater in the CLRV-infected but not virus-free birch pollens germinating in vitro, radiolabelled methionine was incorporated into a protein of m.wt. 55,000 that was precipitated using CLRV-specific γ-globulin.

Dispersal patterns for birch pollen paralleled the incidence of seedling infection in progeny from open-pollinated virus-free trees growing at differing distances from an infected source: no infected seedlings were detected from trees more distant than 6.9m from the nearest source of pollen inoculum.

Infected pollen introduced virus into embryos of seeds developing on virus-free birch trees. Embryos developing on CLRV-infected birch trees that received virus-free pollen differed from virus-free material in being shrivelled and suspended in a loosely fibrillar matrix in which electron microscopy revealed numerous virus-like particles in tubular inclusions. Germination rates of infected seeds were less than healthy; the amount of ELISA-detectable antigen increased while the seeds germinated and the resulting seedlings grew more slowly than their virus-free counterparts, thereby explaining why CLRV was more efficiently transmitted through the microgametophyte than the megagametophyte. Furthermore, after three years in the field at an intensity of 1/cm the population structure of birch seedlings changed drastically: the percentage of infected seedlings diminished almost to zero from incidences at planting out which varied to 65%. We interpret this to mean that in quasi natural conditions the virus-infected seedlings grew more slowly and were eliminated by the shading and other competitive influences of their more vigorous healthy counterparts.

When unselected seedlings were planted out at greater spacing (c.1 per 50cm) the incidence of infection was unaffected over the three year period. Significantly the incidence of CLRV-infection in mature birch trees was greater in street trees (11/63) than in unmanaged populations (24/765) perhaps reflecting the differing amounts of competition to which these populations had been exposed at the earliest stages in their propagation. It was calculated that CLRV could not be stably maintained in naturally regenerating populations of birch by vertical transmission alone (1, 2).

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EPIDEMIOLOGY OF VIRUSES OF GROUNDNUT AROUND TIRUPATI AND THEIR EFFECT ON YIELD

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Groundnut fields were surveyed over two years for the occurrence and spread of viruses infecting groundnut around Tirupati, Andhra Pradesh, a major groundnut growing state in India. Bud necrosis, yellow spot, veinal chlorosis, peanut green mosaic (isolates), and yellow mosaic disease symptoms and a few uncharacterized virus-like symptoms were recorded. Other viruses like peanut mottle, Indian peanut clump and cowpea mild mottle viruses reported from India (Reddy, 1986) did not occur. Groundnut witches' broom, a mycoplasmal disease, was noted very rarely only in the Kharif season (June-October) 1984 and 1985.

Tomato spotted wilt virus (TSWV) incites both bud necrosis (BND) and yellow spot. The incidence of BND was less in the Kharif (1%) as compared to the Rabi (December-April) (varied from 1 to 10%) season. It occurred first 30-35 days after groundnut seed were sown and its incidence increased up to about 70 days. Frankliniella schultzei and Scirtothrips dorsalis, vectors of TSWV, generally occurred in the terminal groundnut leaf. Their number did not always coincide with the incidence and spread of BND. While the vectors occurred on many other mixed crops, vegetables and weeds, TSWV did not, indicating that the above plants are only reservoirs of vectors. Groundnut bunch type cvs. TMV-2 and JL-24 showed high incidence of BND as compared to local long duration spreading type.

The incidence of yellow spot disease was up to 80% in the Kharif and almost nil in the Rabi season. A strain of TSWV causing this disease is also known to be transmitted by the same thrips species.

Veinal chlorosis was noticed first in Rabi 1985 and again in 1986, but not in Kharif 1985. So far this symptom type is only graft transmissible. It was first noticed about 45-50 days after sowing seed. The diseased plants were randomly distributed in the field. Limited seed transmission tests indicated that the causal agent is probably not seed-borne. Its incidence around Tirupati is less than 1%. But elsewhere (Kurnool and Guntur districts) in Andhra Pradesh its incidence is up to 50-60% (Dr. D. V. R. Reddy, Principal Groundnut Virologist, ICRISAT, Patancheru - 503 324, India) in some fields.

Yellow mosaic, reported to be transmitted by whiteflies, peanut green mosaic and a few other uncharacterized virus-like symptoms were seen rarely in the fields.

Aphis craccivora infested groundnut plants never contained any virus, and probably they have no role in the epidemiology of viruses infecting groundnut around Tirupati.

BND and veinal chlorosis reduced shoot length, the number of pegs and pods, and the dry weight of shoot and pods. The early diseased plants were evenly stunted and had only a few tiny pods. Starch, alcohol soluble sugars and lipid contents were reduced but the protein content increased in kernels from diseased plants as compared to kernels from healthy plants. Gradient slab polyacrylamide gel electrophorectic analysis of kernel proteins of healthy and infected (BND) samples indicated that they are qualitatively the same but differed quantitatively with respect to some bands.

Yellow spot disease probably has no effect on plant growth and yield. In diseased plants only a few leaves showed the symptoms and plants were almost the same height as comparable healthy plants.

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SOME FACETS OF THE ECOLOGY OF PRUNUS NECROTIC RINGSPOT VIRUS IN PEACH TREES IN SOUTH CAROLINA

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Literature on Prunus necrotic ringspot virus (PNRSV) infections of peach trees growing in the southeastern U.S.A. is scarce (1). A considerable volume of work has been completed on the ecology of the virus in cherry, and work on other Prunus species including peach has been completed in some western and northern states. However, climatic differences between areas of the U.S. and differences among the growth habits of peach and other Prunus species, may make direct extrapolation of the information from one region to the other invalid.

In preliminary work with PNRSV in peach trees in South Carolina we have examined the localization of the virus within the tree with the object of maximizing the likelihood of detecting the virus by ELISA or other assays and providing information on the development of systemic infection within the tree. We have also collected data on the rate of re-infection of a healthy planting from external sources and have anecdotal information on the potential rate of spread of the virus within a variety once a focus of infection has been established.

Using direct, double antibody sandwich ELISA with antibodies prepared from antiserum to Fulton's strain G of PNRSV (ATCC PVAS 22, 1982) we have detected the virus in both blossoms and leaves. Trees were sampled over a 2-year period. Samples of blossoms were taken and one month later samples of leaves were taken. This sampling procedure was repeated in the second growing season. In trees where the virus was detected in blossoms, the leaf sample was also usually found to contain the virus. Exceptions to this generalization exist. In a few trees infections detected in the blossom were not detected in leaves developing in the same year but the virus was usually detected in the blossom and leaves in the second growing season. However, in eight trees in which the virus was detected in blossoms in the first year it was not detected in any subsequent assay.

Certain infected trees identified during this work were subjected to a detailed examination to determine the distribution of the virus within the tree. Plans of the individual trees were drawn, leaf samples taken, and the sample sites recorded on the plan. Trees were identified in which the virus was restricted to a single scaffold limb, to individual branches on a scaffold limb or to individual leaves on a single budstick. Examination of these same individual trees during the second year of growth revealed that with some trees the infection had become systemic whereas with others the infection was still restricted to specific areas of the tree. Despite this localization within the tree, we have found that by assaying a combined sample composed of samples of either leaves or flowers from each quadrant of the tree we have been able to detect the virus with a high degree of reliability.

A planting of virus-free peach trees (170 trees in an area of 1.7 acres) was established in 1978 as part of the South Carolina Peach Tree Certification Scheme. The trees in this planting have since been assayed twice a year for the presence of PNRSV in order that infected trees can be eliminated and the budwood and seed supplied from this block can be maintained free of PNRSV. This planting is at least 1500 feet away from the nearest potential source of PNRSV in either peach or wild Prunus species.

At the present time 145 trees remain in the block. The losses represent an annual rate of re-infection of less than 1% together with some spread from initial foci of infection.

One variety, Tennessee Natural, the seed of which is used to provide rootstocks, blooms at a later date than any other material in this planting. In 1984 a single tree in a row of 13 trees of Tennessee Natural was determined to be infected with PNRSV by using graft inoculation to Shiro-fugen flowering cherry. The infection was not detected until late summer. The tree was removed but in 1985 the remaining 12 trees were found to be infected with PNRSV and were eliminated. Assuming that the first tree identified to contain the virus was the initial focus of infection, we interpret this high rate of transmission in a specific variety to be due to a relatively few trees being "worked" by a large population of bees while there was no other flowering material available in the area at this time. In practice the considerable potential for the spread of this pollen-borne virus within monocultures of peach varieties, once the initial focus of infection has been established, is probably reduced by the considerable number of trees that are available to a population of bees visiting an orchard.

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WEED HOSTS OF ARABIS MOSAIC VIRUS IN HOP GARDENS

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The hop strain of Arabis mosaic virus (AMV-H) is rather distinct from most other strains of this virus. Because AMV-H is a component of the 'nettlehead' disease, it is necessary to eliminate this virus from all hop gardens. The number of sensitive host plants seems very low, compared with the AMV-type strains, and is nearly restricted to the hop plants. Not much information is available about its presence in naturally occurring weeds or about the virus reservoirs.

A survey was done about the natural occurrence of AMV in weeds from hop gardens compared with weeds from other origins, for instance from regions without hops. The possibility of using ELISA for such epidemiological work is discussed.

WEED HOSTS OF ARABIS MOSAIC VIRUS IN BELGIAN HOP GARDENS

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Arabis mosaic virus (AMV) is an important factor in hop culture because of its proven or suspected role in several virus diseases of hops, such as 'nettlehead,' 'barebin' (spidery hop), 'split leaf blotch' and other virus-like diseases recently observed in some hop gardens (2).

By serology, most isolates of AMV from hops seem closely related to the type strains. But their narrow host ranges and the faint symptoms produced by the hop isolates make the hop strains AMV(H) unique. In our surveys over the last several years, a mean infection rate of about 30% was detected even when most plants lacked symptoms (4,5). AMV is also present in other crops of local importance such as Begonia (3), but in these crops only isolates resembling the type strain are found.

This report deals with the results of a 3-year study on the infection of different weeds from hop gardens with AMV type strains and/or the unique hop strains.

Except for transmission by nematodes (Xiphinema spp.) and by vegetative propagation, not much is known about the epidemiology of hop strains of AMV nor of the importance of weeds as a virus reservoir.

For a long time it was thought that the hop strain only infected hops and some rare weeds such as Urtica dioica. To detect the hop strain in other hosts, such as weeds in the neighborhood of hop gardens, it is necessary to have for these AMV-strains a good differential host or detection technique, such as ELISA, with an adequate supply of antiserum.

Table 1 gives a list of the different weeds occurring in hop gardens. Most of them generally are not perennial in the Belgium climate but can form rhizomes which overwinter and in this way can be significant as virus reservoirs.

Table 1. Weeds found in Belgian hop gardens.

Matricaria recutita L.	Poa annua L.
Elymus repens (L.) Gould	Polygonum aviculare L.
Polygonum persicaria L.	Stellaria media L.
Plantago lanceolata L.	Trifolium repens L.
Veronica agrestis L.	Dactylis glomerata L.
Convolvulus arvensis L.	Chenopodium album L.
Urtica dioica L.	Rumez abtusifolius L.
Urtica urens L.	

The detection with ELISA and the absence of the typical AMV symptoms on Chenopodium quinoa indicated that the hop strain was probably present in some of these weeds in hop gardens.

The absorbance values from different weeds collected in hop gardens were compared with that from weeds collected outside of the hop-growing region. There were significant differences between the values for Poa annua L. and Elymus repens (L) Gould.

Virus transmission from hop to the weeds must occur through nematodes. Xiphinema is present but is scarce in the soils of the hop-growing regions in Belgium. Furthermore, since AMV is known to be transmissible through weed seeds, plants from these seed could be an important source of the virus. The role of root transmission in disease spread should be investigated (1).

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OCCURRENCE OF APPLE MOSAIC VIRUS AND PRUNUS NECROTIC RINGSPOT VIRUS IN ROSES IN SONNENBERG ROSE GARDEN, NEW YORK, USA

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Visual symptoms and ELISA were used in field surveys to determine the occurrence of virus infections in field grown roses. Surveys were conducted at Sonnenberg Rose Garden located in Canandaigua, New York. Approximately 2,500 rose plants of different cultivars were contained in the garden. Most of the cultivars were All-America award winners from the American Rose Society. Visual surveys of viral symptoms on rose foliage revealed that more than 30% of the roses in the garden expressed symptoms of virus infection. Symptoms include mosaic, line pattern, ringspot, chlorotic lesions, distortion, puckering, and vein-banding. Apple mosaic (ApMV) and prunus necrotic ringspot (PNRSV) viruses have been reported to be associated with these symptoms.

The objectives of this research were: 1) to determine the association of virus(es) with the observed symptoms; 2) to identify the virus(es) involved; and 3) to determine the frequency of occurrence of the virus(es) throughout early-, mid-, and late-summer season.

Visual assessment. In 1983 and 1984, the visual assessment was determined by examining rose leaves for viral symptoms expressed on each individual plant in the garden. Representative results of selected cultivars are shown in Table 1.

ELISA. Arabis mosaic (AMV), strawberry latent ringspot (SLRV), ApMV, PNRSV, and rose mosaic (RMV) viruses have been reported to be associated with the rose mosaic complex. Antisera to AMV, SLRV, ApMV, PNRSV, and RMV were kindly provided by Drs. R. W. Fulton and B. J. Thomas. In 1984, none of the collected rose samples reacted to antisera against AMV and SLRV. ELISA results on ApMV and PNRSV antisera are summarized in Table 2.

O.D. readings were usually higher in younger rose leaf tissues. Samples from rose petals and anthers also provided high O.D. ELISA on aphids inhabiting rose shoots gave positive reactions to antisera against both ApMV and PNRSV. Pollen and/or aphids may be responsible for natural infection in the field. No attempts were made to study the virus-vector aspect. However, nematode extraction from the rose garden soil was tried in 1983. Few plant pathogenic nematodes were found in the soil. Field soil samples were collected from 30 locations in the garden and cucumber seeds were sowed in the soil. Cucumber seedlings were observed for viral symptom development and tested with ELISA for the presence of ApMV and PNRSV after 6 wk. This experiment was repeated in 1984. No positives were detected.

In conclusion, visual assessment on viral infection on rose was not reliable since symptom expression fluctuated from 1983 to 1984. Certain rose cultivars were more resistant than others. Rose petals and young

rose leaves gave higher O.D. readings than older rose leaves. Viral symptoms observed in Sonnenberg Rose Garden were associated with the presence of ApMV and PNRSV as determined by serology.

Table 1. Visual assessment of viral symptoms on a few selected cultivars in 1983 and 1984.

Cultivars	No. assessed		% Positive symptoms	
	1983	1984	1983	1984
Queen Elizabeth	295	296	42.0	30.4
Scarlet Knight	224	89	46.9	49.4
JFK	352	362	5.4	52.8
Fragrant Cloud	177	136	72.3	43.4
Cherish	59	58	1.7	0.0
Mr. Lincoln	106	115	62.3	8.7
Nearly Wild	86	86	0.0	0.0
Honor	55	59	3.6	52.5

Table 2. Detection of apple mosaic virus, prunus necrotic ringspot virus, and rose mosaic virus in rose samples collected from Sonnenberg Rose Garden, New York, in 1984 and 1985.

Time	No. tested	% Positive		
		ApMV	PNRSV	RMV
1984				
Late July	169	4.1	46.8	1.2
Early August	164	0.0	25.6	0.0
Late August	150	2.7	16.7	1.3
1985				
Early July	222	6.8	13.5	-
Early August	246	11.4	20.3	-
Early September	145	7.6	14.5	-

CHEMICAL CONTROL OF APHIDBORNE VIRUSES

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Chemicals used to control the spread of aphidborne viruses can be divided into three main types: oils, pyrethroids, systemic/fumigant aphicides. Future chemicals may also utilize repellents.

Non-chemical alternatives include the use of reflective mulches to repel or attract away aphids from crops, barrier crops, means of crop hygiene such as roguing and isolation, and use of times or places when vectors are rare. However, these alternatives are costly in either materials, labor or losses caused by growing crops in sub-optimal conditions and this restricts us in most circumstances to chemical means of control.

Oils. Mineral oils can control the spread of viruses transmitted by aphids in the non- and semi-persistent manner. The mechanism is unclear; however, it does not rely on killing the aphids, and oil may interrupt the transfer of virus particles on aphid mouthparts or prevent the establishment of infection. To be effective, oils have been applied at 7.5-15 l/ha, usually as a 1-2% aqueous emulsion every 1 to 2 wk; repeated spraying is needed to protect new foliage. The most effective oils are paraffinic; aromatic, napthenic and vegetable oils are less effective. Oils already mixed with an emulsifier, perhaps already emulsified in a small amount of water, are commercially available. The emulsifier may affect the ability of a mineral oil to control virus spread either directly, by affecting spray characteristics and by affecting rainfastness. However, little published work is available on this aspect.

The main disadvantages with the use of oils are that they need to be applied frequently, may not be rainfast, have been associated with phytotoxicity and increases in the incidence of fungal disease, may be incompatible with certain pesticides and confer few benefits other than virus control. The main advantages of oils are that they seem to represent little hazard to either operators, environment or consumers and there is no evidence of resistance.

Pyrethroids. The pyrethroids commonly used in agriculture intoxicate rapidly by contact; they have no appreciable fumigant action The usual sequence occurring within a minute or two of aphids being placed on a leaf treated with a lethal amount of pyrethroid is that they cease probing, appear agitated and aphids may walk or fly from the leaf. Within another few minutes they become uncoordinated and then paralyzed.

Aphids may have opportunity to probe a leaf before they are intoxicated, so aphids carrying a non-persistent virus may be able to inoculate it but they do not feed long enough to inoculate semi- or persistent viruses. Similarly, aphids alighting are not able to acquire a semi- or persistent virus but may be able to acquire a non-persistent

one; however, they are usually incapacitated before they can transmit it to another plant. Thus, pyrethroids can prevent all stages of transmission of semi- and persistent viruses, can prevent within-crop spread of non-persistent viruses but, unlike mineral oils, are unlikely to give much protection against inoculation of non-persistent viruses acquired outside a treated crop.

Like oils, pyrethroids need to be applied frequently to protect new foliage. They are usually applied as an aqueous emulsion but for control of non-persistent virus it may be beneficial to add mineral oil before emulsifying in water, control being derived from each component and also from an enhanced aphicidal activity of the pyrethroid, probably because it remains more available, dissolved in the oil film on the leaf surface.

Pyrethroids are widely used for the control of barley yellow dwarf virus in autumn-sown cereal crops, one or two sprays being sufficient to protect the crop until winter stops further immigration; pyrethroids are particularly effective for this as their toxicity generally increases at lower temperatures. Their use to control non-persistent viruses is only a recent commercial practice.

It is difficult to determine which pyrethroids are most cost-effective because they differ considerably in price and recommended (for insect control) rates; a subject particularly requiring more research is whether particular pyrethroids have special properties (such as extra-fast knockdown ?) which make them especially effective as virus control.

Perhaps the two main disadvantages of pyrethroids are that they may not (evidence is conflicting) stop viruliferous aphids inoculating treated plants with non-persistent viruses and that they are insecticides which, although of extremely low mammalian toxicity, may kill beneficial insects, select for insecticide resistance in insect pests, and kill fish. There is also a risk, although no examples seem to have been reported yet, that sublethal residues may increase virus spread by causing insects to move more. This also seems to be an interesting research subject.

Systemic/fumigant aphicides. Aphicides, notably organophosphates and carbamates, have long been used to limit the spread of semi- and persistently transmitted viruses. In ones with fumigant action, this generally lasts for only a few days following application and helps ensure good kill of resident aphids. Systemic aphicides may persist for several weeks or months, depending on the aphicide and environmental conditions, and maintain control of resident aphid populations. The main abilities of these pesticides in virus control is to prevent semi- and persistent viruses spreading within a crop by killing resident aphids. Non-persistent viruses, which are generally spread by migrant alates making brief probes are usually not controlled by these aphicides, and there are records of their increasing their spread, perhaps by increasing aphid movement. These aphicides also give little protection against inoculation of semi- and persistent viruses by viruliferous immigrants, aphids inoculating plants before they have imbibed a lethal dose of sap.

The main advantages of these aphicides is that most are cheap, their systemic action protects new foliage without the need for repeat treatment and their fumigant and/or systemic action ensures good kill of aphids even in furled leaves and on abaxial leaf surfaces. Their main disadvantages are that they give little protection against non-persistent viruses, or semi- and persistent viruses carried by viruliferous immigrants, and their mammalian toxicity may require special precautions for operators and consumers. There may also be resistance to them, as occurs commonly in Myzus persicae, but this is a hazard shared with pyrethroids.

Repellents. Repellents tested for control of aphidborne viruses can be divided into those based on aphid alarm pheromone, on chemicals derived from plants and on synthetic organic chemicals.

The main component of the alarm pheromone of most aphids is E-β-farnesene. Its ability to repel aphids using very low doses would seem to make it ideal for virus control but its successful use remains tantalizingly distant. It is a volatile chemical, and a major difficulty seems to be how to release it over a long period. This has also complicated the interpretation of negative experimental results, as it is difficult to distinguish whether failure was the result of inadequate application or because the pheromone is inappropriate for virus control. One successful technique has been to produce less volatile derivatives but it is unclear whether aphids recognize these as alarm pheromones or whether the derivatives are active in their own right.

Repellent chemicals often form part of the natural defense mechanism of plants against insects. Polygodial, derived from Polygonum hydropiper is repellent to aphids and has diminished transmission of non-, semi- and persistent viruses in the laboratory. The main obstacles to its use on crops are that it is phytotoxic and has short persistence. However, this promising virus control suggests that there must be more suitable candidates available amongst plant-derived repellents, and it may be possible to extend this range further by chemical synthesis. Polygodial probably acts by contact but certain plant volatiles such as carvone and linalool can affect alatae even before they alight; these would seem to present another chemical mechanism to protect crops against aphidborne viruses.

Finally, certain carboxylic acids have been found to repel aphids and to diminish transmission of semi- and persistent viruses, although they were of no benefit for controlling non-persistent viruses and were phytotoxic. However, they further emphasize the range of chemicals available for exploitation.

ECOLOGICAL AND BEHAVIORAL INVESTIGATIONS OF APHID VECTORS OF POTATO VIRUS Y IN NEW BRUNSWICK

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To be an effective virus vector an aphid must not only be present on a crop but move from plant to plant or from field to field. Studies at the Fredericton Research Station center on the dispersal of vectors, more particularly two of the potato colonizing aphid species (the anholocyclic green peach aphid, Myzus persicae, and the holocyclic buckthorn aphid, Aphis nasturtii), and how it may affect their role as vectors of potato virus Y (PVY).

Recent replicated field tests at Fredericton (Boiteau, unpublished) established that the green peach aphid has a temperature threshold for flight take-off of 16-17°C and a unimodal diurnal flight periodicity peaking during the morning and early afternoon with a mean flight time at 12:25. A preliminary analysis of the daily temperature variations in New Brunswick for the period mid-June to mid-August suggest that 9.94 ± 4.84 SD hours per day are suitable for flight take-off by M. persicae. However, the flight periodicity of the green peach aphid is skewed toward the morning with 75% of its flights between 06:00-14:00 leaving in fact a period of only 4.74 ± 2.77 SD hours suitable for flight take-off. On the average, the first winged aphids reach the province July 26-29 and increase to significant numbers around August 8-11 (1). By mid-August, the daily period suitable for flight starts to decrease and is minimal by the end of the month. These data indicate that the green peach aphid, with an intrinsic vector effectiveness of 56% (3), can be an important vector of PVY only during the period July 26-mid-August.

The buckhorn aphid has a temperature threshold for flight take-off of 19°C and a unimodal diurnal flight periodicity peaking in the afternoon with a mean flight time at 15:16. An interval of 5.76 ± 3.91 hr/day is available to A. nasturtii for the flight take-off, a period similar to M. persicae. Our data indicate that in spite of their different temperature thresholds for flight, temperature should not be a modifying factor of their respective intrinsic vector potential. Buckthorn aphids can, however, colonize the potato crop in mid-June spending as much as 8 wk on the crop vs 3-4 wk for the green peach aphid during the growing season. This longer period of contact with the potato could make the buckthorn aphid a more important vector than its intrinsic effectiveness of 19% (3) may suggest.

After crop colonization, most of the aphid population consists of apterous forms. Based on data for PLRV, it has often been presumed that apterous aphids are intrinsically more efficient vectors than alatae. Recent studies at Fredericton suggest that this cannot be generalized between viruses or test plants. On potato, Macrosiphum euphorbiae alatae are more efficient vectors of PVY (4.5%) than apterae (0%) but on tobacco, alatae are less efficient vectors of PVY (23%) than apterae

(30%) (4). Similarly, A. nasturtii alatae are less efficient (23%) than apterae (27%). In the case of M. persicae, alatae are more efficient (64%) than apterae (47%) (3).

Early in the season, regardless of their intrinsic effectiveness, apterous aphids are not likely to be important vectors of PVY because of their low numbers and the very open canopy. Closed canopies probably favor the movement between plants. The behavior of the aphids is probably the next most important factor. It has been generally observed, for example, that the buckthorn aphid is reluctant to change position on the plant decreasing its probability of interplant movement compared to the green peach aphid. This "behavioral mobility" of the aphid can be stimulated by external factors such as predators and insecticides.

A growing body of studies has demonstrated that pyrethroids can reduce PVY spread by their sublethal effects on aphid movement and feeding behavior. At Fredericton, we showed that carbamate and aldicarb have similar properties. M. persicae and M. euphorbiae that survived the insecticide were restless but had a significantly reduced ability to probe and to fly (2). In another test (Lowery & Boiteau, unpublished), the sublethal effects of two pyrethroids, two organophosphates and one carbamate were studied on the green peach and the buckthorn aphids. The pyrethroids increased movement in both aphid species but only in the green peach aphid for methamidophos and pirimicarb. Almost all the insecticides tested decreased the probing frequency except for pirimicarb and methamidophos which increased probing slightly in the buckthorn and the green peach aphids, respectively. There were no apparent differences between apterae and alatae. These observations confirm that not only the pyrethroids have behavioral modifying effects capable of affecting PVY spread and establish that such effects vary between aphid species.

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THE EFFECTS OF APPLICATION OF PROTECTIVE ROW COVERS TO CONTROL INSECT INFESTATIONS AND VIRUS INFECTION ON FIELD GROWN WATERMELON IN SONORA, MEXICO

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A watermelon Citrullus lanatus (Thunb.) Matsum and Nakai cv. Improved Peacock field plot was established in a commercial cucurbit production area of Sonora, Mexico where dramatic yield reduction and/or total crop destruction have occurred as a result of infection by plant viruses. Prior diagnosis of infected cucurbits from the area indicated that the viruses most often responsible for severe disease situations were the aphid- (Myzus persicae L.) and whitefly- (Bemisia tabaci Genn.) transmitted zucchini yellow mosaic virus (ZYMV) and watermelon curly mottle virus (WCMoV), respectively (Brown and Goldstein, unpublished). The field plot was established to test the efficacy of two row crop cover materials as protective barriers against insect infestations and thus virus infection. Though cover materials are commonly applied to afford cold protection to early spring planted crops, they are usually removed when the danger of frost is past. The time of removal generally occurs shortly before or coincides with the arrival of insect vectors. Our objective was to remove the row covers from control ('uncovered') plants after the last frost, as in commercial watermelon production, but to allow the covers to remain on treated ('covered') plants until time of flowering. The hypothesis was that the covered plants would: 1) develop more rapidly in a protected environment, and thus mature and flower sooner, and 2) be protected from virus infection longer than uncovered plants. With this dual advantage, it was postulated that late virus infection (relative to plant maturity) would be less detrimental to plants and losses might be decreased.

Seed was sown December 26, 1985 at 1 m intervals in rows 3 m apart and fertilizer applied (30 kg urea and 60 kg phosphorus/ha). Rows were numbered and cover treatments applied to randomly selected 5 m row segments. Ten replications of five plants/rep were protected with either plastic (Vispore 5042, Ethel Co., Vis-Queen Div., 37350 Blacow Rd., Fremont, CA 94536) or polyester (Reemay, DuPont; Kenbar, 24 Gould St., Reading, MA 01867) row cover materials. The remainder of the field and ten control replications of 5 plants/rep were covered with plastic exclusively for cold protection, and the field was irrigated. Seedlings emerged 11 days after planting (emergence = week 1) at which time two yellow insect sticky traps were placed at different locations in the field to monitor insect activity. Traps were replaced weekly for 12 consecutive weeks, the number of trapped aphids and whiteflies counted, and counts for the two traps averaged. At week 4, cold protection covers were removed from control rows and the remainder of the field.

Protective plastic and polyester covers remained on treated plants until time of flowering, or week 10. Two wk after protective covers were removed (week 12) and at weekly intervals through week 18 (harvest), data were collected for each plant within the three treatments (plastic, polyester and uncovered control) and included 1) symptom readings (1 = curl, 2 = mottle, 3 = fruit symptoms, and 4 = stunting), 2) presence of flowers and/or fruit, and 3) presence of aphids or whiteflies. At weeks 14 and 18, leaf samples were collected from each plant and analyzed for the presence of ZYMV and WCMoV. Diagnoses were accomplished by corroborating data from field symptom readings, bioassay to greenhouse maintained and inoculated diagnostic indicators, and dot immunoassay tests utilizing virus-specific primary antibodies and horseradish peroxidase-conjugated secondary antibodies. Data were utilized to estimate the relative percent virus infection in the field at 2-wk intervals for weeks 12-18. The estimates at weeks 12 and 16 were based exclusively on field symptom readings. Following fruit set, fruit was thinned to two per plant as in commercial watermelon production. Ripe fruit was harvested during a 3-wk period from the end of April through May 13 (week 20), weighed, and graded. Data were analyzed using one way analysis of variance and least significant difference to separate means at $P = 0.05$.

The effects of the extended length of protection on plant health and fruit production were three-fold. First, both plastic and polyester plants were larger, more lush, flowered 2-4 wk sooner, and set more fruit earlier than uncovered plants. Second, virus infection (based upon symptom development and diagnoses) occurred at least 2 wk later in covered vs. uncovered plants, but by week 16 infection levels were similar in all treatments (ZYMV = 63-73% and WCMoV = 58-70%) (Table 1). Minimal virus spread occurred beyond week 16. Third, the average yield (kilos/rep) was significantly higher in covered vs. uncovered plants, and the average weight per fruit was significantly greater for plants protected with plastic than for polyester covered or uncovered plants (Table 2). Quality (grade) and number of fruit per plant were not significantly different among treatments.

Sticky trap data indicated that aphids reached peaks of activity (1000/trap) by week 4 and whiteflies (250/trap) by week 6 (3 wk and 1 wk prior to frost protection removal, respectively). Insects continued to be active throughout week 12, after which traps were not replaced. Aphids and whiteflies infested 47% and 15% of the controls, respectively, the day following removal of plastic and polyester protective materials (week 10). Population levels of aphids and whiteflies fluctuated throughout the remainder of the season, and after week 12 levels were parallel among covered and uncovered plants.

Table 1. Percent (%) infection by watermelon curly mottle virus (WCMoV), zucchini yellow mosaic virus (ZYMV) or both viruses (W & Z) of plastic or polyester covered and uncovered watermelon plants

Week	Virus	% Infection		
		Plastic	Polyester	Uncovered
12	WCMoV	0	4	30
	ZYMV	0	6	30
	W & Z	0	0	14
14	WCMoV	42	39	61
	ZYMV	24	41	51
	W & Z	20	20	29
16	WCMoV	65	58	70
	ZYMV	65	75	63
	W & Z	45	47	43
18	WCMoV	72	68	90
	ZYMV	70	77	65
	W & Z	56	53	61

Table 2. Fruit yield and quality data for plastic or polyester covered and uncovered watermelon plants

	Plastic	Polyester	Uncovered
Average number fruit/rep[1]	4.8a	5.1a	3.9a
Average fruit yield (Kilos)/rep	18.3a	18.9a	13.0b
Grade #1 (Kilos/rep)	10.5a	10.5a	6.8a
Grade #2 (Kilos/rep)	7.9a	9.6a	5.6a
Average weight (Kg)/fruit	3.8a	3.7ab	3.3b

[1]For each row, means followed by the same letter are not statistically different at P = 0.05.

EPIDEMIOLOGY AND INTEGRATED CONTROL OF WHITEFLY-TRANSMITTED VIRUSES OF PHASEOLUS VULGARIS L. IN ARGENTINA

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Argentina is the main exporter of dry beans (Phaseolus vulgaris L.) in Latin America. The main bean production regions span an area of over 200,000 ha in the northwestern provinces of Jujuy, Salta, Santiago del Estero and Tucuman. Bean production in Argentina increased from 40,000 tons in 1970 to 200,000 tons in 1980, in response to production shortages suffered by several Latin American countries, and the availability of fertile virgin land. This rapid increase in bean production was closely followed in the provinces of Santiago del Estero and Tucuman by an expansion of the area planted to soybean (Glycine max L.). It was in these two provinces where the 'achaparramineto' disease of beans, characterized by severe dwarfing, first appeared in 1977. Between 1977 and 1981 this disease affected approximately 20,000 ha of the white-seeded bean variety Alubia predominant in the area. The bulk of the 140,000 ha of Alubia planted in the province of Salta was not affected since soybeans were not cultivated on a large-scale in this province before 1980. The presence of unusually high populations of whiteflies were consistently associated with the 'achaparramiento' disease in affected Alubia plantings. The main host of the whitefly (later identified as Bemisia tabaci) was soybean. By 1981, 80% of the Alubia crop in the provinces of Tucuman and Santiago del Estero was replaced by Negro Comun, a mixture of black-seeded varieties tolerant to 'achaparramiento.' At this time, a geminivirus transmitted by Bemisia tabaci was partially characterized from 'achaparramiento' affected-Alubia plants. The virus was similar to the causal agent of 'dwarf mosaic,' first described in Brazil, and currently known as 'bean chlorotic mottle.' The soybean area continued to expand, mainly in the province of Salta, and reached a total area of 140,000 ha in northwestern Argentina. By 1983 the 'achaparramiento' or 'chlorotic mottle' disease had appeared in the province, particularly in the Anta region where soybeans and beans were first cultivated side by side. The same year, a second whitefly-transmitted virus disease, bean golden mosaic, was observed in northwestern Argentina, causing a more generalized mosaic in genotypes previously reported as tolerant or resistant to bean chlorotic mottle. Whether this new disease is the consequence of the adaptation of the chlorotic mottle agent to beans or the introduction of a new virus is not known yet. The main epidemiological factors determining the incidence and spread of these diseases in Argentina are: 1) the occurence of dry (10-20 mm/month average rainfall in March-November) and warm (13 to 26 C) conditions favorable to the development of large whitefly populations; 2) the planting of soybeans (November-December), a suitable host for Bemisia tabaci; 3) the maturation of the soybean crop when the bean plants are recently emerged, thus, causing a migration of whiteflies to the bean crop at a very susceptible growth stage; 4) the presence of the virus in ubiquitous weed hosts; and 5) the existence of susceptible bean genotypes. Despite these favorable epidemiological

factors, beans continue to be commercially cultivated in northwestern Argentina due to the implementation of an integrated control approach comprising: 1) the zoning of bean and soybean fields, 2) the selective use of insecticides at sowing or germination time, and 3) the use of new tolerant bean varieties with superior yield and commercial characteristics.

THE CACAO SWOLLEN SHOOT VIRUS ERADICATION CAMPAIGN IN GHANA

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Eradication measures are used extensively in attempts to control or at least contain pests or pathogens of diverse crops in many different countries. Plum pox in various parts of northern Europe, citrus tristeza in Israel, sugar cane Fiji in Queensland, banana bunch top in New South Wales, peach mosaic in U.S.A., little cherry in Canada and coconut cadang cadang in the Philippines are all examples of virus or virus-like diseases of perennial crops that are subject to control by eradication. This is in some instances carried out by government or state employees or enforced by official legislation.

The most ambitious and most expensive eradication campaign ever mounted has been against cacao swollen shoot virus in Ghana, where 'cutting out' measures have been practiced on a large scale since the 1940s as the only control measure it has been possible to adopt. The enormous scale of the undertaking is not generally recognized, even though it has from the outset largely monopolized the budget, manpower and resources available for cocoa production and agricultural development in the whole country.

Numerous survey parties are employed by government or quasi-government agencies to carry out periodic inspections of all cocoa-growing areas. Outbreaks are then treated and retreated as necessary by cutting out all visibly infected trees. Official compensation is paid to growers for the loss of trees and there is also a replanting grant or treated farms are replanted before being handed back to the original owners. These measures were originally enforced but they are now operated on a voluntary basis and for the last 3 years have been practiced on a very limited scale.

Collated data are available up to the end of 1985, by which time 186.7 million trees had been eradicated and 64% of these were removed in carrying out the initial treatment of newly discovered outbreaks. The number of trees destroyed is equivalent to 124,000 ha at usual spacings and excludes the many millions of trees killed by swollen shoot before they were found by the inspectors.

The eradication campaign has been fully justified and is reasonably successful in five of the six main cocoa growing regions, where almost all the known outbreaks have been treated and where the number of trees destroyed (17.2 million) is small in relation to the total tree population and to the value of the cocoa produced. The situation is very different in the Eastern Region where swollen shoot was first discovered in 1936 and where infection is now rife in many areas. The number of trees eradicated since 1945 totals 169.6 million, yet it is estimated that there is a backlog of 31.2 million infected trees to be

removed. The true situation is likely to be far worse because there must have been much further spread since the last comprehensive survey was carried out in the 1970s. Moreover, recent studies have shown that the survey parties find only about 23% of all the infected trees in a new outbreak because many are missed or in the latent phase of infection. The situation is obviously unsatisfactory and is likely to deteriorate further because less than 0.5 million trees a year are being eradicated in the current phase of the campaign.

The failure of the eradication policy in the Eastern Region is only partly due to the sheer magnitude of the problem and to the difficulties of organizing and supervising such a major undertaking. There has been a lack of continuity in the campaign and much effort has been dissipated in treating and replanting individual farms that are often small and surrounded by untreated or abandoned cocoa containing numerous sources of infection. Reinfection is inevitable in these circumstances and often occurs at an early stage so that many of the affected farms are young and not yet in full production. A reassessment of current procedures in the Eastern Region is long overdue and there is an urgent need for epidemiology studies to determine safe isolation distances, to assess the merits of treating large contiguous blocks and to find methods of deploying to best advantage the resistant varieties now available. It should eventually be possible to develop improved methods of treating and replanting affected areas in such a way that there is little serious risk of reinfection.

DISRUPTION OF APHID TRANSMISSION OF VIRUSES IN CANTALOUPE

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There have long been aphid-vectored viruses in spring cucurbit plantings in the desert regions of Southern California. Recently a new virus, zucchini yellow mosaic virus (ZYMV), has increased in incidence (1,3,4) and extensive losses due to this particular virus are causing concern among cucurbit growers. In the spring of 1984, high numbers of aphids carried these viruses into fields and infected plants were found in an estimated 95% of all melon fields in the area. In addition, disease severity was at an all-time high as 40-50% of the samples taken from the area had ZYMV (3).

The aphid-vectored problem has been labeled as one of the most serious problems faced by growers in the valley where 29,500 acres of mixed melons valued at $57 million were grown in 1984. Latest figures quoted by the Imperial County Agricultura Commissioner noted a decline in cartons of melons packed per acre from 551 in 1982 to 220 in 1985. Most of the decline has been attributed to virus diseases.

Our approach has been to address the virus in a multidisciplinary fashion. The summary by Castle et al. (contained in this publication) shows results from many of the studies in which we are involved. We also have been interested in evaluating ways in which to manipulate the virus-insect-plant transmission cycle in an effort to disrupt transmission.

We have studied the effects of different colored plastic mulches on aphid attraction/repellency and the resultant impact on disease incidence in cantaloupe. Results of aphids trapped in 7.5 cm clear plastic water pan traps [modified from Irwin (2)], indicated that significantly more aphids were trapped over the yellow mulch. The non-mulched plots (control) had intermediate numbers of aphids. Plots with white, black and silver mulches, in that order, had decreasing numbers of aphids.

Total seasonal virus incidence was not influenced by the colored mulches (since all eventually became 100% infected), but the time at which plants became infected was different. The silver mulch treatment became infected about 2 wk after the other treatments. Similarly, the black mulch caused a delay of 1-1.5 wk. Of all treatments, the silver had the highest yield, while the yellow mulch had the poorest yield. All other treatments responded in accordance to the aphid density.

Canopy covers were used in an attempt to prevent aphid feeding on the melon plants. After planting and prior to germination, one of three canopy cover types (Reemav, Kimberly Farms Row Cover, and Agryl) was placed over the plants. Germination time of plants growing under these covers was reduced in comparison to non-covered plants. All covers were removed when plants began to flower in order to allow pollination.

Canopy covers were successful in providing a physical barrier to aphid feeding. No aphids were present on plants under the covers, while the non-covered plots were infested. After removal of the covers, aphid densities were the same in all plots. In the non-covered treatments, virus infection was evident early in plant growth. Virus incidence under the covers was zero until the covers were removed. After a 2-3 wk period, all plants that had been covered became infected rapidly due to the available inoculum source in surrounding plots.

Another attempt to decrease virus incidence was through the use of intercropping with wheat. Increasing the plant biomass available in the field increased the chance that an incoming viruliferous aphid would land on the intercrop instead of the cantaloupe. Landing first on the wheat the aphid would probe and rid itself of the virus (non-persistent, stylet-borne virus) before probing the melon host.

Aphid counts in water traps indicated that the same number of aphids landed in the intercropped and control plots. The virus incidence in the intercropped treatments was the same as in the control plots; however, a delay in the incidence of several days to 1 wk was observed. One possible explanation for the similar virus incidence is that the intercrop had to be disced due to agricultural practices, when the cantaloupe had about five true leaves. If the intercrop could be maintained for a longer time period, then positive results such as those obtained by Toba et al. (5) might be obtained.

A final study evaluated the impact of various planting dates on the virus incidence in the field. Three plantings at 3-wk intervals were utilized. Results showed that, once again, all plots became 100% infected with virus. An interesting observation was that virus symptoms began to be expressed at the time when the plants began fruit set. This indicated that a relationship might exist between the physiological stress on the plants caused by fruit development and the stress placed on the plant by the virus infection.

Our research has provided positive evidence that aphid transmission of ZYMV can be disrupted. Colored mulches (especially reflective) can be used to "repel" aphids and slow the spread of virus incidence. Canopy covers can be used to prevent aphids from feeding on the plants, thereby reducing virus transmission. Intercropping has not been effective in our studies; however, trends indicate that with proper techniques this might provide some delay in virus incidence. Variation of planting date studies has shown that a relationship exists between the virus symptom expression and the reproductive physiology of cantaloupe plants.

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SUPPRESSION OF APHID COLONIZATION BY INSECTICIDES: EFFECT ON THE INCIDENCE OF POTYVIRUSES IN TOBACCO

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Studies were initiated in 1982 on the incidence and spread of tobacco etch virus (TEV) and tobacco vein mottling virus (TVMV) in a ca 1/2-acre experimental plot of tobacco. The initial incidence and subsequent spread of TEV and TVMV followed a distinct and interesting pattern. Fortuitously, the initial incidence of infected plants occurred at the same time (July 1), and in separate areas of the field. Seven TEV-infected plants occurred near the southeast corner of the field, and one TEV-infected plant occurred at the south end. A single TMV-infected plant occurred near the northwest corner of the field. These initial infections appeared to act as sources for virus spread; virtually all new infections for a 3-wk period occurred near these foci. A few scattered new foci occurred in other areas of the field toward the end of this period. These later resulted in secondary spread. However, even after 5 wk, virus incidence was strongly associated with the initial foci. Time of virus spread was strongly correlated with increased colonization and spread of M. persicae.

The data suggested that virus introduction from outside seed sources was sporadic and that the few primary infections which occurred served as sources for spread by M. persicae, most likely alates which made short flights to nearby plants. We then attempted to assess the relative importance of colonizing aphids on virus spread by using insecticides to suppress colonization. To do this, we reasoned that control and insecticide-treated plots should not be adjacent, to avoid spread from virus-infected, aphid-infested control plants into the insecticide plots. The plots also needed to be large enough to minimize border effects.

We identified six locations and established three pairs of plots of ca 1/2 acre each for comparison. The plots in each pair were in similar ecological situations, but separated by 250-1000 yards. Standard procedures for the cultivation of burley tobacco were used. One plot of each pair was treated with insecticide to suppress colonization by aphids (Myzus persicae). Disyston 15 G was applied at the rate of 4 lb per acre of active ingredient immediately prior to transplanting. Orthene 75% EC was applied at the rate of 0.75 lb active ingredient per acre at approximately 2-wk intervals or more often if there was evidence of the initiation of aphid colonies. The experiment was carried out over a 3-yr period, 1983-1985. In order to attempt to compensate for the effect of plot location, the insecticide treatment was applied to the plots on an alternate year basis; the plots treated in 1983 and 1985 were untreated in 1984 and vice versa.

The fields were monitored for virus-infected plants once a week. Newly-infected plants were marked and the infecting virus was recorded.

The symptoms caused by TEV and TVMV are distinctive enough to allow visual discrimination between these viruses and also to distinguish them from the other viruses which sometimes occurred in these plots. For the purpose of this presentation, the combined incidence of the two viruses will be considered.

With two of the paired plots, insecticide treatment was usually effective in reducing or, in years of heavy "virus pressure," delaying, the incidence of virus-infected plants. For field pair #1, virus incidence was reduced in 1983 and 1984, but not in 1985. Field pair #2 had reduced incidence in the insecticide-treated plots in all three years. The results with field pair #3 were, however, highly variable. Virus incidence in the treated plots was reduced in 1985, virtually equal to the control in 1984, and increased in 1983.

The results suggest, not surprisingly, that colonizing aphids may be important in spread of nonpersistent viruses. Their relative importance appears not to be consistent or, at present, predictable. Our results are consistent with previous reports, some of which have found insecticides effective and others ineffective in reducing virus incidence.

EPIDEMIOLOGY AND CONTROL OF TOMATO LEAF CURL VIRUS IN INDIA

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Tomato plants were susceptible to tomato leaf curl virus (TLCV) infection at all stages of their growth. In summer tomato (cv. Pusa Ruby) crop, 94%, 90% and 78% loss in yield was observed when tomato plants were infected 2, 4 and 6 wk after planting, respectively. However, when the plants were infected 10 wk after planting the loss in yield was only 10.18%. The survey to assess the incidence of tomato leaf curl virus in some tomato growing areas of Karnataka, India revealed that the disease incidence varied from 6.4 to 52.2% in Kharif (June-October) and 52.5 to 100% in crops grown in summer (February-May). In general TLCV incidence and vector populations were high in late December to May planted crops and low from late June to early December planted crops. A high positive correlation was obtained between the percentage of TLCV incidence and whitefly populations. In March (summer) planted crop, the disease appeared 2 wk after planting and initially spread was slow but from 5 wk onwards the incidence increased rapidly reaching 100% by 11 wk. In July (Kharif) planted crops, symptoms were first observed 3 wk after planting, increased slowly and reached 58.83% at the end of 14 wk after planting. In November (Rabi) planted crops, symptoms first appeared 4 wk after planting and a maximum of 66.2% incidence was observed 14 wk after planting. High temperature, low or no rainfall and low humidity contributed to the increase in vector populations from January to May. The low whitefly population during the months of June to November was related to high rainfall, low temperature and high humidity. Whitefly populations were positively correlated with maximum and minimum temperature but negatively correlated with relative humidity. The presence of very low populations of whitefly during the cooler part of the year may be attributed to the influence of temperature rather than humidity. Yellow water pan traps (plastic plate of 30 cm diameter) attracted many whiteflies whereas red, blue and green color plates attracted very few numbers. Yellow water pan traps can be conveniently employed for monitoring whitefly populations in the field. Yellow sticky cylindrical traps kept at 45 cm height from the ground level trapped more whiteflies than the traps kept at 150 cm and 300 cm heights.

Whitefly flight activity was observed throughout the day with whiteflies being trapped from morning to evening. TLCV was transmitted by whitefly Bemisia tabaci to Acanthospermum hispidum, Ageratum conyzoides, Bidens biternata, Capsicum annuum, Centratherum anthelminticum, Datura stramonium, Euphorbia geniculata, Galinosoga parviflora, Lycopersicon esculentum, Nicotiana glutinosa, N. tabacum, Physalis floridana and Sonchus oleraceus. Whitefly B. tabaci was observed in nature on 142 plant species belonging to 23 different families. Two hundred and sixty-two tomato lines were screened for TLCV under field conditions during summer seasons of 1983 and 1984. Two lines of L. hirsutum and one line of L. glandulosum were resistant to TLCV. A nylon

net (cage) frame (10 ft long, 3.6 ft wide and 1 to 1-1/4 ft high) was suggested for covering tomato nursery beds to prevent the entry of whiteflies, B. tabaci, carrying TLCV. A combined treatment of nylon net covering for tomato nursery for 25 to 30 days and 3-4 sprays of insecticides, each at 10-day intervals after transplanting, delayed TLCV incidence 3 to 5 wk and increased the yields considerably. Erytmocerus mundus and Encarsia sp. parasitized the third instar and pupae of B. tabaci maintained on cotton in the laboratory. The percentage of parasitization was 8.94 to 16.12.

REINFECTION OF VIRUS-FREE, VEGETATIVELY PROPAGATED TUBEROUS BEGONIA

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The 'multiflora' group of the tuberous begonias has been propagated for generations in a vegetative manner. The genetic heterogenicity, as the result of the intensive selection, makes seedling culture practically impossible. Since this plant is grown outdoors, it is not unusual for plantings to be 100% virus-infected, especially with cucumber mosaic virus. Some years ago the in vitro culture of this group of tuberous begonias became possible. This, combined with thermotherapy (2 months at 36-38 C), allows virus-free plants to be produced (1).

Because begonias are grown outdoors the problem is how to protect healthy plants from reinfection and consequently to set up a reliable method for mass or routine indexing. Immuno diffusion serology with begonia is practially impossible (2). It is evident that the ELISA technique would be an optimum solution for such a problem. In spite of the previous negative experiences with serology, the possibilities of using the ELISA technique for large epidemiological surveys were investigated.

Our objectives were to compare absorbance values between different lots of samples and to determine the rate of reinfection in the field. We also needed to know the effect of different methods of protection and to be able to decide when the infected plants had to be replaced, that is, how long the original stock could be used. Absorbance values of virus-infected plants were clearly greater than those from healthy plants when the test samples were above pH 7. Because crude begonia sap is very acidic (pH 2), samples had to be diluted and a buffer with a high molarity (pH 7.5, 0.4 M) was necessary to obtain the optimal pH. Virus concentration in the sap does not seem to be a limitation since, at the optimal pH, test samples may be diluted several-fold without much decrease in absorbance values.

We demonstrated earlier that highest absorbance values are obtained at the end of the growing season (2). That is also the most important time to sample because sampling at the end of the growing season gives a better indication of the reinfection rate of tubers at harvest. Tests from April to mid-September are possible, but the results will be more difficult to interpret due to the lower absorbance values. Tables 1 and 2 show the absorbance values (E_{405}) obtained with ELISA from two cultivars at two different places at the end of the growing season. It is very obvious that there is a real difference between the mean of the absorbance values of virus-infected plants and in vitro cultured plants. It can be concluded that at the end of the growing season some reinfection had taken place in the groups of in vitro cultured plants.

One of the first results of these tests was that it was possible to state that the use of insect-proof tissue, such as is commonly used in

cultural practice, had little effect as a method for protection from reinfection. This screen was removed too frequently for normal weed control during which time aphids could have encountered these plants. The tests with the application of mineral oil sprays were rather promising.

It seems possible to determine reinfection rates with CMV of lots of virus-free begonia plants by comparing the distribution of absorbance values. It is true that in the spring and summer months absorbance values of even old virus-infected plants are too low to make a reliable determination of infection with only one test. It is not unusual to obtain false negative results from virus-infected plants. It is also difficult to determine the strain of CMV involved with one test on a single leaf.

It is well known that the concentration of CMV is variable from one day to another in many plants. This is also true in the case of begonia. The purpose here was only to check the possibility of following reinfection of a population of virus-free plants during culture in the open field.

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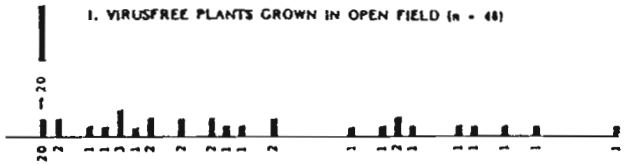
Table 1.

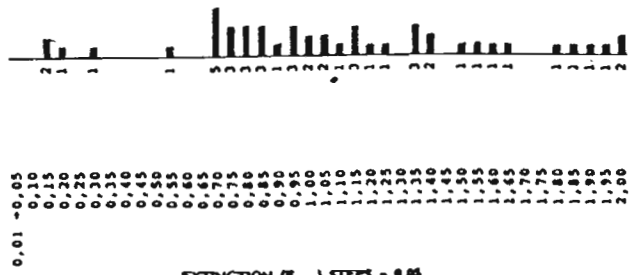
FREQUENCY HISTOGRAMS OF E_{405} VALUES AFTER ELISA ON DIFFERENT BEGONIA TUBERHYBRIDA SAMPLES.
(Date: 10th of October)
cv 'FRAU HELENE HARMS'

1. VIRUSFREE PLANTS GROWN IN OPEN FIELD (n = 48)



2. VIRUSFREE PLANTS GROWN UNDER INSECT-PROOF TISSUE (n = 48)

3. VIRUS INFECTED PLANTS GROWN ON OPEN FIELD (n = 48)

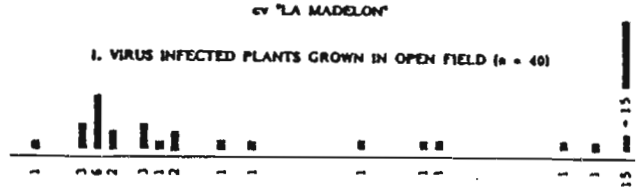


EXTINCTION (E_{405}) STEPS = 0.05

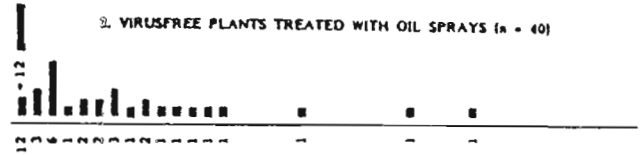
Table 2.

FREQUENCY HISTOGRAMS OF E_{405} VALUES AFTER ELISA ON DIFFERENT BEGONIA TUBERHYBRIDA SAMPLES.
(Date: 10th of October)
cv 'LA MADELON'

1. VIRUS INFECTED PLANTS GROWN IN OPEN FIELD (n = 40)

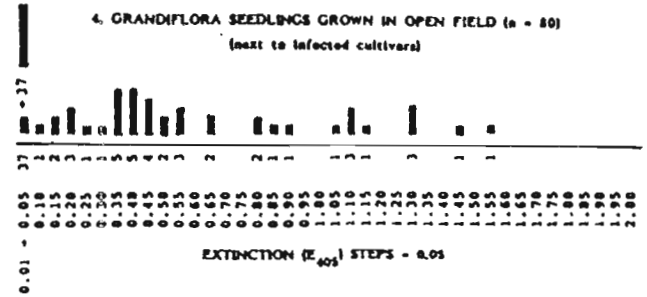


2. VIRUSFREE PLANTS TREATED WITH OIL SPRAYS (n = 40)



3. VIRUSFREE PLANTS GROWN IN OPEN FIELD (n = 40)

4. GRANDIFLORA SEEDLINGS GROWN IN OPEN FIELD (n = 80)
(next to infected cultivars)



EXTINCTION (E_{405}) STEPS = 0.05

CONTROL OF APHID-BORNE VIRUSES WITH OIL SPRAYS

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Oil sprays (JMS Stylet-Oil) have been used for the past 9 yr for control of aphid-borne viruses in vegetable crops. Crops (viruses) include peppers [tobacco etch virus (TEV), pepper mottle virus (PMV), and potato virus Y (PVY)], tomato (TEV), squash and cucumber [watermelon mosaic virus 1 (WMV1)] and watermelon (WMV1 and WMV2) in Florida. For the past 3 yr oil has been used successfully for control of WMV1 in melon and cucumber in the Dominican Republic, Guatemala, Honduras, Jamaica and Puerto Rico.

Oil sprays have been applied mostly with high pressure (28 bar) and Spraying Systems Co. hollow cone nozzles (TX-4 and -5 stainless steel) but also with back pack sprayers in the Caribbean and Central America. Field observations indicate the clear superiority of high pressure applications insofar as both control and phytotoxicity are concerned.

For maximum effectiveness, oil sprays should be started before infections have appeared in the field. In cucurbits we start spraying at 50 percent germination using twice-weekly applications until beds are covered, and weekly sprays thereafter. In slower growing crops (pepper and tomato) weekly sprays are sufficient for control).

Oil is used at a concentration of 0.75 percent. Sufficient gallonage (25-100 gal/acre) is used for thorough coverage to be obtained.

Phytotoxicity from oil has not been a problem on any crop so long as high spray pressure and the recommended nozzles are used. Crops sprayed include the above as well as tobacco, papaya, sweet corn, beans and potato.

Oil is used as a tank mix with fungicides and insecticides. Most insecticides are compatible with oil but care must be taken in selecting fungicides as phytotoxicity can result. Daconil and cataphol are incompatible with oil. Maneb, benomyl, triadimefon and metalaxyl are compatible. Fixed coppers should be avoided because of nozzle erosion problems.

Control of foliar fungus diseases using oil and maneb has been excellent. Diseases controlled include downy mildew, Alternaria, and gummy stem blight on cucurbits. Control of downy mildew has been so good that use of metalaxyl has not been necessary. Copper and maneb are used in combination for control of bacterial leafspot on pepper and tomato. A water soluble copper (copper ammonium carbonate) is used with a flowable formulation of maneb.

The cost of oil for virus control is from $30-40.00 US per acre for season-long spraying.

CONTROL OF PAPAYA RINGSPOT VIRUS BY SEEDLING INOCULATION WITH MILD VIRUS STRAINS IN TAIWAN

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In the past decade, a destructive disease caused by papaya ringspot virus (PRV), a potyvirus, has become the major limiting factor for growing papaya in Taiwan. Several attempts to develop effective control measures, such as escaping infection by planting papaya in the season of low alate aphid numbers, intercropping with a high-stem barrier like corn, eradication of diseased plants in orchards, spraying with mineral oil and systemic insecticides, and protecting young seedlings with plastic bags after transplanting, have proved either ineffective or only of marginal benefit. The severe crop losses, the unavailability of PRV-resistant papaya varieties, the difficulty of eradication, and the restrictive host range of PRV make cross protection an attractive method of controlling this virus.

Cross protection of plant viruses is a phenomenon in which plants systemically infected with one strain of a virus are protected from the effects of infection by a second related strain of the same virus. Large-scale application of cross protection has been reported for the control of tobacco mosaic virus in tomato in European countries and Japan, and for the control of citrus tristeza virus in citrus in South America. The key for these practical applications of cross protection is the availability of a mild virus strain that does not cause severe damage but provides a high degree of protection to the crop preimmunized.

Two PRV mutants, designated as PRV HA 5-1 and 6-1, which cause symptomless infection in papaya under greenhouse conditions, were obtained from nitrous-acid mutagenic treatments at Cornell University in 1982. Cross-protection effectiveness of the mutants was evaluated under greenhouse conditions from October 1982 to April 1983. Either complete or a high degree of protection was observed when PRV HA 5-1 was used to protect papaya against the severe effects of a Hawaii strain, indicating a good potential for the use of the mutants as protectants for the control of PRV.

The potential of mild virus mutants for control of PRV was further evaluated under greenhouse conditions in Taiwan. Neither PRV HA 5-1 nor 6-1 caused severe damage on the major commercial papaya varieties and both strains induced symptomless infection in the test plants of Chenopodiaceae and Cucurbitaceae. This indicated that possible damage to the protected crop and other crops in the vicinity would be minimal. Also, under greenhouse conditions, HA 5-1 and 6-1 provided a high degree of protection in papaya against the severe effect of two prevalent PRV strains of Taiwan. A very efficient method of mass inoculation was obtained by using a spray gun with a standard nozzle of 1.2 nm and pressure of 4-8 kg/cm at 10-20 cm distance. In general, both mutants

meet the requirement as a useful protectant strain and have a great potential for control of PRV by cross protection in Taiwan.

Cross-protection effectiveness of mild mutants of PRV was investigated further under field conditions in Taiwan beginning in the fall of 1983. When the protected papaya were mixed with unprotected control plants at random or row by row under high challenge pressure, unprotected plants showed severe symptoms 2-3 mo after planting and the protected plants showed severe symptoms 1-2 mo later than the control but no economic benefit was obtained because the breakdown happened before fruitset. However, in a solid-block test where the challenge pressure inside the test orchard was minimized by rogueing once every ten days prior to fruitset, protected trees showed 82% increase in yield, resulting in 111% increase in income because of better fruit quality, compared to the control.

Due to the initial success in the field trials, the government proceeded with large-scale planting in the spring and fall of 1984 with 4,000 protected plants (22ha) and 200,000 protected plants (100ha) in the field, respectively. At the end of 1984, the average disease incidence of protected orchards from the spring planting was 31.1%, compared to 82% of that of unprotected controls. The average fruit yield per tree increased from 7.3kg for unprotected trees to 17.9kg for protected ones. The income of the growers from the protected field was 109% more than that from unprotected ones. Similar results of the fall planting were also noticed. The preliminary data of large-scale trials, using the symptomless mutant as a protective strain, indicated a very significant reduction of severe disease incidence and a tremendous increase in the fruit yield of papaya.

After the success in the fall planting of 1984, the Council of Agriculture of the Republic of China expanded the protected orchards up to 200 hectares in the fall of 1985. More than 610,000 papaya seedlings were preinoculated with PRV HA 5-1 or 6-1 and then released to the field. Moreover, more than one million papaya plants (500ha) will be released in the coming fall of 1986.

Using the induced mild virus mutant to preimmunize papaya seedlings for control of PRV may become a routine practice in Taiwan. This will be the world's first case of a successful large-scale application of cross protection to control an aphid-nonpersistently-transmitted potyvirus.

NEARLY UNBIASED ESTIMATION OF NONLINEAR PREVALENCE FUNCTIONS

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The subject for this session is disease prevalence [frequency of occurrence, proportion affected, incidence, or rate, though this last term is often inappropriate as noted by Elandt-Johnson, (5)]; prevalence may be absolute or relative to reference populations and conditions. Investigation of disease incidence is one definition of epidemiology: a narrow definition perhaps, but not so thin as to restrict attention solely to epidemics (epiphytotics in the present context), and quantitative characterization of spatial and temporal patterns of disease prevalence, or of relationships between prevalence and measurable biotic and abiotic concomitants, leads naturally to an integrated view of epidemiology within the crop ecosystem. From this viewpoint there is no meaning to 'plant virus epidemiology'; rather, it is the prevalence of diseases, mostly endemic and with viruses as etiologic agents, in plant populations at risk, that forms our subject matter.

The prevalence functions discussed here are transforms of frequency parameters, such as odds and their corresponding logits, fractional powers, logarithms and angular transforms, in cases of a single frequency parameter θ; but ratios, odds-ratios and their corresponding logit contrasts, in cases of two or more frequency parameters θ_1, θ_2, θ_3, ... There are several motives for interest in such functions. First, for purposes of inference about, or reporting of, those aspects of prevalence appropriate to specific formulations of risk, given observations from either surveys or controlled experimentation, it may be preferable to estimate some function, $g(\theta)$ or $g(\theta_1,\theta_2)$, rather than the θ parameters themselves because that function is a better expression of the inference or of the implications of prevalence variation: for example, the odds-ratio $\theta_2(1-\theta_1)/\theta_1(1-\theta_2)$ when expressing the risk in conditions producing frequency θ_2 relative to conditions producing frequency θ_1. Second, when

attempting to relate prevalence to controlled or naturally observed variables concomitant to, and even prerequisites for, transmission and progress of disease, the relationship may be formulated in terms of response $g(\theta)$ rather than θ. For example, a $\log(\theta)$-linear relation or a $\text{logit}(\theta)$-linear relation may be tenable when a θ-linear relation is not (especially with the constraint $0 \le \theta \le 1$). Third, estimates of $g(\theta)$ may be required when comparing field observations with predictions derived from epidemiological models or simulations.

Estimation of functions $g(\theta)$. Here it is assumed that a sample of N units (plant parts, plants, families, cohorts, fields) yields observed count R of incidents in the diseased/healthy dichotomy scored without ambiguity under conditions in which it can be assumed that R follows a Binomial distribution with frequency parameter θ: $\boldsymbol{E}\{R\} = N\theta$. The maximum likelihood estimator of $g(\theta)$ is $g(\hat{\theta})$, $\hat{\theta} = R/N$, and depending on the nonlinearity of function $g(\bullet)$, may be subject to troublesome bias: $\boldsymbol{E}\{g(\hat{\theta})\} = g(\theta) + \text{Bias}\{g(\hat{\theta})\}$. For an example of such bias see Swallow (8) where $g(\theta) = \theta^{1/k}$ in a context of multiple transfers of pathogen vectors with k vectors per transfer. While bias alone is not a totally disqualifying property of the maximum likelihood estimator, an alternative estimator of similar or improved efficiency and smaller bias would be preferred if available. Henceforth, $S = (N-R)$, $g_j(\theta)$ denotes $\partial^j g(\theta)/\partial\theta^j$, and O_k signifies terms with absolute values that decrease to zero at least as fast as N^{-k} as N increases.

The alternative estimator for $g(\theta)$ presented here takes the form $g(T)$ with $T = (R+a)/(N+b)$ instead of $\hat{\theta} = R/N$; a and b are constants specific to $g(\bullet)$, independent of θ, R and N, and satisfying $b \ge a \ge 0$ so that $0 \le T \le 1$ for all R. When available, this estimator is nearly unbiased in the sense that

$$\text{(i)} \qquad \boldsymbol{E}\{g(T)\} = g(\theta) + O_2$$

under favorable conditions (usually $N(1-\theta) > 1$ and $N\theta > 1$). The form $g(T)$ is an extension of a device suggested by Anscombe (2) who observed that if the same constant a is added to both R and S (equivalent to adding a to R and 2a to $N = R+S$), then $g([R+a]/[N+2a])$ is approximately unbiased for $g(\theta)$ if $g_1(\theta)$ is proportional to $[\theta(1-\theta)]^{-2a}$. This includes the variance stabilizing transform

$g(\theta) = \arcsin(2\theta-1)$ when $a = 1/4$, and the logit transform $g(\theta) = \log[\theta/(1-\theta)]$ when $a = 1/2$, the last result having been given previously by Haldane (7); for another approach using polygamma functions, see Cook, Kerridge and Pryce (4).

Relaxation of the restriction $b = 2a$ yields a larger family of functions $g(\theta)$ for which $g(T)$ is nearly unbiased: included now are all functions $g(\theta)$ with $g_1(\theta)$ proportional to $\theta^{-2a}(1-\theta)^{-2(b-a)}$ as shown in the Appendix.

Properties of $g(T)$. In addition to (i), several properties of estimator $g(T)$ are listed next:

(ii) $g(\hat{\theta})$ is recovered as the special case $a = b = 0$,

(iii) nearly unbiased estimators for Anscombe's family of $g(\theta)$ are recovered as special cases $b = 2a$,

(iv) $g(T)$ shares several properties with $g(\hat{\theta})$: it is range preserving; it is a function of the sufficient statistic R and so is expected to be efficient; it is Normally distributed asymptotically (with N),

(v) central moments $\text{var}\{g(T)\}$, $\mu_3\{g(T)\}$ and $\mu_4\{g(T)\}$ are given in the Appendix to order O_3; this provides for approximate standard errors of the estimates and for comparison of μ_3 and μ_4 with the corresponding moments of a Normal distribution ($\mu_3=0$, $\mu_4=3\sigma^4$).

Useful examples of $g(T)$ for nonlinear prevalence functions. Most of the usable functions correspond to cases $b = a$ or $b = 2a$ or $b = 1$ or $a = 0$.

Negative and fractional powers:

When $g(\theta) \propto \theta^{\lambda}$, with $\lambda < 1$, $g_1(\theta) \propto \theta^{\lambda-1}$ so that $b = a = (1-\lambda)/2$ and $g(T) = [(2R+1-\lambda)/(2N+1-\lambda)]^{\lambda}$. For the function 'odds against', $(1-\theta)/\theta = \theta^{-1}-1$ and $\lambda = -1$ yields the nearly unbiased estimator as $(N-R)/(R+1)$ with variance $\theta^{-2}\{[(1-\theta)/N\theta] + 2[(1-\theta)/N\theta]^2 + O_3\}$. In the context of group testing, both for bulk tests of k seeds for presence of seed borne virus with probability p per individual seed, and for multiple vector transfers of k vectors with pathogen transmission rate p per individual vector, $\theta = (1-p)^k$ and estimation of p invokes

$g(\theta) \propto \theta^{1/k}$: setting $\lambda = 1/k$ yields the nearly unbiased estimator as $[(2kR+k-1)/(2kN+k-1)]^{1/k}$ with variance $(1-p)^2\{[(1-\theta)/N\theta k^2] + \frac{1}{2}[(k-1)(1-\theta)/N\theta k^2]^2 + O_3\}$. For this case it has been shown that bias and mean square error properties of the nearly unbiased estimator are uniformly superior to those of the maximum likelihood estimator (3). Cases involving $g(\theta) \propto (1-\theta)^{\lambda}$ yield $a = 0$, $b = (1-\lambda)/2$ and can be solved by reversing the roles of R and (N-R) in results for $g(\theta) \propto \theta^{\lambda}$. Thus when 'odds in favor' is required, $\theta/(1-\theta) = (1-\theta)^{-1}-1$ and the nearly unbiased estimator is R/(N-R+1) with variance $(1-\theta)^{-2}\{[\theta/N(1-\theta)] + 2[\theta/N(1-\theta)]^2 + O_3\}$. Another function of interest is $g(\theta) \propto [\theta(1-\theta)]^{-1} = [\theta^{-1} + (1-\theta)^{-1}]$: results for θ^{-1} and $(1-\theta)^{-1}$ above can be added to yield the nearly unbiased estimator (N+1)(N+2)/(R+1)(N-R+1), and this is the estimator recommended by Gart and Zweifel (6).

Logarithms and logits:

When $g(\theta) \propto \log(\theta)$, $g_1(\theta) \propto \theta^{-1}$ so that $b = a = 1/2$ and the nearly unbiased estimator is $\log[(R+1/2)/(N+1/2)$ with variance $\{[(1-\theta)/N\theta] + \frac{1}{2}[(1-\theta)/N\theta]^2 + O_3\}$. Similarly, when $g(\theta) \propto \log(1-\theta)$, $g_1(\theta) \propto (1-\theta)^{-1}$ so that $a = 0$, $b = 1/2$ and the nearly unbiased estimator is $\log[(N-R+1/2)/(N+1/2)]$ with variance $\{[\theta/N(1-\theta)] + \frac{1}{2}[\theta/N(1-\theta)]^2 + O_3\}$. For the function $g(\theta) = \text{logit}(\theta) = \log[\theta/(1-\theta)] = \log(\theta) - \log(1-\theta)$, these last two results can be combined to yield the nearly unbiased estimator as Haldane's function $\log[(R+1/2)/(N-R+1/2)]$, but to obtain the variance it is simpler to work directly from $g_1(\theta) \propto [\theta(1-\theta)]^{-1}$ with $b = 2a = 1$, and obtain the variance as $\{[N\theta(1-\theta)]^{-1} + \frac{1}{2}[N\theta(1-\theta)/(1-2\theta)]^{-2} + O_3\}$.

Angular transforms:

When $g(\theta) \propto \arcsin(\theta^{1/2})$, $g_1(\theta) \propto [\theta(1-\theta)]^{-1/2}$ so that $b = 2a = 1/2$ and the nearly unbiased estimator is $\arcsin[(R+1/4)/(N+1/2)]^{1/2}$ as first given by Anscombe (1); the variance is $\{(4N)^{-1} - 2(4N)^{-2}[1 - 1/4\theta(1-\theta)] + O_3\}$. The alternative choice $b = 2a = 3/4$ has the remarkable property that var $\{g(T)\}$, to order O_3, is not a function of θ: $\text{var}\{g(T)\} = \{(4N)^{-1} - 2(4N)^{-2} + O_3\}$. This choice is more in keeping with the intention of a 'variance stabilizing' transform, but the corresponding bias is O_1: $E\{g(T)\} = \arcsin(\theta^{1/2}) + (1-2\theta)/16N[\theta(1-\theta)]^{1/2} + O_2$. Similar results are obtained for $g(\theta) \propto \arcsin(2\theta-1) = [2\arcsin(\theta^{1/2}) - \pi/2]$.

An example. Observations from one of a sequence of experiments conducted by O. W. Barnett in 1977 provide an example of nearly unbiased estimation of $\theta^{1/k}$, $\log(\theta)$ and $(1-\theta)/\theta$. Apterous green peach aphids (Myzus persicae) were permitted a five minute acquisition feed on Alsike clover (Trifolium hybridum L.) infected with pea mosaic virus (PMV-204-1), and then transferred in groups of sizes k = 1, 5, 10, 20 or 40 aphids to individual Alsike clover plants for a four hour inoculation feed. Each group size was repeated twenty times (N = 20 plants) and counts of healthy plants (R) were recorded 3-4 weeks later.

If p denotes virus transmission rate per individual vector, the expected healthy proportion is $\theta = (1-p)^k$ only if it can be assumed that vectors transmit independently, even though transferred in groups, and that test plants are uniformly susceptible and respond independently.

Parameter θ is estimated unbiasedly (and with minimum variance among unbiased estimates) by the ratio (R/N) for each k. But estimation of p via $(1-\theta^{1/k})$, by maximum likelihood or by the nearly unbiased estimator given in the previous section, depends on the assumption of independent transmission, which therefore requires examination. If this assumption is valid then the quantity $\Delta = k^{-1} \log(\theta)$ should be constant for all k [namely, $\Delta = \log(1-p)$]. The nearly unbiased estimator for $\log(\theta)$ does not depend on the validity of the assumption in question: it is $\log[(R+1/2)/(N+1/2)]$ with variance given in the previous section. Thus, for each k, the nearly unbiased estimator for Δ is $\tilde{\Delta}_k = k^{-1}\log[(R+1/2)/(N+1/2)]$ with

$$\text{var}\{\tilde{\Delta}_k\} = [(1-\theta)/N\theta k^2] + 0_2 \ .$$

Here, and subsequently, the symbol ~ signifies nearly unbiased estimate. Estimates of $\text{var}\{\tilde{\Delta}_k\}$ are required in order to judge consistency of estimates $\tilde{\Delta}_k$ with a hypothesized constant value Δ. Nearly unbiased estimates of $\text{var}\{\tilde{\Delta}_k\}$ are obtained from those of $(1-\theta)/\theta$ given previously, namely (N-R)/(R+1). Thus $\tilde{\text{var}}\{\tilde{\Delta}_k\} = (N-R)/N(R+1)k^2$, the square root of which provides the corresponding standard error, $\text{s.e.}\{\tilde{\Delta}_k\}$. The following table contains observed counts R together with $100\tilde{\Delta}_k$ and $\text{s.e.}\{100\tilde{\Delta}_k\}$ for each k = 1, 5, 10, 20, and 40.

Table 1. Results* of group testing for aphid transmission of PMV in Alsike clover.

k	N	R	$100\tilde{\Delta}_k \pm$ s.e.	$\tilde{p}_k \pm$ s.e.
1	20	19	-5.001 ± 5.000	0.0500 ± 0.0500
5	20	16	-4.341 ± 2.169	0.0427 ± 0.0210
10	20	15	-2.796 ± 1.250	0.0276 ± 0.0122
20	20	9	-3.846 ± 1.173	0.0378 ± 0.0113
40	20	5	-3.289 ± 0.884	0.0324 ± 0.0086

*: symbol ~ signifies nearly unbiased estimate throughout except that $\tilde{p}$ is the unbiased estimate when k = 1 (only).

Variation among $\tilde{\Delta}_k$ values is insignificant relative to their standard errors and so the assumption of independent transmission behavior is tenable for groups of sizes $k \leq 40$ aphids. Nearly unbiased estimation of $p = (1-\theta^{1/k})$ can therefore proceed from that given previously for $\theta^{1/k}$:

$$\tilde{p}_k = 1 - [(2kR+k-1)/(2kN+k-1)]^{1/k}$$

$$\text{with } \text{var}\{\tilde{p}_k\} = \theta^{2/k}\{[(1-\theta)/N\theta k^2] + 0_2\} = N^{-1}k^{-2}[\theta^{(2-k)/k} - \theta^{2/k}] + 0_2 .$$

Nearly unbiased estimation of $\text{var}\{\tilde{p}_k\}$ follows from the general formulation for $g(\theta) = \theta^{\lambda}$: -

when k = 1, $R(N-R)/(N-1)N^2$ is exactly unbiased for $\text{var}\{\tilde{p}_1\} = \theta(1-\theta)/N$,

when k > 1, $\tilde{\text{var}}\{\tilde{p}_k\} = N^{-1}k^{-2}\{[(kR+k-1)/(kN+k-1)]^{(2-k)/k} - [(2kR+k-2)/(2kN+k-2)]^{2/k}\}$.

Estimates $\tilde{p}_k$ together with their standard errors are given in Table 1. The appropriately weighted average of the five $\tilde{p}_k$ values yields an estimated transmission rate equal to 0.034 ± 0.006 per aphid.

Estimation of functions $g(\theta_1,\theta_2)$. When the prevalence function of interest involves two or more frequency parameters, θ_1, θ_2, θ_3, ..., a similar estimation device is possible. It is sufficient to consider functions of just two parameters, the extension to three or more being straightforward. Consider first the case where R_1 and R_2 are independent Binomial counts in samples of sizes N_1, N_2 with frequency parameters θ_1, θ_2 respectively, and let $T_i = (R_i+a_i)/(N_i+b_i)$, i = 1, 2. Then there exists a family of functions $g(\theta_1,\theta_2)$ for each of which a_i's and

b_i's can be chosen to yield the nearly unbiased estimator $g(T_1,T_2)$:

(vi) $$\boldsymbol{E}\{g(T_1,T_2)\} = g(\theta_1,\theta_2) + O_2$$

where O_k now refers to $N = \min(N_1,N_2)$. In particular, if $g(\theta_1,\theta_2)$ is separable additively or multiplicatively, $g(\theta_1,\theta_2) = f(\theta_1) + h(\theta_2)$ or $g(\theta_1,\theta_2) = f(\theta_1).h(\theta_2)$, then results from the previous section (for a single parameter) can be combined accordingly. When $g(\theta_1,\theta_2) = \theta_2/\theta_1$ for example, the nearly unbiased estimator is $R_2(N_1+1)/N_2(R_1+1)$ with variance $(\theta_2/\theta_1)^2\{[1 + (1-\theta_1)/N_1\theta_1][1 + (1-\theta_2)/N_2\theta_2] - 1 + 2[(1-\theta_1)/N_1\theta_1]^2 + O_3\}$. The odds-ratio, $g(\theta_1,\theta_2) = \theta_2(1-\theta_1)/(1-\theta_2)\theta_1$, is also separable multiplicatively and the nearly unbiased estimator is $R_2(N_1-R_1)/(N_2-R_2+1)(R_1+1)$ with variance $[\theta_2(1-\theta_1)/(1-\theta_2)\theta_1]^2\{[1+1/N_1\theta_1(1-\theta_1)][1+1/N_2\theta_2(1-\theta_2)] - 1 + 2[N_2(1-\theta_2)]^{-2} + 2[N_1\theta_1]^{-2} + O_3\}$. The logit contrast, being the logarithm of the odds-ratio, is separable additively: $g(\theta_1,\theta_2) = [\text{logit}(\theta_2) - \text{logit}(\theta_1)]$, and so the nearly unbiased estimator is $\log[(R_2+1/2)(N_1-R_1+1/2)/(N_2-R_2+1/2)(R_1+1/2)]$ with variance $\{[N_1\theta_1(1-\theta_1)]^{-1} + [N_2\theta_2(1-\theta_2)]^{-1} + \frac{1}{2}[N_1\theta_1(1-\theta_1)/(1-2\theta_1)]^{-2} + \frac{1}{2}[N_2\theta_2(1-\theta_2)/(1-2\theta_2)]^{-2} + O_3\}$. Similar results are available when R_1 and R_2 are selected counts from a multinomial sample, with the slight complication that R_1 and R_2 are not independent in that case.

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APPENDIX

Moments of g(T). The derivations are based on expectations of functions h(T) expanded in a Taylor series about $T = \theta$:

$$h(T) = h(\theta) + \sum_{j=1}^{\infty} (T-\theta)^j h_j(\theta)/j!$$

Let $Z = (R-N\theta)$ so that $(T-\theta) = [Z + (a-b\theta)]/(N+b)$ and $\boldsymbol{E}\{Z\}$ = zero, $\boldsymbol{E}\{Z^2\} = N\theta(1-\theta)$, $\boldsymbol{E}\{Z^3\} = N\theta(1-\theta)(1-2\theta)$ and $\boldsymbol{E}\{Z^4\} = 3N^2\theta^2(1-\theta)^2 + N\theta(1-\theta)[1-6\theta(1-\theta)]$. Substitution in $\boldsymbol{E}\{h(T)\}$ with $h(T) = [g(T)]^r$ for each of r = 1, 2, 3, and 4, and collecting terms in negative powers of N, yields

(A1) $$\boldsymbol{E}\{g(T)\} = g + N^{-1}B(\theta) + O_2$$

(A2) $$\text{var}\{g(T)\} = N^{-1}\theta(1-\theta)g_1^2 + N^{-2}[2\theta(1-\theta)g_1B_1'(\theta) + \tfrac{1}{2}\theta^2(1-\theta)^2g_2^2] + O_3$$

(A3) $$\mu_3\{g(T)\} = N^{-2}[\theta(1-\theta)(1-2\theta)g_1 + 3\theta^2(1-\theta)^2g_2]g_1^2 + O_3$$

(A4) $\mu_4\{g(T)\} = 3[\text{var}\{g(T)\}]^2 + O_3$

where $B(\theta) = [(a-b\theta)g_1 + \frac{1}{2}\theta(1-\theta)g_2]$. Elimination of the term in N^{-1} from $E\{g(T)\}$, producing the nearly unbiased estimator for $g(\theta)$, requires $B(\theta) = \text{zero}$ so that g must satisfy the differential equation $2(a-b\theta)/\theta(1-\theta) = -g_2/g_1$ with solution $g_1(\theta) \propto \theta^{-2a}(1-\theta)^{-2(b-a)}$. When this is true, (A1), (A2) and (A3) reduce to

(A1′) $E\{g(T)\} = g + O_2$

(A2′) $\text{var}\{g(T)\} = g_1^2[N^{-1}\theta(1-\theta) + N^{-2}2(a-b\theta)^2 + O_3]$

(A3′) $\mu_3\{g(T)\} = g_1^3\{N^{-2}\theta(1-\theta)[(1-2\theta) - 6(a-b\theta)] + O_3\}$

Observe that the only function $g(\theta)$ for which $\mu_3\{g(T)\} = O_3$ has $g_1(\theta) \propto [\theta(1-\theta)]^{-1/3}$, which is in Anscombe's family with $a = 1/6$, but appears to be of little interest.

A SIMPLE MODEL TO FORECAST MAIZE ROUGH DWARF VIRUS EPIDEMICS IN MAIZE

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Maize rough dwarf virus (MRDV) occasionally causes a severe disease to maize in some Mediterranean, Asiatic and South American countries (1). It is vectored, in Northern Italy, by the planthopper, Laodelphax striatellus, whose migrating adults are responsible for infection mainly of spring-sown maize (2).

In this work a predictive model for the infection level in maize fields was developed applying the idea of vector pressure (3) to the combination MRDV-L. striatellus-maize.

The final proportion (p) of infected maize plants in the fields of a crop area was assumed to be related to the product of the density of the planthopper population (d) times the proportion of infective hoppers (i) in the local population ($d \cdot i = VP$). The increment of the final proportion (δp) for each increment of ($d \cdot i$) (δVP) was assumed to be proportional to the percentage of plants not infected (1-p) and to decrease with the decrease of (1-p), so that we could write:

$$\delta p / \delta VP = -h \cdot (1-p)$$

from which, rearranging and integrating:

$$-\ln (1-p) = h \cdot VP + Const$$

With MRDV, where no seed transmision is present, the integration constant (Const) was assumed equal to 0 and the equation became:

$$-\ln (1-p) = h \cdot d \cdot i$$

It was also assumed that the number of insects captured (c) in a standard 'sweep' of a crop area was directly proportional to the local density (d), so that: $d = j \cdot c$

We could therefore write, cumulating (j) and (h) in a constant (k):

$$-\ln (1-p) = k \cdot c \cdot i$$

or, in exponential form: $p = 1 - \exp(-kci)$

The trend of (p) according to the above equation and for different values of k can be seen in Fig. 1.

The linearized form of the equation was used to fit, with the least square method, the data for (i), (c) and (p) obtained during the years 1984 and 1985 in different areas of Northwestern Italy, where the virus is endemic and occasionally epidemic.

The parameter (c) was determined using a backpack motorized suction trap, sucking insects for constant times, with a constant air flow, in the different areas under observation. The number of planthoppers captured was then counted in each sample. The captures were made one week before maize sowing.

The captured insects (all of them or random samples if the number was too high) were tested for infectivity in an insect-proof glasshouse on maize and barley plants at the coleoptile stage. The proportion of infected plants was then transformed into the proportion of infective insects (i) using the maximum likelihood estimator when more than one insect per plant was tested.

The proportion of infected maize plants in the various areas examined was determined in July by visual inspection of at least 4,000 plants in each area. The equation established was:

$$-\ln (1-p) = 0.0545 \cdot c \cdot i \quad (r = 0.9834;\ D.F.:5;\ p < 0.001)$$

As can be seen from the value of the correlation coefficient (r), the agreement between values of (p) predicted by the model and the actual proportions of infected maize plants in the fields for both years was quite good. Moreover, the model with the calculated k = 0.0545 was able to 'predict' the values of (p) in a different area in a previous year (* in Fig. 2).

To become a useful tool for forecasting and preventing MRDV epidemics in maize, the system model needs more validation at the predictive level and the work involved and the time spent in doing the infectivity tests should be reduced. This could be done by using ELISA to detect the virus in the hoppers (4), although the value of (k) might alter slightly.

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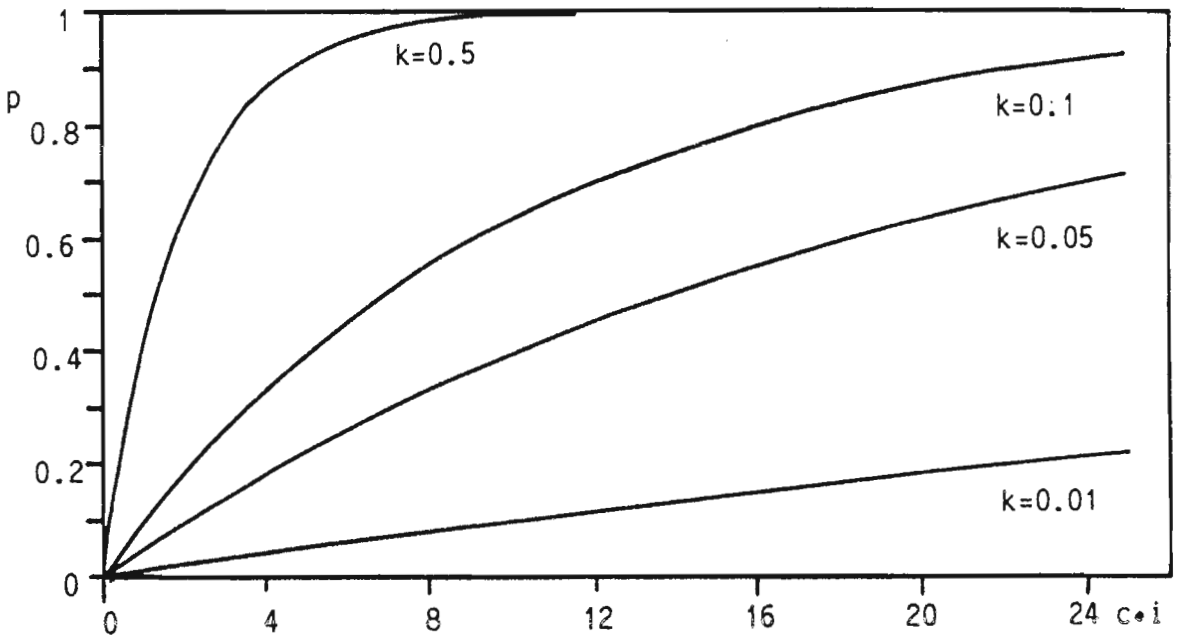

Fig. 1. Trend of $p = 1-\exp(-k \cdot c \cdot i)$ for different values of (k).

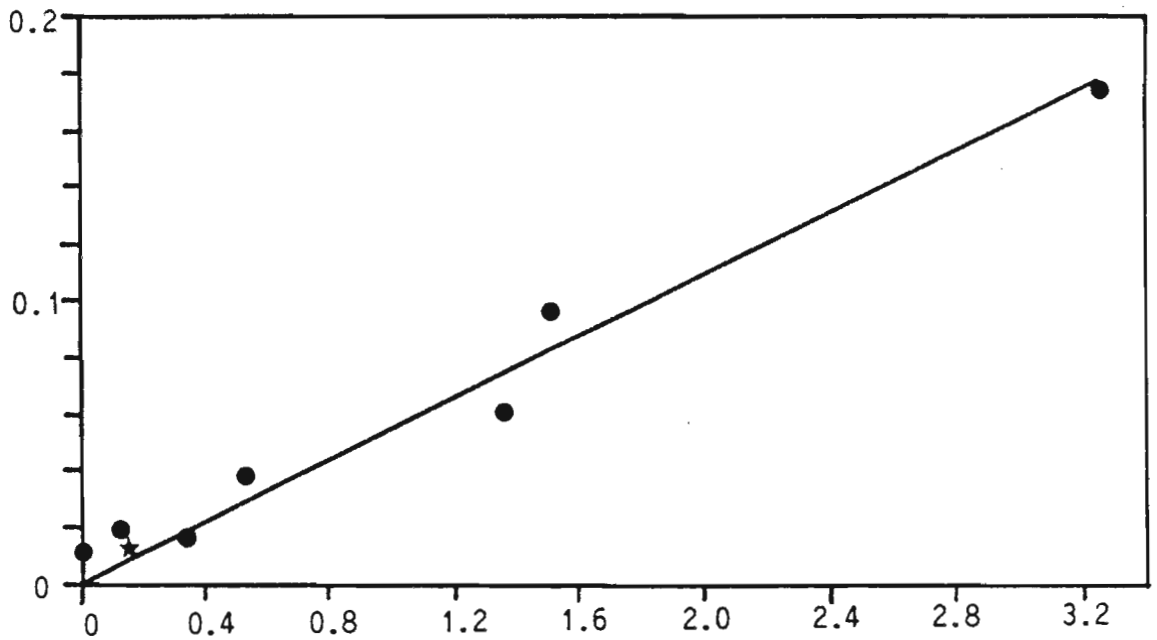

Fig. 2. Experimental values of $-\ln(p)$ as a function of $(c \cdot i)$ and the least square fitted line $-\ln(1-p) = 0.0545\ c \cdot i$.

MODELING THE SPREAD OF POTATO VIRUSES A AND V IN SEED POTATO CROPS

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Presently northern Ireland is somewhat unusual among seed potato producing countries in that PVA and PVV (1) are as common a reason for down-grading crops as is PVY. This situation is largely a product of the virus susceptibility/resistance characteristics of the most popular cultivars. Half the seed potato production in northern Ireland is of a few varieties resistant to PVY but susceptible to PVV and/or PVA.

Another, though lesser contributory factor, is the virtual absence of Myzus persicae, traditionally the vector of PVY and the greater abundance of other vectors, e.g., Brachycaudus helichrysi (Table 1).

These differences and the frequent occurrence of significant early-summer aphid transmission of viruses have prompted us to investigate the spread of non-persistent viruses in the field with the ultimate aim of constructing a model to predict (from aphid population and weather data) the timing and extent of PVA and PVV transmission.

METHODS

An experimental layout designed to monitor aphid populations and virus transmission from PVA- and PVV-infected plants throughout the summer has been operated at two sites since 1984. Data on aphid populations are obtained by water traps and a 12-m high suction trap; virus transmission is monitored by weekly changes of Nicotiana debneyi plants in pots spaced at 30-cm intervals from a double line of PVA- and PVV-infected potato plants. Plots of a susceptible potato cultivar are grown either side of the infector potatoes. Harvesting of random-selected plants evenly divided between 1 July, 1 August, and 1 September provides corroborative data on timing, quantity and gradient of virus transmission.

RESULTS

From a wide range of aphid species trapped, 10 have been selected (Table 1) for inclusion in multivariate analyses of aphid populations/virus transmission relationships because laboratory work at Newforge and the field study of Harrington et al. (2) have shown them to be significant vectors of non-persistent potato viruses. Phorodon humuli has been omitted because it was never found in our aphid traps.

Canonical correlation and correlation matrix analyses of the involvement of each aphid species have not pinpointed relationships between catch figures and virus incidence consistent for both sites but they have provided evidence for A. solani, B. helichrysi and M. euphorbiae being partly responsible for the spread of PVA and PVV.

A third approach, correlating virus transmission with a vector pressure index based on numbers of each species and the proportion that Harrington et al. (2) found to be viruliferous, was no more successful (Table 2). However this index, calculated from suction trap data, indicated that in 1984 maximum vector pressure occurred in June. Timed harvesting of healthy potato plants grown beside the infectors provided supporting evidence; by the first harvest at the beginning of July 80% of total progeny tuber infection had taken place.

Development. So far statistical analyses have been used to determine each aphid species' contribution to total vector transmission without much success. Annual accumulation of data will help with this approach which alone may eventually satisfactorily correlate virus spread with total vector pressure for the whole season. But for development of a model capable of predicting vector pressure throughout the summer, more direct methods of measuring species vector efficiency appear to be necessary.

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Table 1. Numbers of potato virus-transmitting species caught in a suction trap at Newforge in 1984 and 1985 and vector efficiency as ascertained by laboratory transmission studies.

Aphid species	No. trapped[a]		Vector efficiency for[b]		
	1984	1985	PVA	PVV	PVY
Aphis fabae	8	40			
Aphis spp.	35	43			
Brachycaudus helichrysi	603	452	0.2	0.4	0.2
Hyperomyzus lactucae	56	19			
Macrosiphum euphorbiae	123	42	0.3	0.5	0.3
Metopolophium festucae	104	53			
Myzus persicae	13	9	0.4	0.5	0.6
Rhopalosiphum insertum	1084	77			
Rhopalosiphum padi	292	506			
Sitobium avenae	727	759			

[a] Σ weekly catch from 1 June-1 September.

[b] Based on laboratory work in Agricultural Zoology Division.

Table 2. Weekly vector pressure at Newforge and percentage N. debneyi bait plants infected with PVA and PVV

	June				July					August		
	1	2	3	4	1	2	3	4	5	1	2	3
1984												
Suction trap	22.7[a]	24.7	3.3	0.6	3.5	0.6	0.6	1.8	1.5	0.5	0.4	1.2
Water trap	1.2	3.0	3.1	1.2	0.6	3.1	2.4	1.1	0.7	0.6	0.3	0.4
PVA	3	3	7	6	0	1	6	2	0	0	0	0
PVV	0	4	15	6	2	2	4	3	1	0	4	7
1985												
Suction trap	4.8	12.4	14.7	1.7	2.3	0.7	0.2	4.0	0.7	0.5	0.3	0.7
Water trap	0.7	0.3	4.3	1.3	0.2	0.9	0.4	1.1	0.3	0.2	0.1	0.02
PVA	-	-	7	-	1	7	5	5	8	11	5	2
PVV	-	-	0	-	10	6	12	5	7	8	4	10

[a]Vector pressure = Σ (number trapped x vector efficiency index) for species in Table 1. Vector efficiency index = proportion of viruliferous alatae (2).

FLUCTUATION OF THE POPULATION INFECTIVITY OF THE PLANTHOPPER VECTOR, LAODELPHAX STRIATELLUS, OF RICE STRIPE VIRUS AFTER AN INTRODUCTION OF RSV RESISTANT VARIETIES OF RICE

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An epidemic of rice stripe virus (RSV) was traced by measuring two parameters, vector density of the immigrating generation into paddy fields and the population infectivity. Vector density was estimated by a wind-borne tow net 1 m wide and 1.7 m long set 18 m above the ground. Population infectivity was measured by testing infectivity (virulency) individually by the hemagglutination reaction of sheep blood cells sensitized with RSV-antibody. Ten to 15 local populations of the vector, Laodelphax striatellus, in a range of 4 km at Konosu, Saitama were sampled at the overwintering and the first generation. Each population comprised 300 individuals or more. In the previous report (Kisimoto and Yamada, 1986) an epidemic model was proposed as follows:

$$P_n = vP_{n-1} + (1-vP_{n-1})\,[1-\exp(-mwH)]$$

in which P_n is the population infectivity at the n-th generation; v, the rate of transovarial passage; H, the final incidence of infected hills in paddy field; m, the proportion of infected hills at each generation against the final incidence. The formula means that the population infectivity is controlled by two factors, increase due to virus acquisition by non-virulent vectors from infected plants and decrease due to the transovarial passage of lower than 100%. The former is related to incidence of infected plants in the area which is controlled by number of infective vectors immigrating into the paddy field.

Table 1 shows that the epidemic was triggered by a sudden increase of vector density of the first generation in 1977, followed by a heavy RSV infestation on the early transplanted paddy field, such as on 20 May and 1 June. The population infectivity increased thereafter to 20% or greater. The severe level of RSV infection in rice continued even though the vector density decreased to the normal level after 1979. Because of the severity of RSV infection, an RSV resistant variety, Musasikogane, was introduced in 1982. The variety shows strong resistance to virus infection but is susceptible to vector infestation. The population infectivity began to decrease to the pre-epidemic level subsequent to introduction of the resistant variety.

The rate of transovarial passage was estimated as 0.9431 from the population infectivity of the overwintering and the first generation, when no virus sources were available from host plants of the vector. The rate of decrease of the population infectivity from P_1 to P_5 (P_0 of the next year) since the introduction of the resistant variety was shown to be larger than that due only to the rate of transovarial passage,

particularly in 1985 when the average rate of decrease per generation was 0.8880. The reason has not been elucidated yet.

Tabel 1. Epidemic of RSV in Konosu, Saitama, Japan.

Year	P_0	P_1	Vector[1] density	Rate of infection of RSV[2] 20 May	1 June	10 June	% of RV[3]
1973	0.1079	0.1086	941	54.4	17.9	5.6	0
1974	0.0851	0.0710	479	68.2	37.8	8.8	0
1975	0.0771	0.0721	614	39.1	27.8	21.9	0
1976	0.0792	0.0801	134	18.6	14.3	9.1	0
1977	0.0720	0.0664	2094	94.2	75.4	18.8	0
1978	0.1283	0.1271	1591	85.1	87.0	83.8	0
1979	0.1947	0.1624	611	95.6	93.5	92.6	0
1980	0.1897	0.1984	700	94.1	98.9	70.8	0
1981	0.1775	0.1670	576	90.8	93.0	63.9	0
1982	0.2228	0.2035	713	74.2	81.7	43.3	20
1983	0.2279	0.2192	891	95.0	-	47.1	40
1984	0.1692	0.1560	849	95.4	92.1	85.2	55
1985	0.1187	0.0960	228	44.6	42.5	23.5	65
1986	0.0597						

P_0 and P_1: Population infectivity at the overwintering and the first generation.

[1] Total catches of vectors by a tow net at the first generation.

[2] Nearly equal to the % of infected tillers.

[3] % of the acreage growing RSV resistant variety, Musasikogane, in the area.

TEMPORAL ANALYSIS OF TWO VIRUSES INCREASING IN THE SAME TOBACCO FIELDS

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In his classic book of 1963, "Plant Diseases: Epidemics and Control," Vanderplank demonstrated the relevance and utility of analyzing plant disease epidemics as rates of disease increase over time. For diseases with plant-to-plant spread, the logistic equation was presented as a heuristic model for epidemics. Since then many disease epidemics, including several caused by viruses, have been modeled and analyzed using the logistic or related models (3). Usually, pathogens are individually considered, i.e., disease intensity due to a single pathogen is related to time. In many cases, however, more than one virus disease increases concomitantly over time in a crop. One could analyze disease progress for each virus individually with the logistic model, but such an approach fails to incorporate the inhibitory effects of other viruses on the incidence of the studied virus.

An alternative is to model disease increase due to each virus with a set of linked differential equations, e.g., the Lotka-Volterra competition equations (1). With two viruses, indicated with subscripts 1 and 2, the equations can be written as:

$$dy_1/dt = r_1 y_1 (K_1 - y_1 - a_{12} y_2)/K_1 \qquad (1$$

$$dy_2/dt = r_2 y_2 (K_2 - y_2 - a_{21} y_1)/K_2 \qquad (2$$

in which y_1 represents the proportion of plants infected with virus one; dy_1/dt is the absolute rate of disease increase for virus one; and r_1, K_1, and a_{12} are parameters. The rate parameter for disease increase of virus one is r_1, which also represents the maximum relative rate of increase; K_1 is the maximum level of disease incidence for virus one; and a_{12} represents the inhibitory effects of virus two incidence on increase of disease of virus one. Analogous definitions apply to the second equation. The a parameters often are called the competition coefficients. When the a's are zero, there are no inhibitory effects of either virus on the other, and the equations reduce to the classic

Verhulst-Pearl logistic equations. When the K's equal one (i.e., 100% incidence), the logistic equations are identical to Vanderplank's model for compound-interest diseases.

It is not possible to analytically integrate equations 1 and 2 without placing severe constraints on the parameters. Therefore, these equations have not been used frequently to analyze and compare actual data for population growth. One can numerically integrate these equations when the parameter values are specified. Linking a numerical integrator to a nonlinear regression procedure can result in precise parameter estimates (2). In this procedure, initial parameter estimates are specified, the differential equations are then numerically integrated, the error sum of squares is calculated for the goodness of fit between the observed and calculated (predicted) y's, revised parameter estimates are calculated and the equations are once again numerically integrated, and so on. If the procedure is successful, the minimum error sum of squares is reached and statistics describing the parameter estimates can be calculated.

Virus diseases of tobacco, caused by tobacco etch virus (TEV) and tobacco vein mottling virus (TVMV), were studied to test the applicability and utility of the Lotka-Volterra equations. Six plots (~3300 plants each) of tobacco were grown in Kentucky in 1983-85 and the proportion of plants infected with TEV (y_1) and TVMV (y_2) determined at least weekly. In two plots during each year, disease was assessed every 2-3 days. The six plots were arranged in three pairs; one of the plots in each pair was treated with insecticide at planting and throughout the season to prevent or reduce aphid vector colonization.

The Lotka-Volterra equations provided excellent fits to the observed epidemic data. Coefficients of determinaton (R^2) were always greater than 0.90; 75% were greater than 0.975. Parameter estimates varied with year and location, but to a lesser extent with insecticide treatment. The r parameters varied the least of all the estimates; values ranged from 0.1 to 0.3/day. Insecticide treatment reduced r_1 or r_2 in some plots/years but not others. r_2 (TVMV) was greater than r_1 (TEV) in the majority of plots. The maximum disease levels (K's) were as high as 1.0 in some 1984 plots and as low as 0.01 in 1983. In general, K_1 was less than or equal to K_2. The competition coefficients

equaled 0.0 in 70% of the epidemics suggesting that neither virus had a consistent inhibitory effect on the increase of the other.

Parameter estimates and disease progress curves for selected epidemics will be given on the poster.

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EPIDEMIOLOGY OF RICE TUNGRO

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Tungro is the most important virus disease of rice in South and Southeast Asia. It is a disease complex associated with rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) (5, 6, 7, 9). Both viruses are transmitted in a semi-persistent manner by leafhoppers, especially Nephotettix virescens. Leafhoppers fed on RTBV + RTSV infected plants transmitted both RTBV and RTSV together, or RTBV or RTSV alone. When fed on RTSV-infected plants, leafhoppers readily transmitted the virus. RTBV is dependent on RTSV for its transmission by the leafhoppers. RTSV alone once became epidemic in Japan (called rice waika virus) and damaged susceptible japonica rice cultivars (4). RTSV does not cause discernible symptoms on indica rice cultivars. RTBV causes mild "tungro symptoms" and RTSV enhances the symptoms caused by RTBV infection (6).

Current status of tungro in the Philippines. In the Philippines, major tungro outbreaks occurred in 1957, 1962, 1969-1971, 1975-1977, 1983-1984. The year 1971 was the worst so far recorded and the yield loss due to tungro in the Philippines was about 30% which amounted to 456,000 metric tons of rough rice.

Tungro incidence, leafhopper density, infective leafhoppers and cultivars planted were monitored in 40 farmers' fields in 6 provinces from 1973 to 1980 (8). The incidence from June to October in the wet season crop was correlated with the number of leafhoppers and the proportion of infective leafhoppers from May to July. Thereafter, tungro and vector leafhoppers were surveyed from May to July in many provinces for possible prediction of a tungro outbreak.

As cultivars resistant to leafhoppers have been commonly planted in the Philippines, cultivars planted was the major factor that influenced tungro incidence. From 1973 to 1976, cultivars with resistance gene(s) derived from cultivar TKM 6 were commonly planted. Tungro incidence was low on these cultivars, although often high in some other cultivars. IR36 and IR42, which have resistance gene(s) mainly from cultivar Ptb 18, were released in 1976/1977 and were widely planted afterward. Tungro incidence and leafhopper density was very low in all provinces especially in 1976-1979. However, tungro incidences were recorded in IR36 and IR42 in some provinces in 1981 and in almost all provinces in 1982. Nevertheless, IR36 and IR42 still are being planted because of their good characteristics. In 1980, IR50 and IR54 which have resistance gene(s) from cultivar Gam Pai 30-12-15 were released, and have been commonly planted in tungro endemic areas.

Survey of RTBV and RTSV in the Philippines. In the 1983-1985 survey, ELISA was applied to diagnose rice virus diseases (1). Rice tungro was the most important disease, while rice grassy stunt and rice

ragged stunt viruses occurred in rare occasions. Tungro incidence was high in IR36 and IR42, while very low in IR50 and other newly released cultivars.

However, in the 1985/1986 tungro survey, IR50, IR54 and other cultivars which have the resistance gene(s) from Gam Pai 30-12-15 showed high tungro infection in the Southern Philippines.

In most locations, many plants showing tungro-like symptoms contained RTBV and RTSV while many plants without symptoms in the same fields contained RTSV alone. RTSV also occurred in some fields where tungro-like symptoms were not observed. A high proportion of the vector leafhoppers which were collected in the fields transmitted RTSV alone. These results indicate that aside from tungro, RTSV also occurs and spreads independently in the Philippines.

Cultivar reaction to tungro in fields. Cultivars differed in their reactions to tungro infection depending on the disease pressure. At the IRRI farm where lower disease pressure prevailed, susceptible cultivar IR22 had 43% infection based on symptoms (3) (Fig. 1), while moderately resistant IR36 and IR42 had less infection, and resistant cultivars had very low infection rates. At Guimba, in Nueva Ecija province, where disease pressure was high, IR22, IR36 and IR42 as well as IR62 had high infection rates. The results show that cultivars with higher resistance can escape field infection better than those with low or moderate resistance.

Development of RTBV and RTSV in fields. Development of RTBV and RTSV infections in several cultivars was examined in the field. In the wet season when high disease and leafhopper pressures prevailed, tungro-susceptible TN1 and moderately resistant IR36 showed high RTSV infection rates in the initial weeks (Fig. 2). Thereafter, percentage of RTSV-infected plants declined gradually but infection with RTBV + RTSV increased. RTBV + RTSV infection rates increased quickly in TN1 but slowly in IR36. In the dry season when disease and leafhopper pressure was low, RTSV infection was low in the initial weeks and thereafter it increased rapidly on TN1 and IR36. Tungro-resistant IR54 had low infection rates with either virus, with RTSV infection being the higher. These results indicated that RTSV source plants were predominant in the field, while RTBV + RTSV source plants were scarce.

Cultivar reaction to RTBV and RTSV in artificial inoculation. Reaction of IR cultivars and susceptible cultivar TN1 to RTBV/ RTSV infection was evaluated by exposing each seedling to 1 to 30 leafhoppers which had fed on RTBV + RTSV infected plants (2). RTBV + RTSV infection increased when the number of leafhoppers per plant increased in susceptible TN1 and moderately resistant IR36 and IR42, whereas only RTBV infection increased in IR50 and IR54 (Fig. 3).

When the cultivars were exposed to leafhoppers which had fed on RTSV-infected plants, all cultivars tested including IR50 and IR54 showed relatively high RTSV infection. It is not known why leafhopper resistant cultivars which are exposed to leafhoppers carrying both RTBV + RTSV are preferentially infected with RTBV alone.

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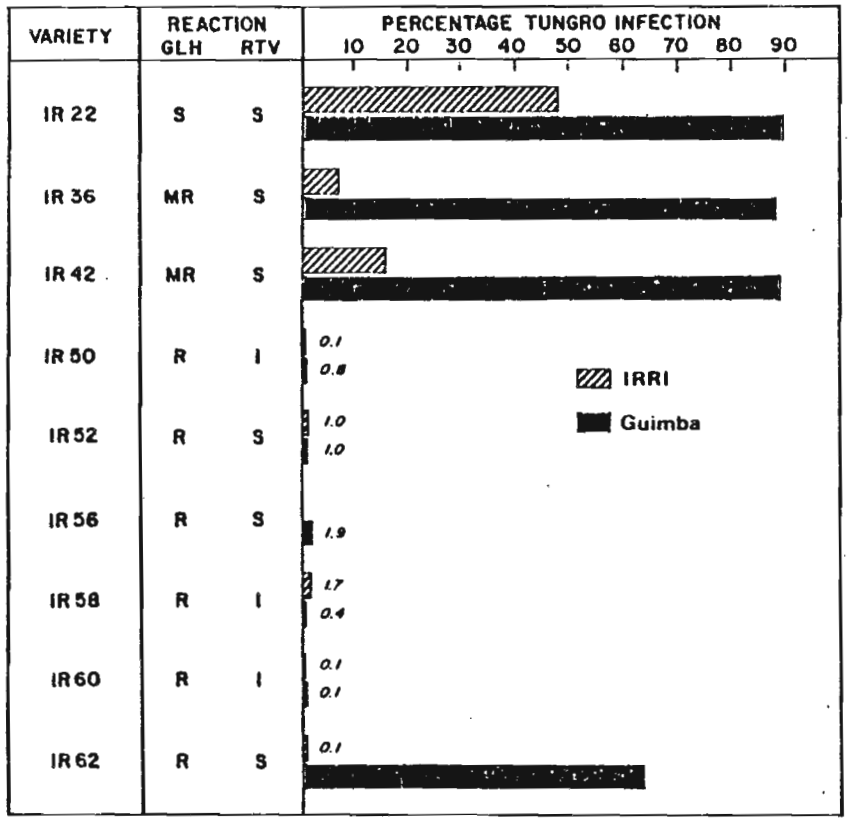

Fig. 1. Tungro disease incidence in IR cultivars with varying level of resistance to tungro disease infection (RTV) and to the vector Nephotettix virescens (GLH) at IRRI and Guimba, Nueva Ecija, Philippines. R, resistant; MR, moderately resistant; S, susceptible; I, intermediate.

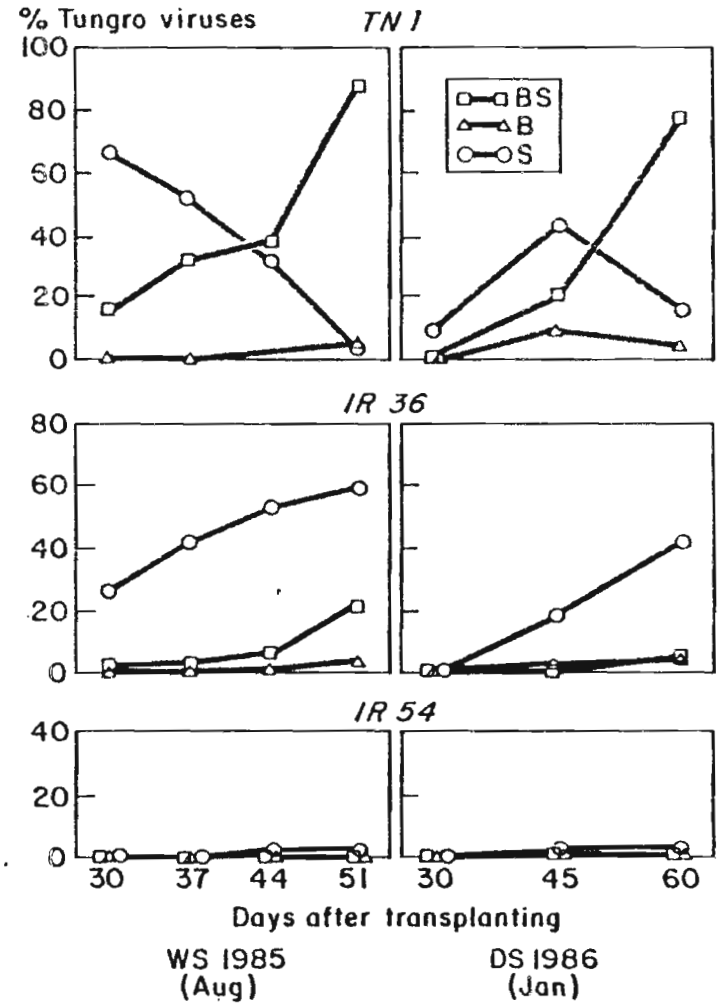

Fig. 2. Development of RTBV (B) and RTSV (S) in cultivars with different levels of vector resistance at different planting time under field conditions, IRRI.

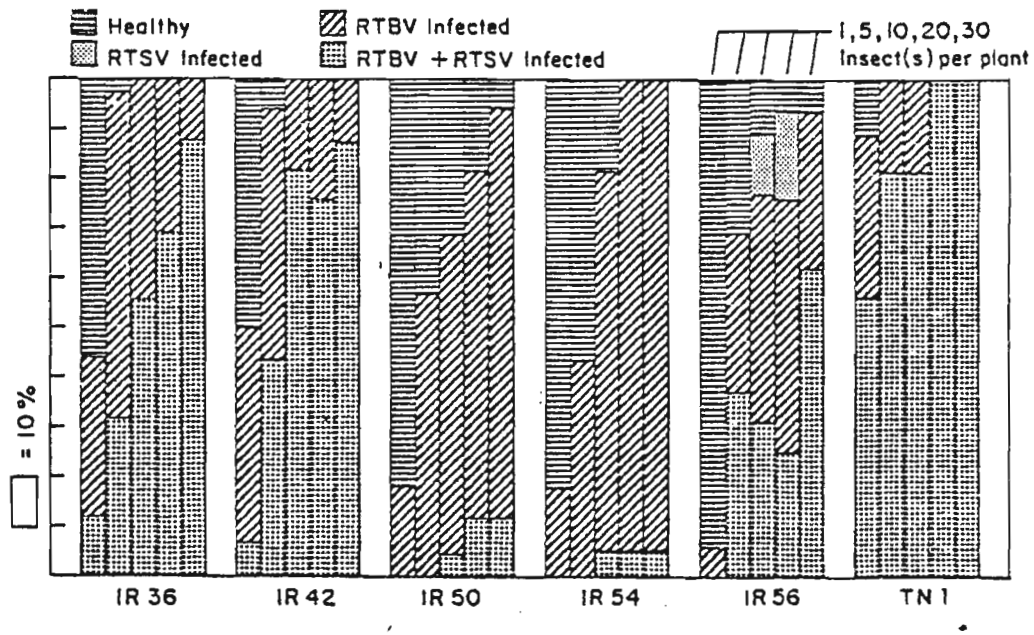

Fig. 3. RTBV and RTSV in rice cultivars inoculated at 1 to 30 per plant by N. virescens which had fed on source plants with RTBV and RTSV.

MYNDUS TAFFINI AND FOLIAR DECAY DISEASE OF COCONUT PALM IN VANUATU

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Coconut plantations in Vanuatu, as in other parts of the Pacific, are aging and becoming less productive. Replanting of the plantations with improved selections has been a primary objective of the Institut de Recherches pour les Huiles et Oleagineux (IRHO) in a number of countries and the IRHO station in Vanuatu was established to develop high yielding lines suitable for use in the region. Improvement is based both on the selection of locally grown lines, and hybridization between parent lines of very different genetic origin (usually dwarf x tall) which have high combining ability. Precocity and productivity are thus improved (3).

Foliar decay disease (FDD). Seed of Green Dwarf, Niu Leka, Malayan Red Dwarf and Rennell Tall were introduced to the IRHO station in 1962 and 1963, and in 1965, 18 months after planting, a wilt disease appeared on the Red Dwarfs, followed by symptoms on Rennell Tall, Niu-Leka, and Green Dwarf (2).

It also appeared in an adjacent 20-month-old Malayan Tall plantation, planted in 1965. The disease was detected in 1968 and 1971 on other islands of Vanuatu, always on the introduced varieties. Up to 90% mortality of Malayan Red Dwarf occurred within 7 yr of planting, whereas the local Vanuatu Tall remained disease-free. A range of susceptibilities among various hybrids was observed (3) and some of the potentially most productive hybrids succumbed to the disease. Symptom remission sometimes occurs, particularly in Rennell Tall. The resistant Vanuatu Tall is still the most commonly planted variety while the most tolerant Vanuatu Tall x Vanuatu Red Dwarf and the quite tolerant Tall x Rennell Tall are recommended as a compromise between tolerance to disease and yield/precocity. Consequently, FDD, a disease of unknown etiology is a limiting factor in coconut palm improvement in Vanuatu.

Symptoms of FDD in Malayan Red Dwarf include yellowing of leaflets on a frond between 7 and 13 positions down from the spear leaf in the crown. The yellowing extends along the frond, to adjacent fronds, and each of these become brown, die and finally hang down through the older fronds which remain green. Younger fronds also become yellow as they reach the mid-position of the crown. Some lateral necrosis of the petioles occurs.

Identification of the vector. Insects collected in the plantation by aspiration were grouped, and placed in insect-proof cages containing Red Dwarf seedlings (4 and 15 months old). As shown in Table 1, infected plants were observed 7-10 mo later only in the cage containing the Cixiid bug, Myndus taffini (4).

M. taffini (Fulguroidea cixiidae) is a new species (1) possibly limited in distribution to Vanuatu. Larvae are found among the old and decaying roots of Bourrao (Hibiscus tiliaceus), which is a common tree on forest verges and in older plantations. The larvae cohabit with ants.

Adults are found commonly on the underside of the bases of coconut palm leaflets. They are most abundant on palms at the edge of the plantation adjacent to the forest, and are rare or absent from sites distant from the forest (4). The vector is now used in tests for resistance of palm selections to FDD.

Distribution of FDD in plantations. A number of surveys have illustrated gradients of distribution of FDD into plantings of Malayan Red Dwarf from boundaries adjacent to forest (2; Dollet, Bonnot and Julia, unpublished results). Increase in incidence is most rapid with the most sensitive lines, and in Red Dwarf x Rennell Tall, the increase was almost linear for about 6 yr after which incidence had reached 53% (2). Myndus taffini was always found in disease foci, and gradients of the distribution of this species could be superimposed on the gradients of distribution of FDD.

Characteristics of transmission of FDD by M. taffini. The large numbers used in the first disease transmission trials did not exclude the possibility that FDD was induced by insect toxins. The minimum number of insects required to transfer FDD given a 24-hr inoculation feed was 2 per plant (1/10 plants infected) with the efficiency of transmission rising with an increase in the number of insects used (e.g. 4 insects, 10%; 8, 30%; 32, 20%; 64, 40%; 128, 80%) (5). The minimum inoculation time for groups of 200 insects was 20 min (2/10 plants infected) (5). Persistence exceeds 2 days (unpublished results). Acquisition trials await development of methods to culture Myndus. These data are consistent with the view that FDD is caused by a transmissible agent.

Search for a pathogen associated with FDD. Hypotheses for a fungal, bacterial, mycoplasma or nematode etiology for FDD have been discarded (5). As no other host species of FDD are known, attempts were made to directly extract molecular components from diseased Malayan Red Dwarf which may be specifically associated with the disease.

Isolation of a disease specific DNA by several different nucleic acid extraction procedures supports a hypothesis viral etiology for FDD (6). The DNA is single-stranded, and both electron microscopy and 2-dimensional polyacrylamide gel electrophoresis have shown that circular molecules are associated with this fraction (Randles, unpublished results).

The molecular weight of the circle is approximately 0.5×10^6. The DNA occurs in low amounts, but an assay using 5% polyacrylamide gel electrophoresis and silver staining now allows detection of DNA in palm leaf samples within 24 hr of commencing the extraction procedure.

A diagnostic test based on the detection of DNA therefore seems feasible for ecological studies on distribution and spread of FDD. Attempts to purify a virus from infected tissue have sometimes yielded virus-like spheres of various sizes (20-50 mm diameter) but the multiplicity of protein bands in these preparations and the cross reaction of antiserum prepared against these preparations with healthy palm extracts have led to the conclusion that insufficient purification has been achieved (Randles, unpublished results).

Moreover, because of the present strategy of attempting to correlate specific components with disease to determine etiology, the processing of large numbers of samples for electron microscopy is less economical than pursuing the use of DNA as a diagnostic indicator of infection. Although the DNA is structurally similar to that of geminiviruses no geminate particles have been observed.

CONCLUSION

FDD is a serious constraint to improvement of coconut palm production in Vanuatu. So far it is not known whether the pathogen occurs outside the archipelago and so whether it presents a risk to replanting programs in other parts of the Pacific.

It is relevant to this meeting that an appreciation of disease epidemiology provided some of the first clues on the etiology of FDD. Gradients of FDD distribution into plantations, apparently originating from wild bourrao, and coincidence of these with the distribution of M. taffini in plantations led to the demonstration that this species is the field vector of FDD in Vanuatu. Detection of breeding populations of M. taffini on bourrao roots is consistent with bourrao acting as a primary focus on the vectors for infection, and thus experimental control measures can now be directed towards eradication of this species. Progress has also been made toward implicating a virus as the cause of FDD, and this should eventually allow virus hosts, reservoirs, and other possible vectors to be identified, and allow the epidemiology of fDD to be modeled.

ACKNOWLEDGMENT

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Table 1.

Insects introduced	Total number introduced	Number of diseased palms
Control	0	0/26
Mixture of species, exlcuding Myndus	30,000	0/26
Jassids (various)	7,500	0/26
Delphacids (various)	6,000	0/26
Cixiids (Myndus taffini)	80,000 +11,000	24/26
Insects collected on understory grass Digitaria sanguinalis with striate mosaic disease	21,000	0/26

TRANSMISSION OF MYCOPLASMA-LIKE ORGANISMS BY CICADELLIDAE IN GLADIOLUS PLANTS

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Asters yellows in gladioli (Gladiolus sp.), originally described in the USA as "grassy top," was first found in Europe in 1962 (in southern France) and in 1979 in Italy (1). Recently, in the spring of 1984, a severe disease associated with the presence of mycoplasma-like organism (MLO) was observed and studied in gladioli cv. Rose Supreme (R.S.) in Emilia-Romagna (northern Italy) (2). The gladiolus plants showed marked growth reduction, yellowing of leaves, tip necrosis and emission of 2-3 or more buds per bulb. Our investigations revealed that the bulbs planted for flower production were probably already infected with MLO, thus having contracted the disease in the previous year (1983) during the swelling phase (Fig. 1).

Considering the importance of gladiolus cultivation in Italy, we decided to investigate further into the epidemiology of this gladiolus disease, to find: 1) the wild plant "natural hosts" of MLO-gladiolus; and 2) the natural vectors (Cicadellidae) of MLO from gladioli to wild plants and vice versa.

1) Wild plant "natural hosts" of MLO-gladiolus. During the spring of 1985, we observed wild plants showing "yellows" and symptoms probably caused by MLO in the same field where infected gladiolus cv. R.S. has been cultivated: Convolvolus arvensis L. - small yellow/antocyanin colored leaves and growth much reduced (Fig. 2); Cirsium arvense L. - chlorotic diseased leaves ("yellows") which were pointed and wrinkled from base to top; Capsella bursa-pastoris L. - marked growth reduction with small leaves and flower phyllody.

The use of optical fluorescent microscopy did confirm the presence of MLO in sieve tubes of these three wild plants. Grafts were also made to transmit MLO from C. arvensis to other MLO-indicator plants: Lycopersicon esculentum Mill. (cv. Marmande), Tagetes patula L. and Zinnia elegans Jacq. which were planted and allowed to sprout in a greenhouse. The symptomatology thus obtained in the MLO-indicator plants was probably caused by MLO-gladiolus.

We reached the conclusion that wild plants, especially the perennial weeds, deserve as much attention as possible as alternative hosts for MLO-gladiolus.

2) The natural vectors (Cicadellidae) of MLO from gladioli to wild plants and vice versa. In the summer of 1985, some MLO-indicator plants (T. patula, Gomphrena globosa L., tomato, Z. elegans, Vinca rosea L.) were planted in three sites of the field, near C. arvensis and C. arvense, naturally infected with MLO, and constantly checked for the

Fig. 1. Field with Gladiolus sp. cv. Rose Supreme: stunted MLO-infected plants and healthy plants.

Fig. 2. Dwarfed leaves and growth reduction of plants of Convolvolus sp. caused by natural MLO-gladiolus infection and healthy plants.

presence of leafhoppers (Cicadellidae). As some MLO-indicator plants showed symptoms of probable infection, several leafhoppers (nymph and adult) were collected in the field and used for transmission experiments from infected Cirsium sp., Convolvolus sp. and/or gladiolus to healthy plants (under cages).

The following insects were identified by Prof. C. Vidano (Istituto di Entomologia agraria e apicoltura, Torino, Italy): Empoasca vitis (Gothe), Emelnoviana mollicula (Bohem.), Psammotettix alienus (Dahlb.), Jassargus sp., Agallia laevis Rib., Adarrus sp. Empoasca sp. and Agallia sp. are known to be natural vectors of MLO (3). The presence of Psammotettix (= Delthocephalus) alienus in Italy is of particular interest. This leafhopper is frequent in Czechoslovakia, Rumania and Poland. Ploaie et al. (4) report P. alienus Dahlb. as an MLO-vector to wheat, barley and oats in nature (Rumania).

We didn't find Macrosteles quadripunctulatus (Kbm.) or Euscelis plebejus (Fall.), possible vectors of MLO-gladiolus, in France (5). However, during our study we collected a large number of P. alienus; the transmission experiments to determine if this leafhopper is the real MLO-gladiolus vector in Italy are still in progress.

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CHARACTERISTICS OF THE TRANSMISSION OF SPIROPLASMA CITRI BY THE LEAFHOPPER CIRCULIFER TENELLUS TO TURNIP

Catherine Eastman and Jacqueline Fletcher

The mollicute Spiroplasma citri, which infects members of almost 20 plant families, is most widely known as the causal agent of stubborn disease of citrus in many Mediterranean countries and the western U.S. In 1981 it was identified also as the causal agent of brittle root disease of horseradish (Armoracia rusticana) in Illinois (central U.S) (2, 4). The beet leafhopper, Circulifer tenellus, is considered to be the most important natural vector of S. citri in the western U.S. This insect is also an experimental vector of brittle root isolates of S. citri to horseradish (2) and has been linked circumstantially with some past brittle root epidemics.

As a preliminary to work on the epidemiology of brittle root, laboratory tests were conducted to determine the characteristics of transmission of a horseradish isolate of S. citri by C. tenellus. Because horseradish is vegetatively propagated, highly variable through individual grower selection of planting stock, and virtually 100% contaminated with one or more mosaic viruses, turnip (Brassica rapa) was selected for use in these basic transmission studies.

Materials and Methods. C. tenellus were taken from a colony established with leafhoppers collected in 1979 from horseradish fields in southwest Illinois and reared on sugar beet plants. S. citri isolate BR-6 was obtained from a diseased horseradish plant from the same area in 1980 and has been maintained in a series of turnip plants via leafhopper inoculation.

Unless plant age of infection (AI) was the treatment variable, turnip plants were used as sources of S. citri 13-22 days after inoculation and as test plants 14-21 days after seeding. Following inoculation, sources were held in a growth chamber at 27:22 C with a photoperiod of 16L:8D. The acquisition access period (AAP) and latent period (LP) portions of each test were spent under the same conditions, while inoculation access periods (IAP) were spent in an insectary at 25 C under continuous illumination. Nymphs were used at the start of each AAP. During the IAP, insects were caged singly on test plants in all experiments; 70-100 insects were tested per treatment, usually in equal numbers of males and females. Insects fed only on healthy turnip or sugar beet plants were used as controls. Test plants were held in a greenhouse for development of symptoms. Plants exhibiting chlorosis and stunting of young leaves were rated as positive for S. citri; a percentage of test plants with and without symptoms, control plants, and plants with questionable symptoms were checked by isolation of spiroplasmas or by ELISA to confirm the reliability of visual ratings.

To evaluate the effect of length of the AAP on the ability of C. tenellus to transmit S, citri, nymphs were caged for specified periods (45 min to 12 h in one test series, 1-21 days in another) on infected turnip and were moved to healthy sugar beet plants if needed to complete

a 21-day period measured from the start of the AAP. Then these insects were caged on test plants for a 2-day IAP. For the LP tests, insects were checked for their ability to transmit S. citri to test plants 3-35 days after the start of an AAP on infected turnip. The insects spent all or part of this time on infected turnip (with a maximum AAP of 14 days) and were held on sugar beet plants if needed to complete the allotted time prior to their confinement on test plants for a 2-day IAP. To examine the effect of length of the IAP, insects were given a 14-day AAP on infected turnip and 7 days on sugar beet plants prior to being caged on test plants for the specified IAP. IAP tests were done with periods of 5 min to 12 h or with periods of 1-6 days. To determine the influence of age of infection (AI) in plants used as pathogen sources on leafhopper acquisition and transmission of S. citri, nymphs were given a 4- to 5-day AAP on sources of a specified AI and then held on sugar beet plants for 16-17 days prior to being caged on test plants for a 2-day IAP. In two of these tests, spiroplasma titers in plants with AI similar to those of plants used as sources for leafhopper acquisition were determined by ELISA (1). Data on percentage of plant infections were analyzed using the SAS Anova procedure (5). Means were compared using the Waller-Duncan k-ratio t-test (5).

Results and Conclusions. C. tenellus transmitted S. citri to test plants after a minimum AAP of 45-90 min. Highest percentages of infected plants were achieved with insects given AAP of 5 days (71, 63, or 54% plant infection in three tests). With longer AAP, numbers of plant infections were slightly to significantly lower; leafhoppers given a 21-day AAP were always very poor vectors.

Nymphs were unable to transmit S. citri successfully after a 3-day LP; the minimum LP in four tests was 7 or 10 days. Highest plant infection rates (45,51, 41, or 65%) were noted when insects were tested after a 17- to 21-day LP, with rates slightly to significantly lower after an LP of 28 or 35 days.

C. tenellus transmitted S. citri to test plants after a minimum IAP of 15 min. In one test, the percentages of infected plants in treatments involving IAP of 2-6 days (80-90% infection) were significantly higher than that following a 1-day IAP (66% infection). In a second test, however, rates of plant infection following IAP of 1-6 days (38-48% infection) were not significantly different.

Plants with AI of 7 days made poor spiroplasma sources; only 6-19% of the test plants developed infections. In two experiments test plant infection rates were highest (63 and 85%) when leafhoppers had fed previously on sources infected for 17-21 days; in the third test plants with AI of 23 days made the best sources. Plants with older AI (27-37 days) were usually poorer sources. In one of two tests for which spiroplasma titer information is available, population levels of S. citri peaked in plants with an AI of 18 days and remained almost as high thereafter; while test plant infection rates were correspondingly highest (63%) after exposure to insects given an AAP on sources with an AI of 17 days, numbers of test plant infections were significantly lower, however, when plants with an AI of 27-37 days were used as sources. This experiment was conducted late in the year, and the number

of test plants developing infections may have been lower than expected because of conditions sub-optimum for symptom expression. In the second test spiroplasma levels were highest in plants with an AI of 23 or 33 days; plants with these AI also made the best sources (68 or 61% infection, respectively).

Data from these tests, although variable, indicate that C. tenellus can be a much more efficient vector of S. citri than has been indicated in previous reports. Rates of transmission of the BR-6 isolate from infected to healthy turnip plants by single insects were as high as 90%. In contrast, Liu and colleagues (3), for example, obtained rates of infection of 2-4% in Madagascar periwinkle (Catharanthus roseus) test plants exposed to single C. tenellus microinjected with a California isolate of S. citri obtained from diseased C. roseus; rates of 66-80% were obtained only with groups of 10-20 insects per test plant. More work is needed to elucidate the possible role of pathogen isolate, leafhopper biotype, and source and test plant species, among other factors, in determining differences in vector capability reported for this insect.

Of the 16 tests in which equal numbers of male and female C. tenellus were used, overall percentages of plant infections were always 2-16 points higher when males were used as vectors. In seven of these tests, infection rates in plants exposed to males were significantly (P=0.05) or highly significantly different (P=0.01) from those in plants exposed to females; in another test these differences approached statistical significance (P=0.06). The biological significance of this finding with regard to the epidemiology of diseases incited by S. citri remains to be determined. At the very least, it indicates that laboratory tests investigating the vector capabilities of C. tenellus should be conducted with equal numbers of each sex or with one sex alone.

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PEREGRINUS MAIDIS AND MAIZE VIRUSES AND SPIROPLASMAS IN SOUTHERN FLORIDA

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In 1979 and 1980 severe epidemics involving three viruses and one spiroplasma caused significant economic losses in fall-planted maize (Zea mays L.) in southern Florida (1). The following pathogens and their vectors were identified from field samples: corn stunt spiroplasma (CSS) and maize rayado fino virus (MRFV), transmitted by Dalbulus maidis DeLong and Wolcott; maize stripe virus (MStpV) transmitted by Peregrinus maidis Ashmead; and sugarcane mosaic virus strain B transmitted by several aphid species. In 1983, maize mosaic virus, which also is transmitted by P. maidis, also was discovered to be part of this complex.

In 1981 experiments were initiated to monitor the temporal and geographical incidence of the maize pathogens and their vectors in southern Florida, and to develop specific and reliable assays for diagnosing virus-infected plant hosts and viruliferous insect vectors of these pathogens.

Incidence of maize pathogens and insect vectors in southern Florida. Forty individually-potted maize plants were placed biweekly at a single location to monitor the seasonal incidence of maize pathogens. Plants were exposed for 2 wk, sprayed with insecticide and returned to the greenhouse for observation. MStpV was by far the most common pathogen detected, and the incidence of MStpV was greatest in late summer and fall. CSS was fairly common in late summer and fall trap plants but no other maize pathogens were detected (Table 1).

Trap plants also were used to assess geographical distribution of MStpV in three southern Florida locations during a 26-week period from September 1982 through February 1983. The locations and MStpV incidence were Homestead 46/520, Fort Lauderdale (100 Km northeast of Homestead) 5/520 and Belle Glade (200 Km north of Homestead) 3/520.

Sampling for leafhoppers and planthoppers with emphasis on Peregrinus maidis, the planthopper vector of MStpV, was done by sweep net and yellow sticky card traps. Areas sampled included mixed grass stands, bermuda grass and commercial maize fields near Homestead, Florida. Total leafhopper and planthopper populations were highest in the summer months of June through September. P. maidis represented only a small and sporadic percentage of the total insects trapped. Graminella spp., which are inefficient vectors of CSS but not MStpV, represented the largest percentage of trapped insects. Based on these data and on field observations, neither sampling method gave an accurate reflection of the P. maidis population. Field observations showed P. maidis to be a sedentary stem-colonizer which most likely would not be

collected by sweep nets. Also high numbers of P. maidis were often found inside the leaf sheath on maize and Rottboellia exaltata (itchgrass) plants, these insects also would not be collected by sweep nets.

Serological detection of MStpV and MMV in plants and P. maidis. Polyclonal antisera were prepared against MStpV virions, MMV virions, and the major MStpV noncapsid protein (NCP), which is a major component of MStpV-infected maize. All of the antisera were specific, none reacted with healthy plants or with maize plants infected by other viral pathogens or CSS. Besides maize, four additional plant hosts for MStpV and one for MMV were confirmed serologically. Rottboellia exaltata, a widespread and common weed in southern Florida, was found to be a host for MStpV, MMV, and P. maidis.

Serological tests for MMV and MStpV in viruliferous P. maidis were done by exposing laboratory-reared P. maidis to either MMV- or MStpV by plant acquisition or injection. Individual insects were tested serologically for viral antigens. MMV insects were detected in individual P. maidis but only after an incubation period which was affected by acquisition method. MMV was detected by DAS-ELISA in 58% and 78% of individuals that acquired MMV by acquisition from infected plants or by injection, respectively. Not all of the P. maidis that were ELISA-positive for MMV transmitted MMV in our tests.

MStpV also was easily detected in individual P. maidis that were exposed to MStpV-infected but not healthy plants. However, in contrast to plant assays, only antiserum to the MStpV capsid protein could be used successfully for detecting MStpV-viruliferous P. maidis. We obtained no evidence that the MStpV noncapsid protein is present in significant amounts in MStpV-viruliferous P. maidis.

Ecological strategy and possible controls. P. maidis, MMV and particularly MStpV are established in southern Florida. Year-round hosts for the viruses and vector are abundant. These include R. exaltata and year-round volunteer maize.

The possibility exists that MStpV might be controlled in the high cash value seed maize crops of southern Florida by using insecticides to control P. maidis. Currently methomyl is applied every 3-4 days to the seed corn crop, but it is ineffective against P. maidis in laboratory tests 24 hr after spraying. Other insecticides such as carbaryl and metasystox-R showed greater activity towards P. maidis in laboratory studies. Also, natural populations of P. maidis are infected with at least one insect virus which does not infect maize or other plant species tested. The effects of this virus on P. maidis are presently unknown.

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Table 1. Seasonal MStpV incidence in trap plants near Homestead, Florida, 1983-1985.

Year	J	F	M	A	My	Jun	Jul	Aug	S	O	N	D
1982	2	4	0	7(4)*	-	5(1)	30(4)	30(10)	13(2)	8	11	-
1983	2	1	1	-	-	2	13	19(5)	12	14	13	7(3)
1984	1	2	1	-	2	4	5	6	3	5	3	1
1985	1	3	0	0	0	0	5	17	5	12	5	2
Total	6	10	2	7	2	11	53	72	33	39	32	10

*Numbers in parenthesis show plants infected by CSS.

CHARACTERISTICS OF VELVET TOBACCO MOTTLE VIRUS TRANSMISSION BY ITS MIRID VECTOR

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The mirid Cyrtopeltis nicotianoe transmits velvet tobacco mottle virus (VTMoV), a virus found in central Australia that possesses an unusual viroid-like RNA component (4). Three other viruses with a similar viroid-like RNA component, Solanum nodiflorum mottle virus (SNMV), lucerne transient streak virus (LTSV) and subterranean clover mottle virus (SCMoV) were used to test mirid specificity, and of these only the serologically related SNMV was transmitted. These viruses have many properties in common with viruses belonging to the sobemovirus group (1,3). Both southern bean mosaic virus, the type member of the group, and sowbane mosaic virus, a possible member (2), were transmitted by the mirid although a number of other viruses with small polyhedral particles were not. Thus the association is quite specific and it has been shown that feeding is required for transmission.

Mirids can acquire VTMoV in 1 min and transmission efficiency increases with increasing acquisition time. Up to 50% of the mirids tested were shown to transmit after a moult (pre-adult to adult). Transmission still occurred when newly moulted adults were denied access to their shed cuticle, thus precluding the possibility that virus was acquired by probing contaminated components of the cuticle. Furthermore, non-viruliferous mirids given 24-hr access to either moulted cuticles from viruliferous mirids or cages previously occupied by viruliferous mirids did not transmit VTMoV. The evidence supporting transstadial transmission implies that the virus persists and thus circulates or accumulates in the mirid.

Experiments were done to determine persistence rates, examine factors affecting persistence and determine whether the virus propagates or simply circulates in the mirid. VTMoV can be retained by the mirid for up to 10 days and is transmitted intermittently and only occasionally on successive days during its period of persistence. Mirids which have acquired VTMoV for 2 days can inoculate plants in 2 hr and transmission efficiency increases with increasing inoculation time. However, when mirids acquire for only 1 hr, or when mirids fast for 16 hr after acquisition, transmission efficiency is significantly reduced. This suggests that the potential to transmit is lost more rapidly than one would expect from a persistent association.

Information about propagation of VTMoV in the insect was obtained by monitoring the rate of clearance of the virus from its vector using both transmission and ELISA assays. If it was shown that virus was completely cleared from the insect, one could suggest that the virus was not multiplying in its vector. Viruliferous mirids were fed on tomato plants (a virus immune host), for 3 days followed by 1 day inoculation feeding on healthy Nicotiana clevelandii. This cycle was repeated for

17 days to test the rate of virus clearance from the insect and it was found mirids stopped transmitting between 5 and 9 days after acquisition. Viruliferous mirids fed on tomato after acquisition of VTMoV, were assayed daily by ELISA and were shown to be free of virus 8 days after acquisition. It may be significant that in both trials, most mirids were free of VTMoV after 2 days and thereafter just a few mirids remained viruliferous for up to 8 or 9 days. Thus it appears that the virus does not multiply in its vector.

Evidence for circulation of VTMoV in the mirid still rests with the transstadial transmission trials. Theoretically a virus that circulates in its host should be transmitted after direct introduction of the virus into the insect haemocoel. Thus microinjection of a virus solution labeled with ^{32}P was done and 2 out of 60 mirids transmitted. Low transmission levels may be due to a dose factor and current experiments are being done to determine minimum virus levels required for transmission.

Further work will involve localization of the virus in the vector, with emphasis on virus distribution in the few insects that consistently retain virus over longer periods than most other insects. This phenomenon has been observed in the inoculation, persistence, clearance and injection trials and could possibly be explained by a combination of non-persistent and circulative transmission. Alternatively the high rates of transstadial transmission indicate the virus and vector may be associated semi-persistently in which VTMoV is retained at a site not lost during ecdysis of the insect.

Transmission of characteristics:

Acquisition threshold	1 min
Inoculation threshold	2 hr
Transstadial transmission	50%
Persistence	10 days

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PREDICTION OF THE PEAK APPEARANCE OF RICE GREEN LEAFHOPPERS IN WEST BENGAL

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Rice green leafhopper is an important pest of rice in West Bengal as it is the vector of tungro virus disease of rice which is an endemic disease of this state. The spread of this disease is related to the increase in the population of the leafhoppers. The population usually increases from August reaching a maximum during October-November. The population and rainfall data suggested the potential for using rainfall to forecast the time of maximum hopper populations (1, 2, 3).

Data for trapped leafhoppers and rainfall were collected through different research projects of the Indian Council of Agricultural Research, New Delhi. The leafhoppers were trapped using a modified Rothamsted-type light trap made of a metal frame holding glass pieces with 100 W tungsten bulb. The trap was 50 cm high with an opening 44 cm in diameter. It was supported on a metal table 76.5 cm high. The narrow part of the trap was placed in a central hole in the table. On the undersurface of the table surrounding this central hole, a metal chamber was made for placing the collecting vessels. The leafhoppers were collected daily after each night. The leafhoppers trapped during August to November were considered for analysis because most were trapped then. Daily rainfall data were obtained from the field observatories of the plant virus center, Bidhan Chandra Krishi Viswavidyalaya, Directorate of Agriculture, Government of West Bengal, or Central Soil Salinity Research Institute, ICAR at Canning, West Bengal as the case may be. Data for 7 years (1976, 1977, 1979, 1982, 1983, 1984, and 1985) were collected from the Plant Virus Experimental Field at Kalyani (22°50'N 88°20.0'E). Data for only one year (1984) were collected from the Seed Multiplication Farms of the Directorate of Agriculture, Government of West Bengal at Bongaon (25°2.4'N 88°49'E), Bagda (23°13.2'N 88°49.7'E), Hanskhali (23°20.4'N 88°37.4'E), Karimpur (23°58.2'N 88°37.4'E) and Central Soil Salinity Research Institute, ICAR at Canning (22°19.2'N 88°41.3'E).

When the moving average of trapped leafhoppers for 15 days during September to November and the corresponding moving total rainfall during July, August, and September were compared, most signficant correlations were found at different lag periods varying from 48 days to 74 days (Tables 1 and 2). Correlation studies were made with the lag period observed for each year/location following the same equation. Regression analysis was done for each year using the regression line of $Ye = a + bx$, where a and b denote the population constants and Ye= expected leafhopper catch corresponding to any value of x, the moving total rainfall. The fit of the equation was tested by analysis of variance for each year/location. The regression line obtained was used to predict the number of leafhoppers or the peak appearance of leafhoppers with respect

to a particular value of x or rainfall. These predictions were then compared with the actual data.

The regression lines calculated for individual years at Kalyani and different locations during 1984 were found to be similar (Tables 3 and 4). When the individual equations were tested by analysis of variance, the value of F in all these cases has been found to be significant both at 1% and 5% level of critical difference. The respective value of 'F' for 1976, 1977, 1979, 1982, 1983, 1984 and 1985 at Kalyani are 15.79, 29.5, 10.36, 31.67, 10.45, 76.84 and 14.75 and at Canning, Bongaon, Bagda, Hanskhali and Karimpur are 23.64, 14.31, 34.96, 21.35 and 19.37 respectively. The results confirm that the appearance of trapped leafhoppers in October is statistically related to the occurrence of rainfall in August-September. There always occurs a lag period between the occurrence of rainfall and the appearance of leafhoppers which may be used for predicting the population development of leafhoppers. But the lag period differs from year to year and to some extent from location to location. This variation indicates the involvement of additional factors in the monsoon rainfall-leafhopper incidence relationship. These factors are yet to be determined for the development of a practical predictive model.

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Table 1. Lag days between the peak rainfall and peak leafhopper catches obtained by shifting method with 15 days logarithmic moving average of trapped leafhoppers and 15 days moving total rainfall in different years at Kalyani, West Bengal.

Year	Lag days	Correlation co-efficient	Degree of freedom
1976	74	0.5475	38
1977	66	0.666	38
1979	70	0.3867	32
1982	59	0.6804	38
1983	60	0.4742	37
1984	48	0.8250	37
1985	60	0.5231	37

Table 2. Lag days between the peak rainfall and peak leafhopper catches obtained by shifting method with 15 days logarithmic moving average of trapped leafhoppers and 15 days total rainfall in 1984 at different locations in West Bengal.

Location	Lag days	Correlation co-efficient	Degree of freedom
Canning (22°19.2'N 88°41.3'E)	49	0.63	37
Bongaon (23°2.4'N 88°49.7'E)	59	0.53	37
Bagda (23°13.2'N 88°41.7'E)	48	0.73	31
Hanskhali (23°20.4'N 88°49.7'E)	68	0.61	37
Karimpur (23°58.2'N 88°37.4'E)	62	0.59	37

Table 3. Regression lines with respect to lag correlations obtained between the 15 days logarithmic moving average of trapped leafhoppers and moving total of rainfall during 7 years at Kalyani, West Bengal

Year	Calculated equation	Degree of freedom
1976	Ye=2.084 + 0.1336x	38
1977	Ye=2.028 + 0.1064x	38
1979	Ye=1.7814 + 0.2377x	32
1982	Ye=0.9937 + 0.0938x	38
1983	Ye=1.0355 + 0.0926x	37
1984	Ye=1.181 + 0.0797x	37
1985	Ye=1.3461 + 0.023x	37

x = 15 days moving total rainfall in cms. Ye = Log (1+Ve) where Ve = expected number of trapped leafhoppers, averaged over 15 days.

Table 4. Regression lines with respect to lag correlations obtained between the 15 days logarithmic moving average of trapped leafhoppers and moving total of rainfall in 1984 at different locations in West Bengal.

Location	Calculated equation	Degree of freedom
Canning	Ye=0.3778 + 0.098x	37
Bongaon	Ye=1.162 + 0.0988x	37
Bagdah	Ye=2.00 + 0.0207x	31
Hanskhali	Ye=2.18 + 0.0193x	37
Karimpur	Ye=1.593 + 0.062x	37

PATTERNS OF VECTOR ACTIVITY RELATIVE TO X-DISEASE SPREAD

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Surveys of the leafhopper fauna of cherry orchards in central California have repeatedly shown that species capable of vectoring the X-disease agent (a presumptive mycoplasma-like organism) are rare relative to most common leafhoppers (1). The importance of different vector species in the spread of X-disease has been related to their relative abundance in orchards. We sought to document the activity of the known vector species, as assayed by yellow sticky traps, to cherry orchard cultural practices and nearby crops or vegetation. We also mapped the occurrence of X-disease in the same orchards to assess the correlation of disease spread over a 3-yr period to leafhopper activity in preceding years.

The most common vector species detected were Colladonus montanus, Euscelidius variegatus, and Fieberiella florii. C. montanus was most prevalent in orchards near fields of mature sugar beets or where curly dock (Rumex crispus) or various clovers were abundant. Only adults of C. montanus were found in sugar beets, and laboratory tests indicated that sustained survival and reproduction were poor on beet. Euscelidius variegatus was located most often on grasses or weeds in or near orchards. F. florii appeared to move into orchards from certain ornamental shrubs such as Ligustrum, Pyracantha, Buxus and others. Studies of movements of F. florii from shrubs into orchards revealed mostly short range dispersal (10-50 m). After midsummer, F. florii reproduced on cherry in some orchards. C. montanus was much more dispersive, especially after leaving harvested beet fields.

C. montanus trap catches were correlated (Spearman's rank test) with subsequent X-disease spread in 2 of 3 years for orchards with Prunus maheleb rootstocks. Simple linear correlations or those which included orchards on P. avium rootstocks were not statistically significant. This is perhaps explained by the rapidly (<1 yr) lethal reaction of cherry on P. mahaleb to X-disease, whereas disease expression on P. avium rootstocks requires more than 1 yr. Three orchards that had high numbers of F. florii also had unusually high rates of spread of X-disease. There was no significant correlation of E. variegatus activity with subsequent X-disease incidence. In orchards that had high numbers of F. florii, the incidence of X-disease declined rapidly with distance from nearby ornamental shrub hosts, further supporting the conclusion that F. florii was substantially responsible for the spread of X-disease in these circumstances. Where F. florii reproduces on cherry, nymphs would be molting into adults during July-August, when leafhopper transmission from cherry is at an optimum (3).

C. montanus were tested for natural infectivity by exposing field-collected leafhoppers to celery test plants, a sensitive indicator plant for X-disease. Over a 3-yr period, less than 1% of the C. montanus

tested transmitted to celery. There was no evidence from laboratory tests that sugar beet (2) or curly dock were suitable acquisition hosts for transmission of the X-disease agent to cherry. Burr clover (Medicago hispida) was an excellent host for transmission by C. montanus to celery. Because C. montanus feeds on cherry only as an adult, weed hosts such as burr clover may be important sources of X-disease inoculum for transmission by this leafhopper.

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THE ROLE OF FLYING AND COLONIZING APHID SPECIES IN THE EPIDEMIOLOGY OF NON-PERSISTENT VIRUSES IN ANNUAL CROPS

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Non-persistent viruses (NPVs) are the cause for severe epidemics in many annuals. Damage is in reduction of yields, in lowering the commercial value or in expenses invested to reduce these losses. Loss is also caused by reinfection of virus-free high quality propagation material. In many cases the cost reflects the crop value, thus more is known on food crops such as potato, corn and soybeans than on other annuals.

In the present paper we describe spread of NPVs as affected by non-colonizing and colonizing aphid species. Spread of CMV was monitored among the plants of two crops (peppers and gladioli), which were grown in the same location and season at Bet-Dagan. This allowed comparisons of the role of aphids in virus spread.

NPVs are known to be of a low specificity for their aphid vectors. In many cases, the number of aphid species reported represent those which were available for the test rather than the actual spectrum of vectors. Indeed, many species were found vectors among live-trapped aphids (1,4). On the other hand, of those found to transmit, only a few were responsible for more than 2/3 of the total transmission (1,2,4). In all three locations, flying aphids entering the crop took part in the spread. However, the epidemic situation was different in each. In Illinois, primary infection was present in the plot as soybean mosaic virus is seed-borne to a certain extent. There was no colonization in the plot (Irwin, personal communication). Thus, incoming aphids served primarily for secondary spread. In France, primary infection was introduced by incoming aphids. Thus, incoming aphids were probably responsible for the introduction of virus, although the incoming aphid species was the colonizing melon aphid Aphis gossypii. In Israel, no seed-borne virus sources were available in the pepper plots. Two non-colonizing aphid species, namely Aphis citricola and A. gossypii, were responsible for the primary infection at the beginning of the season, and for part at least of the secondary spread. Later, colonization of peppers took place by Myzus persicae and Macrosiphum euphorbiae. At that time, secondary spread was accelerated.

It is suggested to group aphids which contribute to infection in three behavior classes:

Visiting, non-colonizing aphid species. This term refers to species that land and probe on the plants in the plot, but take off almost immediately after probing. This behavior was noticed in Israel for the green citrus aphid Aphis citricola on peppers and probably also on cucurbits.

Settling, non colonizing aphid species. This term refers to species which land, probe and feed on the crop for hours and sometimes

for days. However, they do not reproduce on it. This behavior was recorded for A. gossypii on peppers in Israel.

Colonizing aphid species. This term describes species which fly, land and produce several generations on the crop, and where mixed populations of larvae, apterates and alates coexist.

The role of each of the groups mentioned above in the spread of NPVs is dependent upon the presence of sources of viruses. Several natural sources were ascertained for both cucumber mosaic virus (CMV) and for potato virus Y (PVY) in Bet-Dagan. For PVY, they were all in the solanaceae. Among those found, Hyoscyamus desertorum, Hyoscyamus aureus and Solanum vilosum, as well as from pepper and tobacco, was determined in the laboratory. A similar test was carried out for Beta vulgaris and Portulaca oleracea in addition to pepper, cucumber and tobacco, as potential sources for CMV. As expected, M. persicae and A. gossypii were found the most efficient from most sources.

Experimental plots in Bet-Dagan served to study the role of flying and colonizing aphid species in spread of CMV and PVY in peppers and the spread of CMV in gladioli. The two plots were in immediate vicinity.

Transmission of CMV to gladioli was different from that observed for pepper. None of the aphids trapped alive by suction were capable of inoculating gladioli. At the same time and location live-trapped aphids successfully vectored the virus to pepper. We also noticed that spread of CMV was possible only if infected gladioli were present in the plot.

The explanation for this phenomenon was obtained from laboratory transmission tests, using non-gladioli sources of CMV (tobacco, cucumber and pepper). Indeed, no transmission to gladioli was obtained if virus was acquired from these sources. Aphids given acquisition access feedings on these non-gladioli sources, could readily inoculate other hosts. In addition, it was found that M. euphorbiae was the principal aphid species found to colonize gladioli. This same species was also the most efficient in transmission of CMV in the laboratory either in confined inoculation access feedings or in free-access feedings. It should be added that the number of M. euphorbiae trapped by suction was much lower than expected from their relative occurrence on the gladioli leaves (Ali et al., unpublished results).

A similar lack of transmission by live-trapped aphids was recorded in 1982 in Kentucky (Raccah, unpublished results), where M. persicae was prevalent in large numbers on the tobacco leaves, including numerous alates; however, their number in suction traps were by far misrepresented. Also there, none of the aphids trapped by suction were capable of transmitting either tobacco etch virus (TEV) or tobacco vein mottling virus (TVMV) to tobacco test plants despite the fact that there was plenty of virus inoculum.

A possible explanation for this behavior is that colonizing species do not take off to a height that will be drifted into the turbulence produced by the suction traps. Therefore, alternative trapping methods

seem necessary in situations where colonizing species play the major role in spread.

A third case where colonizing species seemed to be the principal vector for infection was examined this last summer in a commercial zucchini plot at Lachish, in the South of Israel. The plot was sown on March 15, 1986, germinated within 10 days, at a time when the flight activity of A. citricola was at its peak. The distance from electrical power supply did not allow the use of suction traps. However, green tiles and yellow pans were exposed in the plot. Few zucchini yellow mosaic virus (ZYMV) infections were recorded on April 23. However, the infection rate of the plot remained low for more than 3 weeks after the peak of A. citricola subsided and after the appearance of the first symptoms. Increase in infection was noticed first with the appearance of the first A. gossypii in flight. However, massive secondary spread only occurred when the melon aphid heavily colonized the crop at the end of May. The lag section of the temporal progress curve indicated that in this case roguing the infected plants at the beginning of the infection could have resulted in a decreased final rate. Therefore, this procedure will be tested in the next season.

Attempts to model the spread of CMV and PVY as a function of the aphids involved was constructed on the basis of our findings (3). The contribution of visiting and colonizing aphid species to the spread of the two viruses was considered.

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THE EPIDEMIOLOGY OF APHID-BORNE VIRUSES IN CUCURBITS IN A DESERT AGRO-ECOSYSTEM

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Research was conducted in the Imperial Valley of California, situated in the south-central part of the state bordering Mexico. This is an irrigated desert agro-ecosystem with hot summers and mild winters, a site of diverse year-round agriculture.

Our epidemiological studies have focused on cantaloupe, which constitutes a large proportion of the cucurbits grown in the valley. Studies reported herein were conducted in melons planted in the spring, the time of the year at which peak aphid populations are present. A complex of aphid-borne viruses typically infects fields, but two potyviruses [watermelon mosaic virus-2 (WMV-2) and zucchini yellow mosaic virus (ZYMV)] are particularly important in terms of economic impact and levels of incidence.

Our approach toward understanding the cantaloupe-virus pathosystem has been to identify the principal components that interact to bring about the disease cycle. Efforts have been directed toward defining what we consider to be the four fundamental components: 1) virus; 2) aphid vectors; 3) the melon crop; and 4) alternate hosts of the virus. With insight into the dynamic parts of the system, it should be possible to examine the stochastic processes that drive the component interactions leading to virus epidemics in the valley.

One objective of this research has been to correlate the incidence of virus infection with aphid flights. We have attempted to quantify two basic parameters: 1) the number of viruliferous aphids alighting per unit area of cantaloupe canopy, and 2) the proportion of infected plants in the field. To measure the first parameter, two techniques have been employed. The first involved the use of horizontal ermine lime-colored water traps (1) placed in the field. The second technique used an aerial screen positioned at the upwind edge of the field to trap aphids for assaying on caged test plants. The second parameter was measured by monitoring a cohort of cantalopue plants through time for the occurrence of virus symptoms. Weekly samples from these plants were collected to be analyzed by ELISA for virus incidence. This two-fold virus evaluation provided a qualitative measure of the virus types in the field as well as a quantitative measure of the inoculum potential in the field for possible secondary spread of the viruses.

Results up to this time have been inconclusive. We have not been able to identify the primary vectors responsible for initially transferring the virus into the field. In the spring of 1985, 2822 alate aphids were assayed on 14 calendar dates beginning at the time of stand establishment. There was not a single infection that resulted from the assay prior to the first occurrence of infection in the field. After

about 30% of the plants were infected, viruliferous aphids were trapped. There were five species of aphids that proved to be field vectors of the two viruses. These were Myzus persicae (Sulzer), Acyrthosiphon pisum (Harris), Acyrthosiphon kondoi (Shinji), Lipaphis erysimi (Kalten), and Rhopalosiphum padi (L.). Studies from the ELISA tests are not completed at this time.

In other research, laboratory studies on the transmission efficiencies of four commonly occurring aphid species were conducted. Local isolates of WMV-2 and ZYMV were used. Notable differences occurred between aphid species for both viruses (Table 1). It was interesting to observe that ZYMV was transmitted more efficiently than was WMV-2 by M. persicae and A. gossypii. A. pisum, on the other hand, was more efficient transmitting WMV-2. Integrating this information with 1984 and 1985 aphid densities in the field indicated that M. persicae presents the greatest vector pressure [defined by van Harten (2)]. A. pisum and A. kondoi are common in the field and may account for substantial vector pressure.

One of the curious features of this system is the pattern of virus spread in terms of phenological occurrence and percent incidence that is consistent from year to year. Cantaloupe fields normally are virus-free for many weeks, even though large numbers of aphids are present, and then in early to mid-April the virus incidence increases from 0 to 100% in a 2-wk period. These observations have prompted us to conduct growth chamber experiments to determine the influence of temperature on symptom expression. Chambers were programmed to simulate a diurnal temperature profile for the dates of February 20 and April 10 based on 30 yr average temperatures in the Imperial Valley. The mean time to symptom expression for all test plants was only slightly longer at the lower temperatures. Therefore, it does not appear that there is a temperature-dependent latency preventing the occurrence of symptom expression in infected plants.

We recently have begun research to identify plants that serve as alternate hosts for the virus. This research consists of collecting plants in the field and using ELISA to determine the occurrence of virus. At the present time, we have not been able to isolate virus from any of the species that have been collected.

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Table 1. Summary results of aphid transmission studies.

Aphid	WMV-2		ZYMV	
	Infection rate	% infection	Infection rate	% infection
M. persicae	31/180	17	52/140	37
A. gossypii	23/144	16	32/88	36
A. kondoi	5/144	3.5	0/88	0
A. pisum	10/64	16	2/56	3.5

EPIDEMIOLOGY AND VECTORS OF PLUM POX VIRUS (SHARKA) IN NORTHWEST ITALY

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Plum pox virus (PPV), or 'Sharka', was detected for the first time in Piedmont, northwest Italy, in the summer of 1982. Virus infection was limited to apricots, although the area also included orchards of peaches and plums (1). Of about 15,000 plants screened during 1982 and 1983, 1,400 (10.7%) were infected. Although the orchards more severely affected (infection 20%) were destroyed, further field inspections during the summer of 1985 in 95 apricot plantations revealed that virus spread had increased, ranging from 0.1 to above 40% in different orchards, with most frequent incidences between 2.6-10%.

Diagnosis. Immuno-sorbent electron mircroscopy (ISEM) and double antibody sandwich ELISA were used either together or alternatively to diagnose PPV in both field samples (stone fruit leaves, fruit, twigs, etc. and herbaceous plants) (1,2) and plants experimentally inoculated by aphids. Two antisera to PPV were used, one supplied by Dr. L. Box, Wageningen, and another by Dr. R. Casper, Braunschweig. The two serological techniques appeared equally sensitive in detecting the virus in apricots, giving 100% positive reactions with leaves showing symptoms. In apricots without symptoms as well as in symptomless leaves from infected plants, PPV presence could be demonstrated by both serological methods only erratically. When infected, N. clevelandii, routinely used as a virus indicator, showed symptoms of variable intensity; a 100% positive correlation was observed between the presence of these and the results of serological diagnosis.

Field investigations. Besides apricots, PPV was also found in the myrobalan plum and wild peach rootstocks of infected apricots, in very few young peach trees, and in one volunteer Prunus damaschina. It was never found infecting cultivated plums, ornamental Prunus spp. or about 120 wild herbaceous plants of 16 different species, growing in or around the PPV-infected orchards.

Aphids were practically absent in the large, industrial orchards, due to intense spraying with insecticides. Colonies of the following aphid species were, in contrast, frequently found on several Drupaceae grown in family gardens, in spring and autumn: Hyalopterus pruni (apricots), Brachycaudus helichrysi (prunus spp), Myzus cerasi (Prunus serrulata), M. ornatus (ornamental plums), M. persicae and M. varians (peach trees), and Phorodon humuli (P. pissardi, myrobalan plum). They were cultured in the glasshouse, under experimental conditions, and used for transmission experiments.

Aphid transmission. The ability to transmit PPV of 5 stone-fruit-infesting aphid species (B. helichrysi, H. pruni, M. persicae, M. varians, P. humuli) plus Aphis craccivora was investigated with three different virus donor/test plant combinations: (i) from the spring

vegetation of peaches and apricots to two-year-old apricots cv. Tonda di Costigliole, grown in a screenhouse, and to glasshouse-grown N. clevelandii, used as controls; (ii) from N. clevelandii to N. clevelandii, in the glasshouse; (iii) from peach to young 'CF 305' peach seedlings grown in the glasshouse.

None of 51 screenhouse-grown apricots inoculated by aphids from peaches and apricots, has so far shown evidence of PPV infection, about 2 years after the inoculation. Twenty-five N. clevelandii plants inoculated in the same experiment were also not infected with PPV. Periodical checks on the apricot trees are continuing. From N. clevelandii to N. clevelandii, PPV was transmitted by single individuals of H. pruni (3/7), M. persicae (18/20), M. varians (1/10) but not by A. craccivora (0/10), B. helichrysi (0/20), or P. humuli (0/20). From peach to 'GF 305' peach seedlings, PPV transmission was achieved by B. helichrysi (3/5), M. persicae (3/4), M. varians (4/5), and P. humuli (4/5).

Some virus/vector relationships were studied by using N. clevelandii as a test plant and M. persicae as vector. The inoculation experiments (total 420) were done in a climatic chamber (± 0.5°C), using one aphid per plant. Following the acquisition feeding of 1 min, M. persicae transmitted PPV to N. clevelandii in 72% of cases at 18°C, and in 30% at 26°C while, with 10 min acquisition feeding, it transmitted the virus to 28% of plants at 18°C, and to 2% of plants at 26°C.

The maximum retention of infectivity in fasting aphids, previously exposed to PPV acquisition, was 8 hrs. However, the proportion of individuals able to transmit decayed rapidly after a post-acquisition fasting period of 2 hrs.

Seed infection. The presence of PPV in both apricot seed kernels from infected fruit with symptoms, and young seedlings grown from other seeds of the same batch was checked by ELISA and ISEM. The results were as follows: (i) PPV presence was detected in 72% of 225 seeds of the cv Tonda di Costigliole, and in 90% of 220 seeds of the cv Bulida; (ii) to detect PPV in seeds, ELISA was 5-6 times more sensitive than ISEM; (iii) 180 apricot seedlings of both cvs, obtained from 250 seeds from plants with symptoms, grown in steam-sterilized soil in the glasshouse did not show PPV symptoms or give positive serological reactions, 1 month after their emergence. Eight months later, 50 such seedlings were re-tested as above and found still PPV-free. These results appear in contrast with those on seed transmissibility of PPV in Hungarian apricot cvs, where significant seed transmission was reported (3).

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CARLA-VIRUSES IN GERMAN HOPS

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The presence of rod-shaped viruses in German hops has been known since 1958 (4), but their effect on hops was in dispute for a long time, as they were found to be present in plants displaying symptoms of various types like "crinkle disease" or "infectious sterility." Electron microscopy was for a long time the only means to detect these viruses, but length measurements of the particles did not allow differentiation between particles of what we call today hop mosaic virus (HMV), hop latent virus (HLV) and American hop latent virus (AHLV) (1,2).

In a survey all nine hop-growing regions of Germany were examined for the presence of the three viruses mentioned. The samples were collected mainly in 1978-1980. The infestation with HMV reached from 64% in the hop-growing region Pfalz to 83% in the Tettnang regions, while HLV was found in 31% of the samples tested in Baden to 75% in Spalt. AHLV has not yet been detected in the German hop growing regions, but was found in hops introduced from America in 1978. These plants were kept in a breeding garden far away from the hop-growing areas.

Table 1 gives the average values for all samples and gardens examined in Germany. The figures point out the wide distribution of HMV (79% of the more than 3,000 samples and 98% of the hop gardens) and HLV. The latter has a lower infestation rate as far as the samples are concerned (51%) but 82% of the hop gardens were infected.

HLV could not be associated with any symptomatological deviation from the ordinary shape and appearance, while HMV was the cause of "mosaic disease," but in sensitive varieties only. Two German varieties were found to be insensitive: "Hersbrucker spät" and "Rottenburger Späthopfen," the latter out of cultivation now. Brewers Gold, in general a tolerant variety (97% HMV incidence), often shows yellow spots attributed to the variety character. In certain years these spots become more distinct resembling mosaic patterns. But no stunting or reduction in flowering could be observed in this variety.

Compared to cultivated hops, escaped and wild hops showed a much lower virus infestation (Table 1). The differences between these groups were significant except for HLV in escaped and wild hops, representing probably a common level of natural infection. The low HMV incidence in wild hops with only 2% of the 600 samples tested may lead to the suspicion, when compared to 79% infection in cultivated hops, that HMV is not a genuine hop virus, but when introduced into plantations it is able to spread quickly by plant contact, as demonstrated with grafting experiments, by infectious implements during cultivarion procedures as demonstrated with mechanical transmission and by aphid vectors. All three viruses were transmissible by Phorodon humuli and Myzus persicae. Aphis

fabae, also tested as vector for HMV, failed to transmit the virus. The dissemination of infected planting material may explain the overall distribution of HMV and HLV.

In sensitive "Hersbrucker spät" a correlation was demonstrated between severity of mosaic symptoms and extinction reading in the ELISA test. Plantations where this variety is in close juxtaposition to tolerant ones are soon characterized by typical disease gradients at the edges of the sensitive plot next to tolerant ones: "Hallertauer mittelfrüh" at the left and "Hallertauer Gold" at the right. The different anount of spread may be explained by the different infestation rates of the tolerant varieties in this particlar region with 89% for "Hallertauer mittelfrüh" and only 24% for "Hallertauer Gold."

Many farmers still tend to replace infected or killed "Hersbrucker spät" singly by plants of tolerant varieties, thus creating further problems: the introduction of HMV carriers into the plot and impurity as far as the variety is concerned, with problems during harvest and/or when selling the impure yield. The fact that in Bavaria, where most of the hop-growing regions are situated, the acreage of sensitive plantations expanded from 2973 ha in 1978 to 4915 ha in 1982 (3) may give an idea of the problems occurring.

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Table 1. CARLA-Viruses in German hops.

	% Infection					
	HMV		HLV		AHLV	
	sites	samples	sites	samples	sites	samples
Cultivated hops	98	79	82	51	0*	0*
Escaped hops	37	25	38	18	0	0
Wild hops	4	2	29	16	0	0

*At one site outside the hop-growing regions, four individual plants of the introduced clone USDA 21055 were infected.

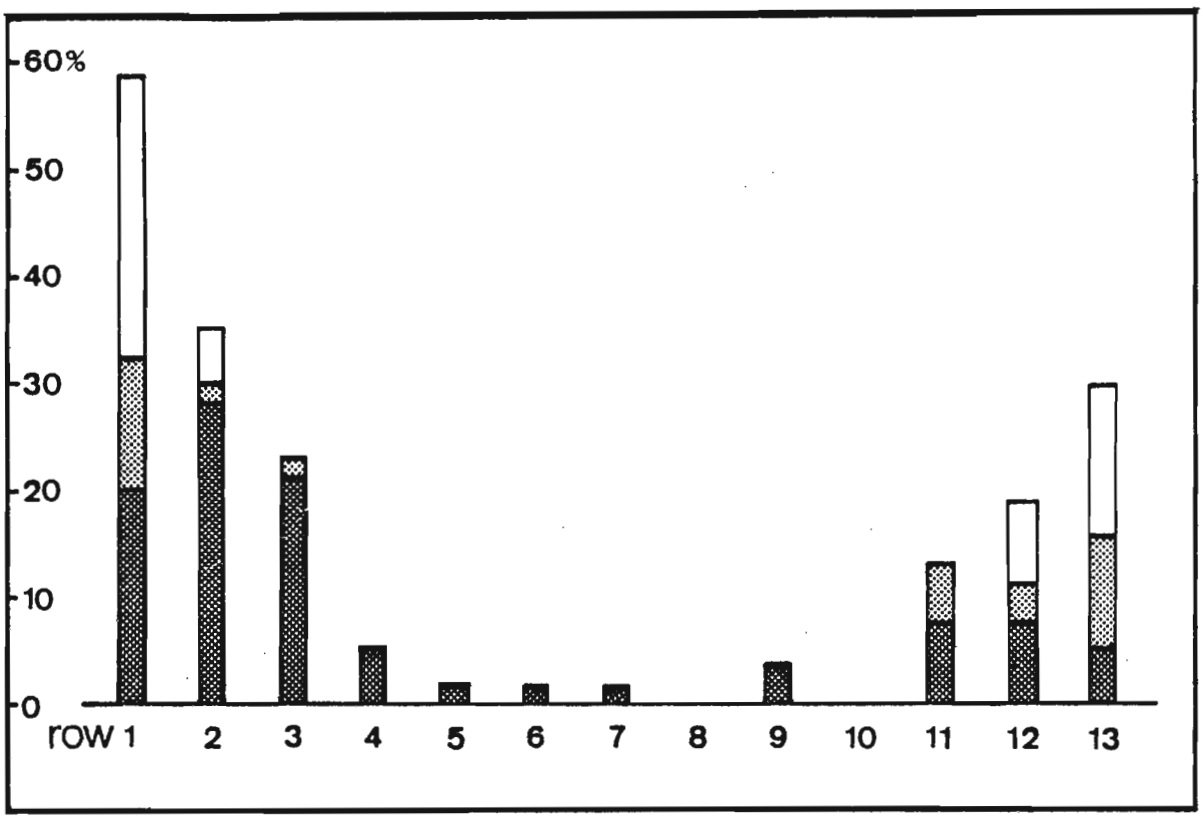

Fig. 1. Mosaic disease incidence in a hop garden in the hop-growing region of Hersbruck. Dark = plants displaying mosaic symptoms; medium = dead resp. grubbed plants; white = recognizably replanted plants. Adjacent to row 1 of Hersbrucker spät: Hallertauer mittelfrüh. Adjacent to row 13: Hallertauer Gold.

THE EPIDEMIOLOGY OF POTATO VIRUS Y IN ENGLISH POTATO CROPS

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Vectors of potato virus Y (PVY) in English potato crops are being identified by catching winged aphids on a net placed downwind of an infected crop (1) once a week throughout the growing season, confining each aphid on a tobacco seedling to test whether it could inoculate PVY and then identifying each aphid (2).

In 1984, 6769 aphids were caught and 165 transmitted PVY. Brachycaudus helichrysi, Myzus persicae, Phorodon humuli and Aphis spp. accounted for 90% of transmissions and B. helichrysi alone for 52% of transmissions. In 1985, B. helichrysi, P. humuli and M. cerasi accounted for 63% of transmissions and B. helichrysi was again the major vector, causing 29% of transmissions. Of the main vectors, only M. persicae colonizes the potato crop. This experiment will be continued in 1986.

In Britain and parts of Continental Europe, flying aphids are sampled routinely using suction traps situated throughout the area. It is intended to combine information gained on vector efficiencies with these data on species abundance to assess the amount of virus spread in crops both to assess health of seed crops and the correct timing for control measures to be applied.

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THE ROLE OF MIGRATORY APHID FLIGHTS ON NONPERSISTENTLY TRANSMITTED SOYBEAN MOSAIC VIRUS EPIDEMICS

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Soybean mosaic virus (SMV) presents a constraint to soybean production in parts of the world where early season infections lead to major reductions in seed quantity and quality (4). SMV will become important in other areas if factors leading to early season spread of the pathogen become more favorable. Seed transmission and its importance in the movement of the virus over long distances and between seasons, spatial distribution of initial inoculum foci, aphid vectors of the virus, their species, abundances, and flight timings, the propensity of each vector species to transmit SMV, genetic variations in host plant and virus, and timing of infection relative to crop phenology are major factors that contribute to SMV spread.

Seed transmission is the most important single factor in the dispersal of SMV. Soybean seed, shipped long distances over relatively short time intervals, accounts for the fact that SMV can be found wherever soybeans are grown and has made SMV the most widespread of the viruses infecting soybean. Seed transmission accounts for the carryover of virus from one season to the next. Because fields are often sown from single seed sources, the distribution of infected seedlings within given fields results in a mosaic of soybean fields, each with a randomized distribution of initial inoculum, but each with a potentially different inoculum level than that of its neighbors.

The only natural spread of SMV during the growing season is through transmission to noninfected plants by certain aphid alatae that are transient within the field. Timing and abundance of the several vector species account for disease progress in time and space. Aphid landing rates are measured by mosaic green pan traps set within soybean fields (5,6). Fig. 1 shows the SMV simulation model with the flow of aphids into the catch trap. The simulation model itself is explained in Ruesink & Irwin (9).

In the existing model for the seasonal progress of SMV, the probability of a healthy plant becoming infected during a given 24-hr period depends on the number of source plants present, the total number of plants present, and the measured landing rate of each aphid species. Because host plants are presumed to be randomly distributed within the field, there is no spatial component to the intrafield model. The conceptual model considers only intrafield buildup of the disease and assumes that neighboring fields have comparable or lower levels of SMV infection.

Forecasting the buildup and impact of SMV necessitates a knowledge of vector species composition and movement, obtained through monitoring

daily aphid landings. This must incorporate their intrafield, interfield, area-wide, and long-distance movement patterns. Transmission propensities are species specific (5). Absolute landing rates of any given species combine plant-to-plant movement and long-distance migration. Aphids moving within fields spread the virus in proportion to the number of source plants available, whereas it is assumed that immigrants carry no SMV. In other virus-vector systems such as the persistently transmitted maize dwarf mosaic virus (Richard J. Zeyen, personal communication), the pathogen may be carried long distances by migrating vectors. The present model includes logic to handle the two components of landing rates, but suitable monitoring methods to resolve them are lacking.

Our conceptual aphid movement model, where the environmental inputs are identified and measurable and the outputs realistically forecast the timing, abundance, and impacts of the incoming pests (3), consists of a horizontal translation component and a vertical movement component. Because aphid flight speed is low compared with that of wind under most conditions, the model assumes that aphid horizontal movement is controlled by air movement. The vertical component of the model considers voluntary and involuntary ascent and descent, under the control of biological and meteorological factors, respectively.

This model incorporates an objective back-trajectory analysis technique (10) developed from a predictor-corrector streamline routine (1) for the horizontal component. Three-hourly wind data are interpolated on a regular grid of 100 km spacing from 12-hourly upper air soundings. Back trajectories for 12- or 24-hour periods are computed from these interpolated data for the desired level corresponding to the elevation of the migrating aphids and, when combined with flight energy analyses, provides the potential for much improved resolution of source regions under all meteorological conditions. The reliability of these back trajectories is dependent upon the spatial and temporal sampling of wind speed and direction (3), especially for prefrontal zones.

A knowledge of the aerial distribution of migrating insects, including their elevation, density, spatial organization, and relationship to meteorological parameters, is crucial to the development of the wind transport model. Helicopter-mounted aerial collectors were developed and proved reliable and usable under most weather conditions. They accurately sampled absolute volumes of air, allowing the computation of realistic insect densities and partitioned samples by time and elevation. Specimens collected were undamaged, suitable for identification, and usable for biological assays.

Collections were made to heights of 2000 meters, with aphids being collected from as high as 1200 meters. We found that migrating aphids preferred prefrontal conditions of moderate to strong southwesterly flows of air, and that they are usually concentrated in distinct layers apparently associated with temperature inversions and wind maxima.

The vertical movement component of the model simplifies reality by dividing the troposphere into four layers (Fig. 1). The lowest layer represents the aphid pool on plants. The layer immediately above the

crop canopy contains the aphid pool within the surface boundary layer (about the lower 10-20 m). The layer of air in which turbulence and surface effects dominate and that is capped by inversions during aphid migrations is called the planetary boundary layer and typically is about 100-1000 m deep. The uppermost layer represents the aphid pool that has become involuntarily uplifted by convection into the free atmosphere above the planetary boundary layer.

Maximum aphid movements occur between the pool of landed aphids, the layer in which SMV is actually transmitted to plants, and all other layers. In our model, individual aphids voluntarily leave this layer by local take-off or migration take-off behavior. An aphid located within the plant canopy can ascend to only the surface and planetary boundary layers. Aphids reach the free atmosphere only by convective uplift from either of these two boundary layers, but not through behavioral motivation. Aphid landing is complex and can conceivably result from behavioral and physical actions.

Aphids that occur in the surface boundary layer are considered to be in a local or short-duration movement mode and will land but will not move into the planetary boundary layer. This pool is responsible for the short, plant-to-plant, intrafield movement patterns and is directly accountable for much of the virus spread within a field.

We believe aphids occurring in the planetary boundary layer are true migrants, having arrived from resident specimens that are in migratory dispersal (7), from aphids that are passing over the area, or from aphids that are terminating their long-distance flight and are descending. Our results indicate that movement from this layer to the pool of aphids in the plant canopy is largely cued by the environment and dictated by the depletion of fuel reserves within the body of the individual aphids.

Our conceptual model for the vertical component takes into account voluntary transport of the aphids during their ascent and descent. Furthermore, we consider the involuntary ascent from lower levels when convective currents produce vertical wind velocities in excess of an aphid's maximum flight speed. Meteorological models of air movement predict aphid movement, and their rate of settling from the free atmosphere is determined entirely by their aerodynamic properties and air movement.

We are studying flight activity of one of the major vector species of SMV in central Illinois, the corn leaf aphid, Rhopalosiphum maidis (Fitch) (Homoptera:Aphididae) (2). The relationship between flight duration and fuel utilization has been characterized under laboratory conditions. Under field conditions we can discriminate between resident and immigrant specimens and can estimate flight duration. Long-distance migration is most probable when flight initiation occurs between the ages of 0.5 and 1.5 days (8).

Predicting intrafield spread of SMV requires a knowledge of species composition and daily landing rates. Discrimination of flight activity improves predictive capabilities because different virus transmission

probabilities result from resident and immigrant specimens. This requires a knowledge of aphid movement patterns and flight energetics. Conceptually, aphid movement consists of horizontal and vertical components. We use an objective back-trajectory analysis technique, supported by measures of aerial densities and elevation of aphids, to determine the horizontal component. The vertical component conceptually features four pools of aphids in the troposphere: landed aphids which can move into the surface or planetary boundary layers; the surface boundary layer pool consisting of aphids in local, infield flights; the pool in the planetary boundary layer in migratory flight; and those involuntarily moved into the free atmosphere.

We hypothesize that the aphid pool in the surface boundary layer is responsible for infield spread of SMV; aphids landing directly from the planetary boundary layer do not contribute until they commence local flight activity. We are currently focusing our research activities towards the testing of this hypothesis.

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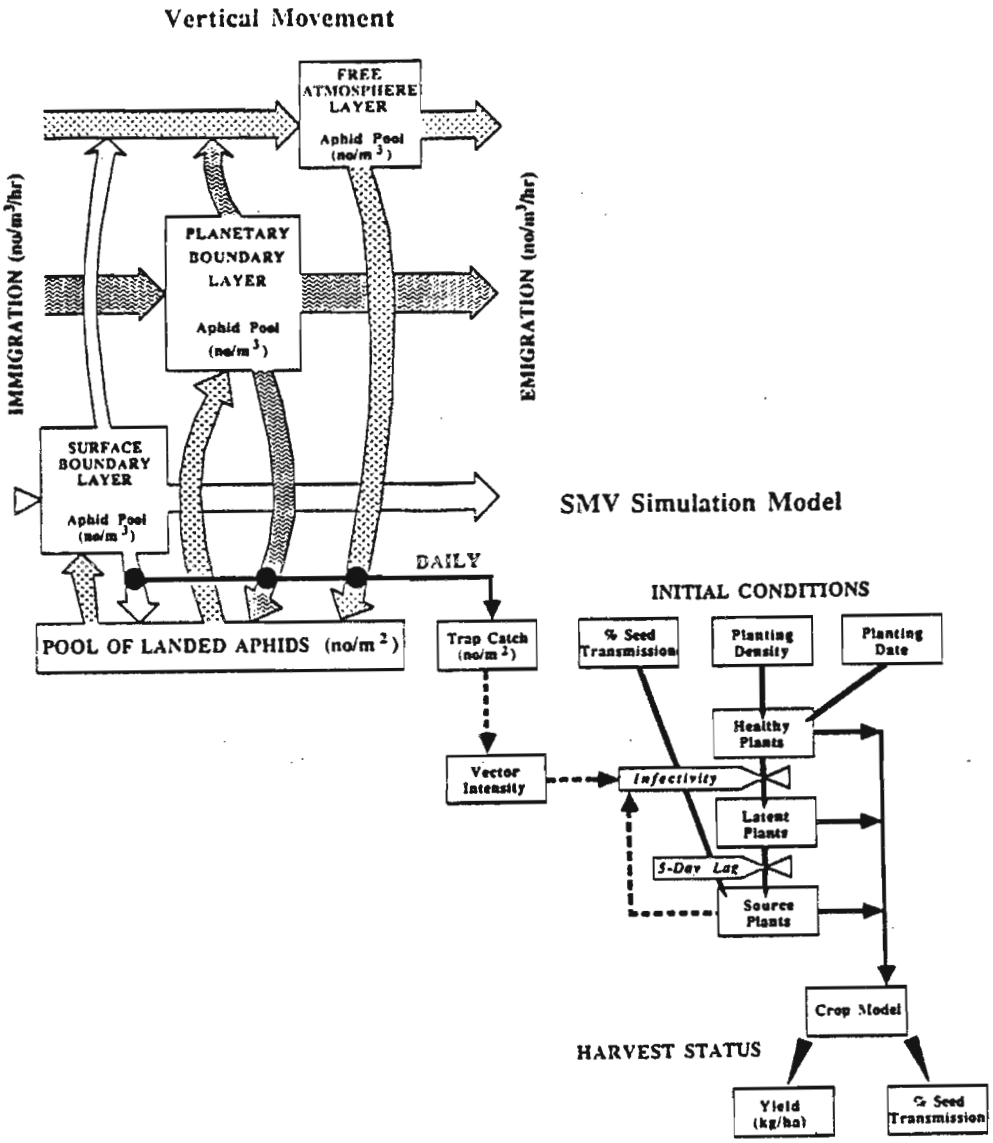

Fig. 1. Flow chart of aphid movement, illustrating component aphid pools of actual trap catch, and simulation model of SMV progress in a soybean field with yield and seed transmission outputs.

VIRUS BAIT PLANT TRAPPING AND APHID SPECIES ASSOCIATED WITH FORAGE LEGUMES IN MISSISSIPPI

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Alfalfa mosaic virus (AMV), bean yellow mosaic virus (BYMV), clover yellow vein virus (CYVV), cucumber mosaic virus (CMV), peanut stunt virus (PSV) and red clover vein mosaic virus (RCVMV) are aphid-transmitted viruses which cause important diseases of clovers (Trifolium spp.) and other forage and food legumes. To improve our understanding of the epidemioloy of these diseases, a long-term bait plant study of aphids and viruses associated with clovers was initiated at Mississippi State in 1982. Arrowleaf clover (T. vesiculosum), crimson clover (T. incarnatum) and white clover (T. repens) known to be susceptible to these viruses (unpublished results of Southern Regional Research Project S-127, Forage Legume Viruses), were used. Seedlings were grown in an insect-free greenhouse, transplanted individually to 1-gal cans and placed in the field (at 6-8 wk of age) for 1 wk. Twelve plants of each species were exposed each week (36 total). Plants were positioned with their crowns at the soil line by placing their containers down inside sunken metal sleeves. Plants were arranged in three groups of four plants each for each species. Plants within each group were placed 2 m apart at the N, E, S, and W compass points, around an ermine-lime-green water pan aphid trap (9 total) containing 50% ethylene glycol. Aphids were collected weekly, preserved in 70% ethanol and returned to the laboratory for identification. Plants were removed from the field at this time, sprayed to runoff with a combination of contact and systemic insecticides (Malathion and Orthene), held in isolation overnight, then returned to an insect-free greenhouse. Old and new leaves were collected from individual exposed bait plants 2 wk later, placed between layers of moist paper toweling, grouped in sets by species and exposure date, and stored inside sealed plastic bags at -20 C. Leaves were later tested for virus infections by enzyme-linked immunosorbent assay (ELISA). A continuous record was made of the weekly incidence of virus transmissions and associated aphid species. Similar records were made through cooperation with local researchers in Regional Research Project S-127, at the following locations: Raleigh, NC; Gainesville, FL; Experiment, GA; Lexington, KY; Baton Rouge, LA; and Overton, TX. At Mississippi State over 6000 aphid specimens representing 37 genera and 67 species were trapped and identified. Some of the Mississippi data, illustrating the seasonal incidence and fluctuations of the most prevalent aphid species in relation to virus incidence from January 1982 through September 1985, are summarized in Fig. 1. Average annual totals for each aphid species listed in Fig. 1 exceeded the 3-yr total of the most prevalent unlisted species. The incidence of BYMV, a potyvirus which infects the annual clovers (crimson and arrowleaf), and PSV, a cucumovirus which also infects white clover (a perennial) are separated for comparison.

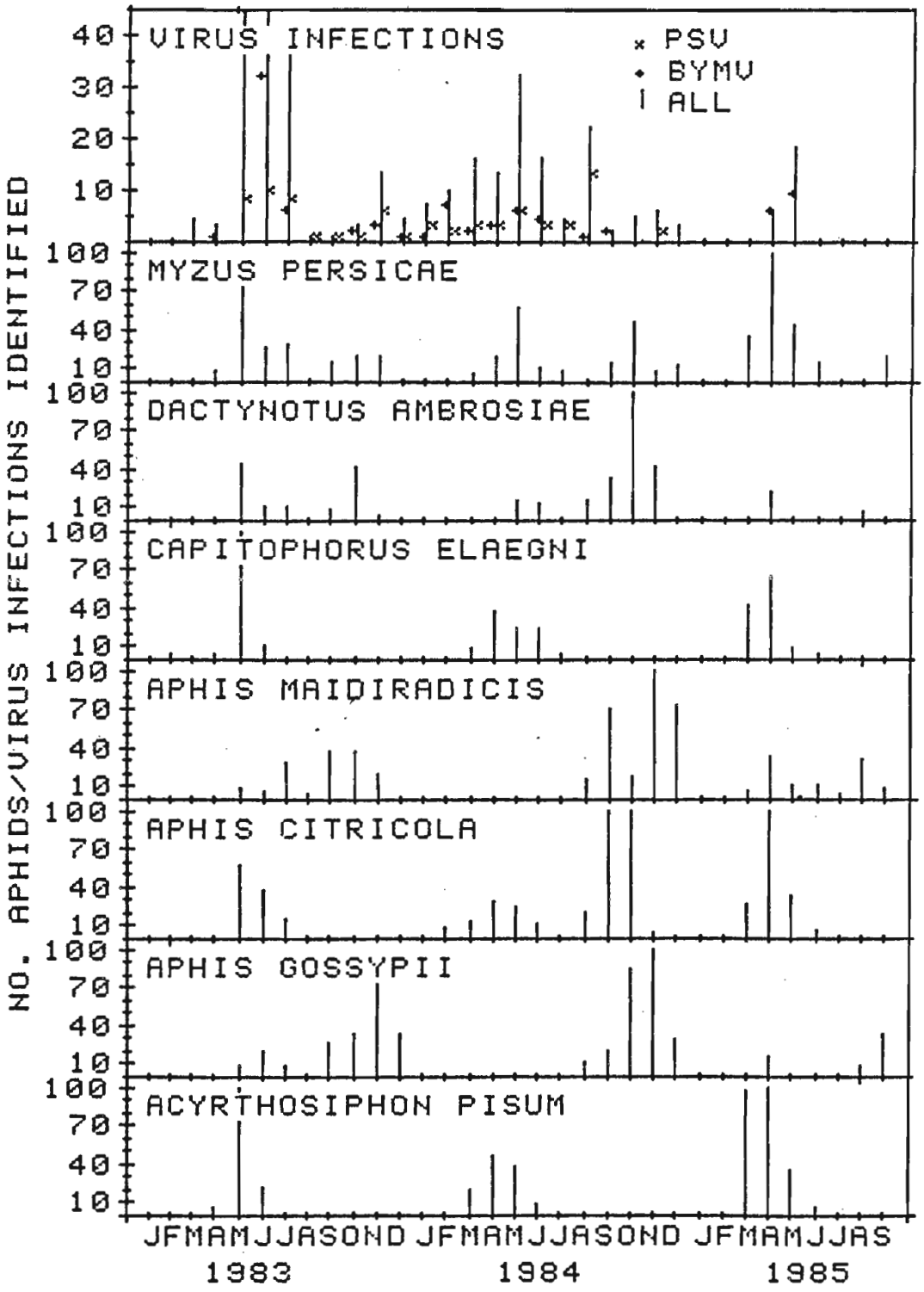

FIG.1. MONTHLY TRAPPING TOTALS, 1983-85

THE OCCURRENCE OF WINGED APHIDS OF DIFFERENT SPECIES AND THE SPREAD OF POTATO VIRUS Y IN POTATO FIELDS

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Some 31 species of aphids have been identified in the literature as vectors of potato virus Y (PVY). However, the determination of the important vector species in a country or in a region must also take into consideration the effect of local phenology on the disease and each aphid species. This abstract is a preliminary report on a study of key aphid vectors of PVY in New Brunswick conducted in 1984 and 1985 using an original technique.

In 1984 and 1985 PVY infected potato plots interplanted with the potato cv Jemseg serving as indicator plant for PVY (2) were used to determine the beginning of the spread of the disease at three locations in New Brunswick. In 1984, PVY was first detected July 20-24 in Grand Falls and Florenceville, and August 9 in Fredericton. In 1985 PVY was first detected July 22-24 at all three sites. The spread in August has traditionally been attributed to the inflights of green peach aphids taking place at that time (1). The earlier spread indicates the possible involvement of other vectors.

To identify these vectors we are sampling the aphid fauna in New Brunswick potato fields using yellow water pans. It is assumed that aphids caught in pans are fairly representative of the fauna that may land in a potato field. Aphids who arrive or increase in abundance at the time PVY starts spreading are presumed responsible and will eventually be tested in the laboratory for their intrinsic vector potential. The study is conducted at three sites to take into account regional variations and is to be repeated over 3 years to compensate for the yearly variations in faunal composition. This abstract reports on preliminary data for 1984 and 1985.

Some 59 different species or group-species of aphids have been collected. Thirty of these are occasional and four consist of the potato infesting species: the buckthorn aphid, the foxglove aphid, the green peach aphid and the potato aphid whose vector potential has already been studied (2,3).

Among the 25 species remaining, the following four have catches well correlated with the apparition of PVY in the field plots: *Hayurtsia atriplicis*, *Rhopalosiphum maidis*, *Acyrthosiphon pisum* and unidentified #17. *A. pisum* is a vector of PVY^n on tobacco and PVY^o on potato according to the literature. However, our tests (Boiteau et al., unpublished) indicate that the New Brunswick pea aphid is not a vector of PVY^o (0/70 transmissions). The transmission efficiency of the clones of pea aphids varies within wide limits for bean yellow mosaic virus. Maybe the clone tested was not an efficient PVY vector. *A. pisum*

remains a potential vector of PVY^{O} in New Brunswick until other clones have been tested. Our testing method was also different than those used by other workers. The vector potential of the other species is unknown.

Also, present at the time of PVY spread, but less abundant, are Capitophorus horni, Chaitophorus sp., Coloradoa rufomaculata (?), Hyperomyzus lactucae, Nasonovia ribisnigri and unidentified #57. H. lactucae can carry the virus but its overall efficiency remains to be established. According to the literature N. ribisnigri is not a vector. The vector potential of the other species is unknown.

Catches of Rhopalosiphum padi, a Rhopalosiphum sp. (#20), Pemphigus spp., Pterocallis alnifoliae and Amphorophora rubi started before the spread of PVY took place suggesting that these aphids play a minor role in PVY spread. R. padi may be responsible for some PVY transmission when very abundant. It has been identified as a vector although a relatively inefficient one. Pemphigus spp. and P. alnifoliae have never been tested for their vector potential. Our tests with Amphorophora rubi (Boiteau et al., unpublished) indicate that it is not a vector (0/35 transmission).

Catches of Aphis idaei(?), Cavariella aegopodii, Dactynotus erigeronensis, Diuraphis sp. and Eriosoma spp. peak before the spread of PVY takes place eliminating them as potential vectors. We also know from the literature that C. aegopodii is not a vector.

The Aphis spp. (#11, 8, 9, 14, 22, 23) remain potential vectors until they have been identified. Many have been shown in the literature to be vectors but their role may be limited by their low numbers at the critical time. Drepanaphis sp. were never abundant and our tests (Boiteau et al., unpublished) indicate that they are not vectors (0/35 transmissions).

In summary, in addition to M. persicae and A. nasturtii, there are nine aphid species suspected of playing a role in PVY spread on potatoes in New Brunswick, including known vectors A. pisum and H. lactucae. Their intrinsic vector efficiency must now be determined for the New Brunswick biotypes.

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APHID TRANSMISSION OF GROUNDNUT POTYVIRUS ISOLATES

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Peanut green mosaic virus (PGMV) has been reported as anew member of the potyvirus group occurring naturally around Tirupati (2). It is transmitted non-persistently by Myzus persicae and Aphis gossypii, but not by A. craccivora. A few more isolates were collected from the farmers' groundnut fields around Tirupati. They are serologically related to PGMV and a few other potyviruses (unpublished data). Three of the isolates produced symptoms on groundnut different from PGMV induced symptoms, but the viruses have similar physical properties. These virus isolates had different host-ranges. They are flexuous rods, and induced pinwheel and cylindrical inclusions characteristic of potyviruses. Based on the symptoms induced by these isolates on a local French bean cultivar, they are tentatively called non-systemic (NS: only local lesions), systemic mosaic (SM: local lesions followed by systemic mosaic) and systemic necrosis (SN: local lesions followed by systemic necrosis). SM and SN isolates, but not the NS isolate, are transmitted by A. craccivora (cowpea), M. persicae, Taxoptera odinae and A. gossypii (1). A. craccivora from groundnut could not transmit these three virus isolates.

In this report the authors present the detailed transmission characteristics of SM and SM isolates by A. craccivora from cowpea. Cowpea aphids, reared from a single adult aphid on healthy cowpea leaves, were subsequently cultured on healthy caged cowpea plants.

Fully expanded groundnut leaves showing severe symptoms and 15 day old healthy groundnut plants were used as virus source and plants.

Aphids were given pre-acquisition starvation periods in glass test tubes for ½, 1, 2, 3, 4 and 5 hr. The percent transmission was greatest with aphids starved for 2 hr for the SN isolate (20%) and for ½-1 hr for the SM isolate (52.5%). However, unstarved aphids also transmitted the two isolates (6.6% for SN; 15.0% for SM). Pre-starved aphids were allowed to acquire virus on virus source leaves for 30 seconds, 1, 2, 3, 5, 10, 20, 30 min., 1, 3, 6 and 24 hr. The percent transmission of the isolates decreased (from 18.3% to 3.3% for SN; 22.5% to 2.5% for SM) gradually as the feeding period increased. Shorter acquisition access periods of 30 sec. (for SN) and 2 min. (for SM) were more effective than continuous feeding.

Inoculation access periods of 2, 5, 10, 20, 30 min., 1, 5, 8, 24 hr were given to viruliferous aphids on test plants. Shorter inoculation periods of 3 and 5 min. gave higher percentage transmission (15% for SN; 12.5% for SM) of the isolates.

The percent transmission of the isolates decreased as the duration of the pre-inoculation starvation period increased, indicating virus

inactivation in the vector during starvation in a glass container. No transmission was noticed with aphids starved for 30 min. for the SN isolate and 10 min. for the SM isolate.

Aphids carrying the two virus isolates were able to inoculate only 1-3 plants out of 11 plants exposed in a series, regardless of the length of the virus acquisition periods (5, 10, 20, 20 and 60 min.). These data indicated that aphids cannot retain these virus isolates for long periods unlike circulative viruses.

Minimum of two aphids/test plant for the SM virus isolate and five for the SN virus isolate were necessary for transmission, and percent transmission increased with the increase in the number of aphids/plant.

Nymphs and apterae of A. craccivora were more efficient in transmitting the two virus isolates than alatae.

Based on the above transmission characteristics, it is concluded that the two groundnut potyvirus isolates are nonpersistently transmitted by A. craccivora from cowpea.

A. craccivora from cowpea did not colonize on the groundnut plants. Cowpea is commonly grown as a mixed crop along with groundnut. Thus cowpea aphids may play a role in the epidemiology of the present groundnut virus isolates as short acquisition and inoculation access periods are sufficient for transmission.

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EPIDEMIOLOGY OF CEREAL VIRUSES WITH SPECIAL REFERENCE TO BROME MOSAIC VIRUS AND CUCUMBER MOSAIC VIRUS

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In South Africa brome mosaic virus (BMV) was first noticed to occur in wheat in 1964 in the Orange Free State and in association with Puccinia graminis tritici on Agropyron distichum growing on coastal dunes in the Western Cape.

In 1978 the aphid Diuraphis noxia was detected for the first time in wheat fields in the eastern Orange Free State, which became the major wheat producing region since about 1970. This is a new invader aphid, previously only known in the Middle East. In recent years its presence was also noted in Ethiopia, Zimbabwe, the Yemen, and Mexico. The aphid is tolerant to cold, dry winter conditions and starts colonizing wheat early in winter. Abnormal symptoms are noticed in D. noxia-infested small grains due to the aphids' phytotoxic effect and the feeding damage caused by the large numbers in which they occur. Observed symptoms include: dwarfing, yellowing, streaking and yellow blotches on foliage, dead ears, cessation of stem elongation at ear emergence, sterile ears or parts of ears, uneven length of shoots and early death. Analysis of affected plants showed that several viruses could be present. BMV was invariably found to be present in high concentrations and appeared to be the most prevalent virus. Subsequent laboratory aphid transmission experiments showed that a complex of viruses consisting of barley yellow dwarf virus, BMV, Rhopalosiphum padi virus and an unidentified filamentous virus were present in field collected plants (1). Later investigations showed that CMV was also present in some specimens (unpublished results). Visual diagnosis of infected plants was unsatisfactory and inaccurate. In mild weather conditions, diseased plants appeared yellow as if infected by barley yellow dwarf virus (5) whereas under dry, warm conditions, foliage died early, thus complicating diagnosis and emphasizing the need for detailed laboratory analysis, i.e, extraction, fractionation, serology, immuno-electroblotting (2) and electron microscopy.

Seed from BMV infected field and laboratory plants contained seedborne virus (seedcoat & embryo) and gave rise to infected seedlings (4). With few exceptions, the infection was latent. Symptoms could be induced by colonizing seedlings with a latent infection with virus-free aphids for 2 days. Virus content was shown to increase 10- to 20-fold in such seedlings. This observation possibly explains the high concentration of BMV in field grown wheat late in the season, whereas virus concentration early in the season (pre-aphid infestation) was usually low (unpublished results). Seedborne BMV was also detected in seed obtained from sources outside South Africa.

In A. distichum BMV was initially found in association with P. graminis tritici. Subsequent studies showed that uredospores originating

from BMV-infected wheat carried large quantities of virus on their surface and could initiate BMV infection when germinating on wheat and barley seedlings (3). Rust pustules developing on BMV-infected wheat (latent infection or symptoms) were smaller and gave the appearance of reduced susceptibility.

In 1984 a severe infection of wheat with CMV was investigated in the Eastern Transvaal causing an estimated yield loss of 40-50%. The virus was identified serologically and was shown to be sap- and aphid-transmissible. Predominant symptoms, in this case and in other CMV-infections in wheat, were the emergence of yellow-white sterile ears at the time of flowering, cessation of stem elongation and strong yellowing of foliage. In conditions of water stress the appearance of sterile white ears is a dominant feature related to the percentage seedborne virus at time of sowing (unpublished results). Prior to ear emergence apparent symptoms were absent. Double infections of BMV and CMV have occasionally been observed and were best diagnosed by whole virus electrophoresis and immuno-electroblotting (unpublished results). The presence of several viruses with similar transmission mechanisms and symptomatology complicates the diagnosis of single viruses and their epidemiologies. Although the D. noxia aphid migrated to other wheat growing regions in South Africa, the infestations were never as severe as in the eastern Orange Free State. It is believed that the virus disease problem can be attributed mainly to the presence of the new invader aphid and that this incidence is another example where the introduction (voluntary) of a vector aggravated the disease condition of a latent virus.

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A SUMMARY OF THE EPIDEMIOLOGY OF AFRICAN CASSAVA MOSAIC VIRUS

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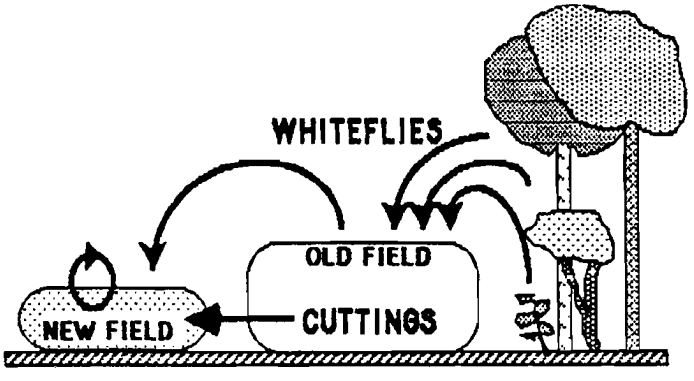
The first aim of the Laboratory of Plant Pathology in Adiopodoumé (Ivory Coast), when it was created by the ORSTOM in 1969, was to describe the predominant tropical viral diseases of the African continent (7). At the end of this preliminary phase of etiology, we decided in 1979 to focus our attention on one of the most serious viral diseases identified in this continent - the African cassava mosaic virus (ACMV).

Justifications. The economic importance of the disease was the determining factor when we decided on the choice of the program. The cassava crop is the most important food crop in Africa. Over 50 million tons of fresh tubers are produced each year. African cassava mosaic disease is not the most spectacular disease of cassava, when compared to bacterial blight, mealy bug, mites and antrachnosis. However, since ACMV occurs each year and is widespread over the whole continent, it is therefore likely to be the most devastating disease of cassava. The first objective of our program is to understand the epidemiology of the disease and to propose sound measures of control.

ACMV is a geminivirus transmitted by the whitefly Bemisia tabaci. Whitefly-transmitted geminiviruses are now known to be responsible for an increasing number of viral diseases in tropical regions. Although large advances in the etiology and pathogen characterization of these diseases have occurred recently, comparatively little attention has been devoted to the epidemiology of the disease. The second objective of our program is to provide some basic knowledge which could help in understanding other whitefly transmitted geminiviruses. ACMV is endogenous to the African continent, however similar symptoms have been described in India, but so far it has not been detected in South America.

Overview of the problem. The disease is transmitted in two different ways, by the whitefly Bemisia tabaci, and by man through the cassava cuttings. Cassava was first introduced into Africa in the 16th century, free of virus but today it is almost 100% infected. What is actually the real important vector - whitefly or man?



The answer to this question is essential because it determines two very different strategies for the control of the disease: 1) if man is the main vector, an educational program should be initiated to improve the distribution and choice of healthy cuttings, 2) if whitefly is the main vector, cultural practices and resistant clones should be developed to lower the impact of the disease.

Statement of knowledge in 1980. From the beginning of the century, the symptomatology of the disease had been extensively described in every country of the continent. The transmission patterns were studied mostly in East and West Africa (4,6,14). Adult and larval stages of whiteflies transmit the disease in a persistent manner, but there is no transovarial transmission. The ethology of the vector was quite unknown, with only a few studies done on the population dynamics of the insect (10,12). Two strains of the virus, a mild and a severe one, have been known in East Africa for a long time (1), and two serologically related strains were recognized (3): one originating from the east of Kenya and the other one from the west of Africa (13). It was only in 1983 that Bock (2) confirmed the geminivirus etiology and proposed changing the previous name of "Cassava latent virus" to "African cassava mosaic virus." Selection programs were initiated in 1947 in East Africa (11) and carried on in Kenya and Nigeria (5,8,9). All these programs produced resistant clones to ACMV, but the type of resistance was unknown. An extensive study of ACMV epidemiology was carried out in Kenya from 1973 to 1983 (1). From these experiments it was concluded that man is the main vector of the disease in Kenya and that whitefly spread was limited. Thus, control of the disease could be achieved simply by a distribution of healthy cuttings combined with some survey of the fields and eradication of newly infected plants. However, the results of this work could not be extended directly to the whole continent and additional studies on ACMV epidemiology needed to be carried on in other countries. For all these reasons we decided, in 1979, to develop a research program on the epidemiology of ACMV in the Ivory Coast.

RESOURCES AND DIFFICULTIES

Plant material. Epidemiological studies are usually based on trials where recontamination of healthy cassava plants is followed. The first difficulty in developing an epidemiological program on ACMV was to find large amounts of healthy material: all the cuttings available were infected due to vegetative propagation of the host and consequent virus transmission. Sophisticated techniques, such as meristem culture or thermotherapy combined with in vitro culture have been successfully applied to cure some cassava clones. However, with these techniques, only limited healthy material could be provided. A natural phenomenon - we called it reversion - occurs in the fields: a percentage of diseased plants give rise to some healthy stems. Although it occurs at a very low percentage, it allowed the selection and multiplication (in special conditions), within 3 years, of six different healthy clones with enough material to plant up to several hectares. In addition, we introduced some healthy resistant clones from Kenya and Nigeria. Our germplasm now totals about 50 clones. These clones from other countries provided us

the opportunity to compare our results with those obtained in different countries.

The virus. In 1980, the geminivirus named "Cassava latent virus" was only suspected to be the causal agent of the disease, so we were not sure until 1983 that we were working with the actual causal agent. The ACMV is difficult to purify and poorly immunogenic, thus the antiserum is not sensitive and the usual serological technqiues are of limited value. The biological assay by mechanical inoculation from cassava to tobacco, even if it were feasible to perform, does not detect all infected clones. All these constraints led us to develop an ELISA test to evaluate the virus concentration. However, the extent of the surveys (several thousand plants are checked each week) explains why field surveys had to rely on symptom assessment. This method is not ideal because, after inoculation, there is a latent period before symptom appearance. The length of this period depends on the clone tested and on climatic conditions. This unpredictable length of the latent period causes some uncertainty about the real level of infection, as it is never certain that a symptom-free plant is also a virus-free plant.

The vector. The difficulties faced with the vector result from obstacles encountered in handling and sampling due to its small size (1 mm long) and from the lack of basic knowledge about its biology and ecology. Species of Bemisia can be recognized only at the pupal stage. So, we can never determine to which species an adult whitefly belongs. The ethology of this vector has not been extensively studied in any region of the world. We overcame these difficulties in studying the movements and behavior of the vector because, on cassava, a very high percentage of pupae are Bemisia tabaci so we could estimate that the adults were present in the same proportion.

METHODOLOGY

Cassava growth is highly dependent on the environment and on the cultural practices. The variability of the cassava growth pattern causes obstacles; laboratory experiments, conducted under controlled conditions to test the influence of factors such as symptom expression or clone susceptibility to whitefly inoculation could be misleading, as the cassava growth is very different from its growth in a field. However, most of the experiments were carried out in the fields. We balanced the difficulty of uncontrolled conditions by conducting many experiments, taking into account many variables and using multivariate analyses.

RESULTS

We present the results of our program in eight different subjects, taking into account the vector, the virus and plant, in the environment of the Ivory Coast.

Ecology of ACMV. The effect of the virus on cassava yield and the effect of the reservoirs on contamination are described. The relations between the "actors" are presented and show a noticeable connection: the greatest number of vectors are feeding on the leaves that are the most

susceptible for acquisition, and which contain the highest virus concentration. Nevertheless, the percentage of viruliferous whiteflies is extremely low: 0.18-0.67%.

Field dispersal of Bemisia tabaci, vector of ACMV. This study describes the different aspects of vector landing, multiplying, moving and leaving the field. It shows the important effect of the wind direction and intensity on these movements. These results explain different aspects of the epidemiology of ACMV.

Spatial pattern of ACMV spread. As a consequence of the vector ethology, the dispersal of ACMV in the fields follows a gradient in relation to the prevailing wind. This gradient remains all along the time of the culture and exists in very different field environmental conditions.

Automatic mapping of the spread of ACMV. The application of the theory of the regionalized variables allows us to explain, describe and map automatically the development of the viral disease. It presents a practical interest in that estimating and mapping the spread of ACMV can be done with a sample of 7%.

Primary and secondary spread of ACMV. Compared to the ACMV secondary spread, the primary contamination is the most important. A practical result of this finding is the implication that removal of diseased plants would not allow the maintenance of healthy plantations in a considered region.

Development of ACMV at a regional level. This study demonstrates that the contamination of different fields is neither exclusively depending on the number of whiteflies, nor on the plant growth of cassava, but also on the environment of the field. The presence of diseased cassava up-wind from the field is the determining factor for its contamination rate.

Temporal pattern of ACMV spread. This experiment, conducted 5 years, shows the annual fluctuation of the inoculum pressure, of the whitefly population and of cassava growth. Temperature is the most important factor acting on all these variables. The interrelations of these variables and of climatic factors were studied and it is possible, within the experimental conditions, to forecast the development of ACMV accurately within 2 months and roughly on a yearly basis.

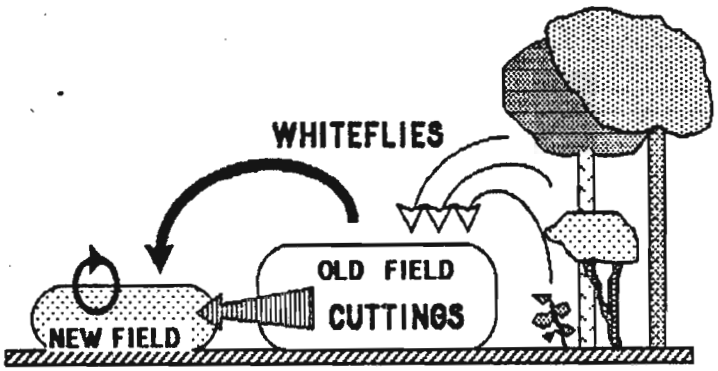
Multicomponent resistance of cassava to ACMV. Field resistance is mostly the expression of symptom resistance, but other components exist. Among them, one is the vector resistance which has never been suspected nor used and which is, furthermore, almost independent from the other components, suggesting that independent genes are involved and allowing new selection schemes for ACMV resistance to be devised.

DISCUSSION-CONCLUSION

One objective of our program was to understand the development and provide knowledge on the epidemiology of whitefly transmitted diseases,

such as ACMV. The disease spread in space and time is now well known and we are able to describe and understand the development of ACMV. The most efficient climatic factor predictors are temperature and wind. Both are acting on the vector and consequently on the disease. Almost all the movements as well as the behavior of Bemisia tabaci, are in relation with the direction and intensity of the wind. We think that these results are a general feature whatever the region considered. The temperature is acting on the population dynamics of the vector and also on the growth of cassava. Though the action of temperature on the growth of the vector populations might be a general feature, the prevalence of this factor, obtained in our region, cannot be extended to other regions without experimental confirmation. In other aspects, experimental results have shown the influence of the plant growth on the susceptibility to the inoculation and on the behavior of the vector.

The crop losses due to ACMV are of considerable importance and could easily justify this study. They are higher in the case of viral transmission through the cuttings than in the case of whitefly transmission. Even if the plantation is recontaminated during the culture, planting healthy cuttings is a positive action with regard to the production. This is in favor of a sanitation program which requires healthy cuttings. The main reservoir of virus and vector is, actually, most probably cassava itself (see figure below). This result also favors sanitation techniques.



ACMV EPIDEMIOLOGY IN 1986

The determination of the most important vector depends on the local conditions; it might be man or whiteflies, or both. In consequence, in each region, it is necessary to determine whether or not it is feasible to grow healthy plantations. The results obtained on the eastern coast of Kenya or in the center of the Ivory Coast support this conclusion, but those obtained in the south of the Ivory Coast show their relativity. This is naturally dependent on the field resistance of the cassava clone multiplied. The cassava resistance to ACMV is multicomponent and, particularly, we have demonstrated the existence of a vector resistance which remains unexploited in the selection programs to the ACMV.

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AFRICAN CASSAVA MOSAIC VIRUS: THE VIRUS, THE VECTOR, THE PLANT AND THE RESERVOIRS

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The ecology of African cassava mosaic virus (ACMV), is peculiar: the disease results from the encounter of a plant originating in South America (2) with a viral pathogen likely native to Africa. This pathogen, a geminivirus, is transmitted by man through the planting of diseased cuttings and by the whitefly Bemisia tabaci. We investigated yield losses in relation to the mode of infection. We studied the relationships between the "actors" involved in the disease spread: the virus, the vector, the plant and the reservoirs.

Yield losses. Dates of symptom appearance were recorded individually for 500 plants in two 1-ha fields planted in October 1982 and July 1983, respectively. Roots were weighed individually 12 months after planting. Results are shown below.

Date of symptom appearance (DAP)	<45	60	90	120	150	180	>195	H
Root weight (kg)								
Field 1 (mean)	1.33	2.13	2.39	2.60	2.85	2.93	2.60	2.70
Field 2 (mean)	1.32	3.42	4.60	3.95	5.26	5.62	5.39	5.0

Preliminary experiments showed that ACMV transmission through cuttings induced symptoms within 45 days after planting (DAP), whereas following whitefly inoculation, symptoms appear later. Highest yield reductions are observed in vegetatively infected cassava. In both trials, infection by vectors, even when it occurred early, had less effect. When infection is by B. tabaci, both experiments indicate that the earlier it occurs, the greater is the yield loss. After 120 DAP, yield of infected plants does not differ significantly from that of healthy cassava (H).

Reservoirs of ACMV. The reservoirs of ACMV were investigated by combining ELISA (4) and transmission tests. Based on these results, only two Euphorbiaceaes Manihot glaziovii and Jatropha multifida are, with a high degree of certainty, hosts of ACMV. However, epidemiological studies suggest that their role as reservoir of virus and vector is limited compared to the cultivated cassava, Manihot esculenta ("Development of the disease at a regional level," same issue).

Virus/vector/plant relationships. On each cassava, leaf position was counted from the youngest unfolded leaf (graded F1) downward to the older leaves(F2, F3...). Leaves F0 and F-1 were younger, smaller in size, and still folded. Maximum surface is usually reached at leaf F4. Surface does not increase further when aging (Fig. 1). On these aging leaves, we have followed:

- The concentration of virus, estimated by ELISA tests (A 405 nm). Maximum concentraton is reached on leaf F1 and virus content then decreases in older leaves. ACMV is not detectable in leaf F7 and in older leaves;

- Whitefly populations were periodically evaluated. The adult whiteflies are gathered on the younger leaves F-1 to F3. Very few adults were detected on the older leaves. Most larvae are located on leaves F5 to F7, as a result of the adult distribution;

- Sensitivity of aging leaves to ACMV has been evaluated by Storey & Nichols (3). They set groups of 100 whiteflies on leaves of different ages and observed the number of plants showing symptoms afterwards (Fig. 1). They concluded that the young growing leaves are susceptible to the disease, whereas the mature ones are not.

The young cassava leaves not only contain more virus but also are more susceptible to infection than mature ones. So the prevalence of Bemisia tabaci on the young growing leaves of cassava will help both the acquisition and inoculation and, thus, the field spread of ACMV. Surprisingly however, the percentage of individual B. tabaci in cassava fields which transmit ACMV, as established by infectivity tests, is usually very low (Fig. 1) when compared to viruses such as cowpea golden mosaic virus where transmission per individual may exceed 70% (1).

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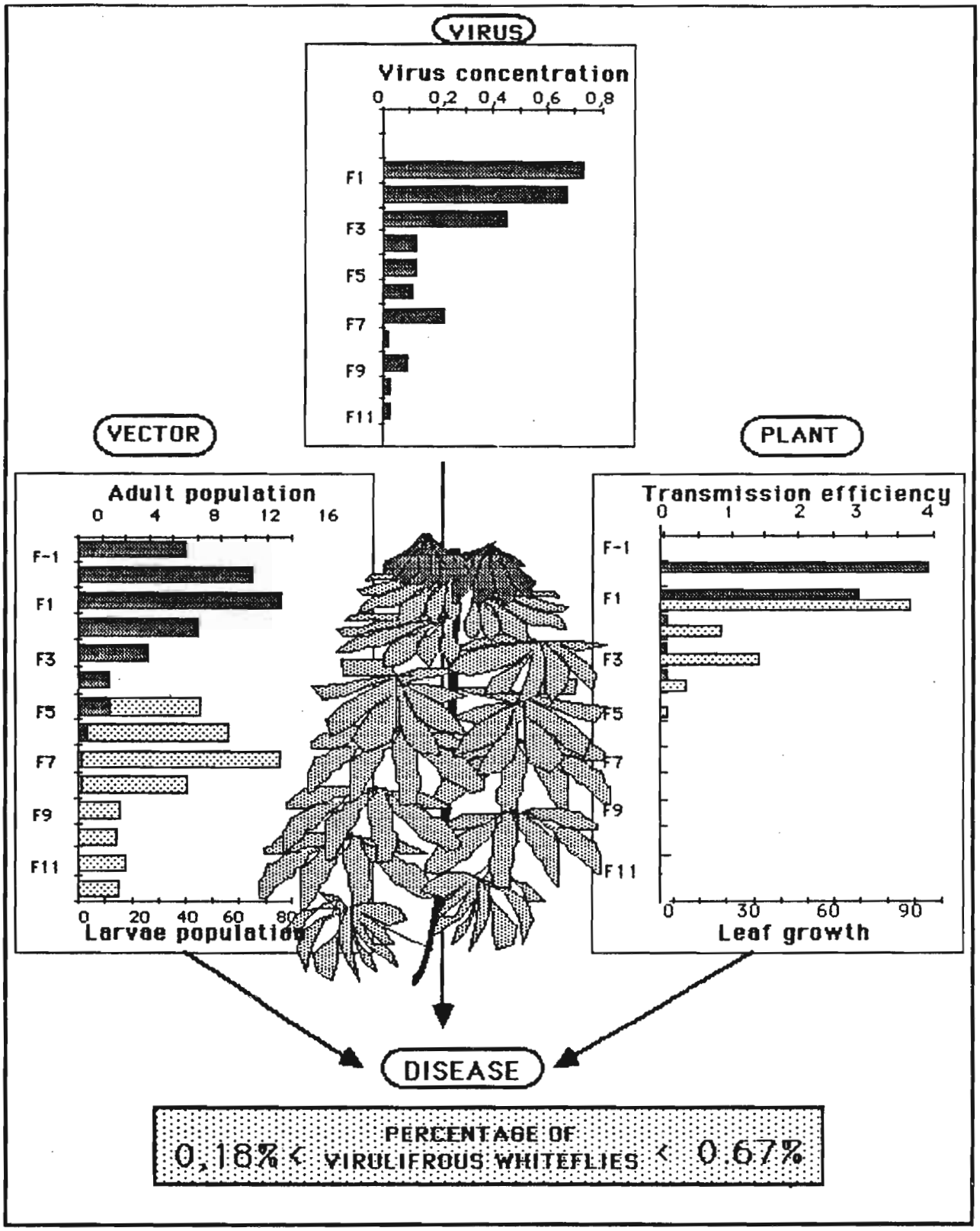

Fig. 1. For aging leaves are indicated: the ELISA absorbances (top of the figure), the number of whiteflies per leaf, adult and larvae (on the left), the sensitivity of the leaves to transmission and the surface leaf growth (on the right). Percentage of viruliferous whiteflies collected in the fields is indicated at the bottom.

FIELD DISPERSAL OF *BEMISIA TABACI*, VECTOR OF AFRICAN CASSAVA MOSAIC VIRUS

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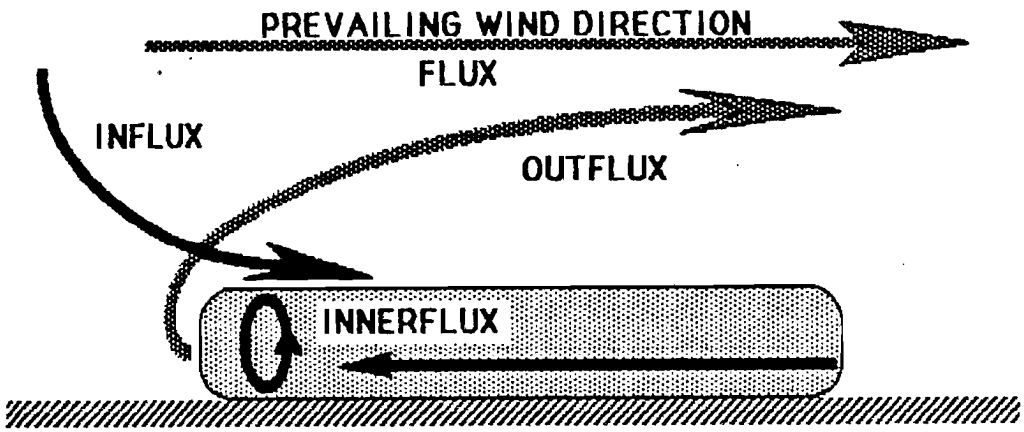
African cassava mosaic virus (ACMV) is transmitted, in a persistent manner, by *Bemisia tabaci* Gennadium (Aleyrodidae). Epidemiological studies have shown that several features of ACMV spatial spread (disease gradients, rates of primary and secondary spread) are likely to be linked to whitefly movements ("Spatial pattern of ACMV spread," "Primary and secondary spread of ACMV", same issue). To define these movements and their relation with infection, we studied whitefly dispersal in a cassava field. This dispersal is composed of four different movements: 1) the flux of whiteflies flying above the field (not studied here), 2) the influx of landing whiteflies, 3) the innerflux including the movements inside the field and the multiplication of the insect, and 4) the outflux of whiteflies taking off from the cassava field.



All four categories of flux occur simultaneously but their relative importance changes during the culture. Furthermore, the climatic conditions (particularly the wind direction and intensity) could obviously influence some of them.

MATERIALS AND METHODS

We planted, with the CB clone, a 0.5-ha cassava field facing the prevailing wind. The trial was planted at the beginning of the dry season to get a high multiplication rate of the insect.

The experiment is based on two main principles: 1) a wide range of insect traps, and 2) the duration of the experiment for 5 months. Some traps screen the air and gather passively the insects whereas others imply their active movement.

The catching techniques used were the following: 1) counting of the adults on 490 plants; 2) counting the larvae on 14 plants; 3) unattractive sticky traps - distributed at four levels (0.5 to 2.5 m) in 18 sites, inside and outside the field; 4) attractive yellow sticky traps: each sticky trap is made of 10 yellow rings (10 cm wide), separated in eight directions and distributed on 10 levels (0.1 to 3.0 m), 12 of these sticky traps were placed in and out of the field; 5) a suction trap, situated 20 m up-wind of the field. The wind speed was registered in 10 points of the field, allowing the detection of a vertical and horizontal gradient in the cassava field.

RESULTS

The comparison of the catches of the different categories of insect traps allowed us to describe the different movements involved.

Influx. The influx appears all along the experiment but, compared with the other movements, was predominant in the first 50 days of the culture.

Innerflux.

a. Population dynamics. It is composed of three different parts: i) a setting phase corresponding to the influx contribution during 50 days, ii) a multiplication phase during 50 days, and iii) a decreasing phase of 50 days. This dynamic was observed in all parts of the field and with all the different traps. A good correlation also exists between the adult and larvae population dynamics (all instars cumulated).

b. Vertical distribution of the vectors. Whatever the stage of plant growth, 90% of the counted adults feed on the five upper leaves. During the plant growth, the insects follow the canopy rise. However, when the canopy is closed (1-1.20 m), the vectors fly in the morning at the apex level, then fly downwards at mid-day and upwards in the evening.

c. Horizontal distribution of the vectors. Whatever the wind direction, whiteflies are scattered in the field following a gradient: the maximum is in the up-wind border and the minimum in the down-wind border. This gradient is always observed even for low or high populations. The number of flying insects is related to the total number of whiteflies present and to the wind speed in that place. Thus the highest whitefly activity is registered in the down-wind blocks in phase i, in the center blocks in phases ii and iii, and as the plants are canopied, the vectors are more active in the up-wind blocks.

d. Flying direction of the vectors. Before the establishment of the canopy, the whiteflies are flying windward, but in the down-wind blocks the wind speed is so low, it enables the insects to fly against the wind. When the canopy is continuous, the vectors keep flying against the prevailing wind, between the ground and the canopy, and windward above. The

results are always the same for any wind direction (N, W or SW) and when it is windless catches happen in all directions.

e. Daily activity of the vectors. We performed eight experiments with catches of 10 to 2000 insects, and all the maxima were recorded between 6 h and 8 h A.M. and all the minima between 12 h and 14 h P.M.

Outflux. The traps placed exactly on the edge of the up-wind blocks show an abnormal increase of the ratio in the beginning of phase iii. It may correspond to the outflux of the vectors against the wind in the canopy (up to the up-wind edge of the field) and windward outside, and above, the canopy of the field.

DISCUSSION

The whiteflies' movements are conditioned to the existence of the "Boundary layer" (1), which depends on the wind speed (2) and on the plant growth. The drastic decrease of the population in the beginning of the third phase cannot be induced by biological or climatic factors, but a change in the insect behavior could account for it. Our observations confirm the hypothesis of a whitefly migration, but we need further proofs.

The distribution of the vectors following a gradient explains the disease gradient observed in all the cassava fields ("Spatial spread of ACMV", same issue). The fact that the horizontal movements depend very much on the establishment of a continuous canopy and that the whiteflies fly against the wind, explains the minor importance of the secondary spread and the up-wind spread around an infected source ("Primary and secondary spread of ACMV", same issue). Furthermore, the canopy establishment coincides with the outflux and thus reinforces the lesser importance of the secondary spread. The huge contamination registered each year in April-May ("Temporal pattern of ACMV spread," same issue) could be understood by the great multiplication of the vector 4 wk before, but these populations need to "migrate" from the old fields to the new ones, as suggested by our results.

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SPATIAL PATTERN OF AFRICAN CASSAVA MOSAIC VIRUS SPREAD

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From 1981 to 1986, the spread of African cassava mosaic disease (ACMV) into several healthy trial cassava fields was recorded. With insect-transmitted viruses patterns of infection depend on the vector movements and on the wind direction (2). So, the distribution of the vector, Bemisia tabaci, was recorded in relation to the wind directions.

Disease distribution. Table 1 indicates for each field, the planting date, the field area, a brief description of the ecological situation, the way of survey, and the disease incidence in the up-wind borders, the center of the field and the down-wind borders. As indicated in Fig. 1 there is a prevailing southwest oriented wind. The patterns of virus incidence show several common features: infection was not homogeneous throughout the fields as the wind-exposed south and west borders had a higher disease incidence than the north and east borders or the center of the field. Following a SW-NE direction there is a sharp decrease of the disease incidence from the up-wind edges, then a plateau around the middle of the fields and eventually an increase towards the down-wind edges (Table 1). These gradients of contamination are established early. Afterwards, there is a tendency for a blurring of the gradients (1).

This pattern of disease spread is a general feature as it was observed in most fields whatever their ecological situation and the year of planting. However, during a five-year program, we observed a few exceptions: 1) in several small fields (0.07 ha) such as Field 6, the gradients were sometimes faint or sometimes not established; 2) in several varietal trials (sub plots of different clones), the pattern of spread was not that observed with fields planted with a single clone; and 3) the presence of a 3-m wide path across field 5 modifies the general pattern as the highest incidence was observed along these inside paths.

Vector distribution. Several kinds of traps were used to study the whitefly distribution in the cassava fields. Yellow water traps and white sticky traps were set at different heights. In addition, sampling of the whitefly population on the plants was carried out. Despite the different ways of catching and counting, the patterns of whitefly distribution share several common features. The distribution of the catches is not homogeneous throughout the field. More whiteflies were trapped and counted near the wind-exposed borders than in the center of the fields or near the down-wind borders ("Field dispersal of Bemisia tabaci, vector of ACMV," same issue).

The vector distribution suggests that airborne whiteflies carried by the south-west prevailing wind alighted preferentially on cassava plots on the up-wind edges of the fields. Several observations suggest that reduction of the wind speed on the borders of the fields allows the

incoming whiteflies to control their flight and to land. (See "Bemisia tabaci cassava field dispersal," same issue). This behavior of the vector would explain the ACMV pattern of spread which is common with other whitefly-transmitted diseases such as okra leaf curl (Fargette & Hamon, unpublished results). The quoted exceptions to the general pattern of spread could be due to unusual wind modifications such as those induced by small fields or by paths in the fields.

When considering the whitefly movements and the position of the fields there are indications that both the reservoirs of virus and vectors are located at some distance up-wind from the field, a distance up to several km being possible.

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Table 1.

Field	Date of planting	Area	Ecological situation	Survey	Disease incidence (%) up-wind	center	down-wind
1	Feb 1982	0.7 ha	Fully exposed to the wind	R*	70	15	40
2	Oct 1982	1.0 ha	Surrounded by a wind break	L**	76	20	37
3	Oct 1982	1.0 ha	Surrounded by the forest	L	86	22	37
4	Jul 1983	0.5 ha	Southwest orientation	R/L	58	18	30
5	Oct 1984	4.0 ha	Fully exposed to the wind	L	54	19	27
6	Each month	0.07 ha	Southwest orientation	R	75	38	17

*Diseased plants were removed. ** Diseased plants were kept and labeled.

AUTOMATIC MAPPING OF THE SPREAD OF AFRICAN CASSAVA MOSAIC VIRUS

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The automatic mapping technique of cartography employed here uses the application of the theory of regionalized variables (2). Some examples of regionalized variables are: densities of human population in a given geographic zone, a mineral concentration in an ore-bearing earth ... The cumulative percentage of cassava contaminated plants is an adequately defined regionalized variable of density.

Let us consider the two following A and B linear sequences of numbers:

A: 1 - 2 - 3 - 4 - 5 - 6 - 5 - 4 - 3 - 2 - 1
B: 1 - 4 - 3 - 6 - 1 - 5 - 4 - 2 - 3 - 5 - 2

In case A we can see an obvious symmetrical structure; in case B the structure, if there is one, is unaccented; however, these two sequences of 11 numbers have the same variance. So these two mathematical values are insufficient to describe the structure and the main characteristics of a natural phenomenon.

The two main characteristics of a regionalized variable are the continuity and the isotropy in the considered space. If the continuity, in general, is unrespected we are in the case of an irregular repartition named "pure nugget effect;" the clearest example being the gold nugget field.

For a local estimation, the structural information needed is totally summarized by the semi-variogram study. Each point of this semi-variogram (G) represents for a given h distance (H), the mean (E) of the squared value of the deviation between the values of the regionalized variable in every point of the space studied [Z(X+h);Z(X)].

$$G(H) = 1/2\ E[Z(X+h) - Z(X)]^2$$

Practically, this semi-variogram is adjusted to a modelized variogram. The different types of adjustment of the regionalized variables are likely to enable the deduction of spreading patterns of, for instance, the mineral element or the species, or the disease considered. In the case of ACMV, the experimental semi-variogram is likely to be adjusted to a straight line, showing a precise gradient effect in the structure of the variable within the considered trials. Furthermore, in the case of oriented variables, it is possible to calculate the semi-variogram in each direction and to find a prevalent direction. In these circumstances, the contamination is essentially a primary contamination (coming from outside the field) ("Spatial pattern of ACMV spread," same issue), following the direction of the prevailing wind and with a border

effect as it was found in field experiments ("Primary and secondary spread of ACMV," same issue).

Knowing the modeled semi-variogram of a given variable it is possible to calculate a local estimation of the regionalized variables from a sample collected experimentally.

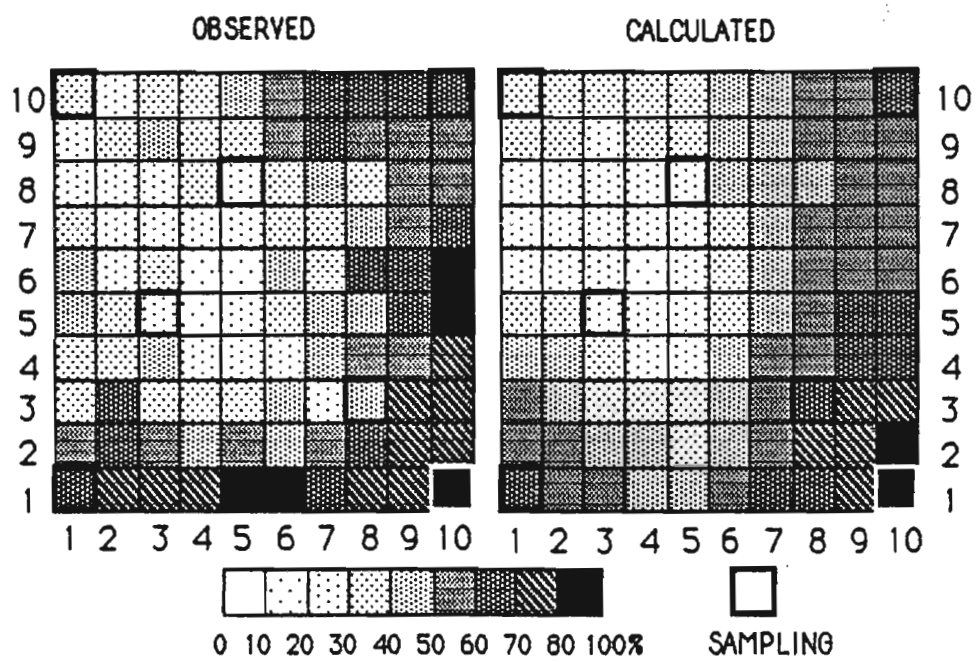
A theory of a local estimation, without any shift, was adjusted by Krige (1). This theoretical method calculates again the values of the sampled points, restoring the distribution in mean and variance; this method is known as the kringing method.

The calculation of a Z(Xo) value in an Xo of any point surrounded by n sampled points is obtained by the formula:

$$Z(Xo) = \sum^{n} Li\ Z(Xi)$$

where $\sum^{n} Li = 1$ and Z(Xi) represents the variable value of a sampled point Xi; Li is the calculated balancing coefficient of the value of the sampling in Xi. The Li values are calculated with the modeled semi-variogram, so that the expected value of the variance, in Xo, is minimum.

The studies comparing the calculated values obtained from a given sample and from a h max distance from which the Xi values are considered to have no more influence upon this Xo calculation, show that in the case of ACMV, a sample of 7% (7 blocks of 25 or 100 plants in a trial of 50 to 100 blocks) and a h max distance near 5 blocks (25 to 50 meters) give the best estimates.



The figure above visualizes the results obtained with the automatic mapping with a cassava field of 1 ha, 6 months after planting, with a sampling of 7%. . The correlation between the observed and the calculated cartography is 0.81. Nevertheless, the knowledge of a border effect, particular to the spread of the ACMV disease, implies that a structured sample collection rather than a random sample collection should be chosen.

The kringing method enables the reduction of about 14 times the field observation work, while correctly giving the necessary structural information needed to study the spread of the ACMV viral disease in the experimental trials.

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PRIMARY AND SECONDARY SPREAD OF AFRICAN CASSAVA MOSAIC VIRUS

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At the field level, disease spread from outside (primary spread) is often distinguished from internal spread within a site (secondary spread) and different methods of control are advised according to which one is predominant (2). Three approaches were applied to study the primary and secondary spread of African cassava mosaic virus (ACMV) transmitted by the whitefly, Bemisia tabaci, under the Ivorian conditions.

ACMV dispersal from a source. Dispersal of ACMV was followed in healthy cassava fields from centrally located, internal sources of 9, 25, 50 and 100 infected plants, which were propagated by cuttings. Fig. 1 indicates the positions of new infections around a 50-plant source 6 months (left) and 7 months (right) after planting. This local spread occurred up-wind, down-wind and laterally. The spread decreased as distance increased from the source. Although the disease incidence increased from the 6th to 7th month, its extent was limited to the first eight rows surrounding the source. This pattern of local spread, which expands somewhat independently from the wind direction, differs from the distant spread originating from outside sources which is strongly down-wind oriented ("Spatial pattern of ACMV spread," same issue). Detailed studies of whitefly movements indicate that, within the canopy, the wind speed is much lower than above. This allows the insects to control their flight somewhat independently of the wind direction ("Field dispersal of Bemisia tabaci, vector of ACMV," same issue).

Spread from internal sources indicates that infected plants in a field contribute to the infection of other plants. So, it is likely that the spread from outside sources leads to establishment of internal sources which themselves contribute to further spread.

Distribution of the diseased plants; aggregated vs random distribution. An attempt to distinguish primary and secondary spread was carried out by studying the distribution of diseased cassava plants. In a 1.0-ha healthy cassava field (100 plots of 100 plants each) the position of the diseased plants was assessed and the date of contamination recorded each fortnight in 18 plots. Nine plots were located in positions where inoculum pressure was high (near the up-wind border) and the other nine where inoculum pressure was low (near the down-wind border). Three methods of analysis which discriminate aggregative from random distribution were applied to study the diseased plant distribution: the number of doublets (3); the binomial distribution; and the convolution method (1). According to the results of these methods, the distribution of the diseased plants is predominantly of the random type.

Disease progress curves. We compared the disease incidence in plots with and without internal sources. This method, although suffering some limitations, indicated that the secondary spread contributes to infection, that its rate is variable from one month to another, and that both spreads are linked to the size of the whitefly population 6 weeks earlier. However, the primary spread was predominant and contributed to over 70% of the disease incidence.

CONCLUSION

Secondary spread does occur and may occur preferentially between adjacent plants. The predominant random primary spread may mask this aggregative spread. From a practical standpoint, the rapid primary spread in the coastal region of the Ivory Coast implies that removal of diseased cassava, although limiting secondary spread, would not suffice to maintain virus-free plantations. This situation is not typical of the entire Ivory Coast, and in areas such as Toumodi ("Development of ACMV at the regional level," same issue) adequate cultural practices including eradication of diseased cassava allowed us to maintain virus-free fields for years.

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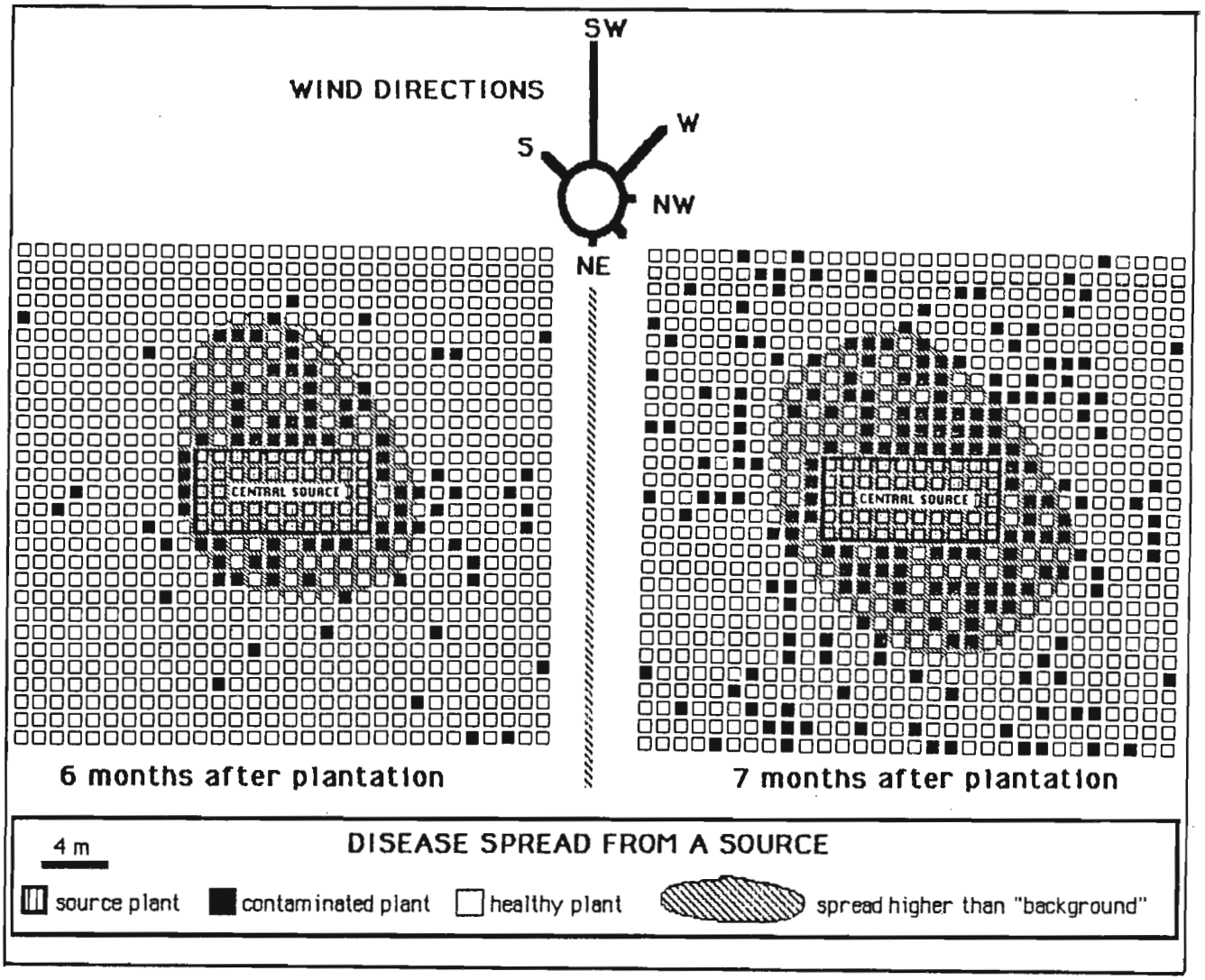

Fig. 1. Dispersal of ACMV from a source, 6 and 7 months after planting. Directions of the winds are indicated.

DEVELOPMENT OF AFRICAN CASSAVA MOSAIC VIRUS AT A REGIONAL LEVEL IN THE IVORY COAST

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The African cassava mosaic virus (ACMV) is transmitted in two modes: by the Aleyrodidae Bemisia tabaci, and by diseased cuttings. The experiments conducted in East Africa concluded that the farmers themselves were the main vector (1), and that the role of the natural vector was minor. The conclusions based on results of epidemiological studies done in West Africa, were that vectors were the main source of virus spread (2,3). In order to determine the role of the vector in different ecological conditions we have conducted, in the Ivory Coast, an experiment at the regional level. The infection dynamics of healthy cassava plants, the vector populations, the ecological and environmental situations of the fields and the plant growth were considered.

MATERIALS AND METHODS

Most of the trials were planted with the CB cultivar (susceptible clone coming from Congo), and we also used the H58 clone (very susceptible clone originated from Malagasy) and the BR clone (Bonoua Rouge, resistant clone from Ivory Coast) among several cassava clones.

The experiments took place in two very different regions of the Ivory Coast: the first one is situated in the two rainy-seasons part of the forest area, in the south of the country (= 2000 mm of precipitation); the second one is situated in the savannah region, in the central part of the country, with only one rainy season (= 1000 mm of precipitation).

In the forest area, we experimented with one cultivar (CB) but in different environmental conditions, during one year. In the savannah region, we compared the H58 and BR clones in two different environmental conditions, during one year. Finally, the two different regions were compared by following reinfestation of fields of several clones during several years or at different planting dates for the same clone. In each region, field areas were varied from 0.06 ha to 1 ha, always oriented in the prevailing wind direction, in order to get a homogeneous infection of the plots (2).

The infection of the plants, the populations of the vector and the plant growth were recorded each month during 9 months. The whitefly populations were estimated by counting the adults directly on the apical leaves of 25 different plants per plot. The plant growth was estimated by measuring the diameter and the height of the principal stem of 25 plants per plot. Infection percentages and whitefly populations were analyzed by comparing cumulative numbers. We have also compared the ratio between the cumulative number of whiteflies per plant and the cumulative percentage of infected plants per plot to get the "Apparent

Transmission Power" (ATP) of the whiteflies with time and in different regions.

RESULTS

Comparison between the forest and the savannah regions. Whatever the year or the clone considered, infection was always more severe in the forest than in the savannah region.

Clone	BR	H57	CB	TA49	H58	BB
Forest region 1982	32	45	82	-	88	81
Forest region 1983	10	25	74	67	84	69
Forest region 1984	-	-	49	-	-	-
Savannah region 1982	3	3	1	-	5	20
Savannah region 1983	1	2	3	1	2	7
Savannah region 1984	-	-	4	-	-	-

Similarly, cassava plots planted at different dates within the same year had higher infection rates in the forest than in the savannah region.

Plantation date	March	April	May	June	July
Forest area 1984	91	58	49	42	50
Savannah area 1984	4	43	11	4	12

Comparison between two sites in the savannah region. We have compared infection rates of two different clones (H58 and BR) in the savannah region. In one case the fields were free of diseased cassava plants up-wind and in the second case the fields were planted in the middle of a huge diseased cassava plantation. In the latter case the infection rate was 25 higher for the BR clone and 40 higher for the H58 clone than in the former. The whitefly number was always higher in the site with the higher infection rate but was not in the same range as the infection rate.

Whitefly number	Savannah 1	Savannah 2	Forest area
BR Clone	2.4	9.5	3.0
H58 Clone	3.7	9.2	4.3

Comparison of different sites in the forest region. Five different 0.06 ha were planted with the CB clone in the forest area, along a south-north axis, beginning near the sea (field 1), and ending 10 km inland (field 5). All the sites were different in the cassava environment and in the diseased cassava area which was swept by the prevailing wind coming from the south-west. A sixth field planted on the research station was considered as a reference (field 6). The highest infection rate was registered in fields 2 and 5, and the lowest in field 1. The highest whitefly population was in field 1 with lower populations in fields 3 and 4. The ATP was similar in all the fields excluding field 1 where it was about 10 times lower. The plant growth pattern could not account for these differences.

DISCUSSION

The differences between the dynamics of infection of cassava fields are variable within the same region and between different regions. Neither the climatic conditions nor the plant growth were predictors of the infection rate. Within a site there is a good correlation between the whitefly number and the infection rate (2,3). However, from one site to another and from one region to another these are not related. Comparing the ATP we distinguished two situations: 1) field 1 in the forest area (ATP = 300) and field 1 in the savannah area (ATP = 1000); 2) all other situations (ATP = 40 to 80). The fields with a high ATP had no up-wind diseased cassava fields, whereas those with a low ATP were surrounded with viral infected cassava fields. These results support the hypothesis that cassava is the reservoir for both ACMV and its vector, Bemisia tabaci.

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TEMPORAL PATTERN OF AFRICAN CASSAVA MOSAIC VIRUS SPREAD

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Disease development of virus diseases with time depends on many factors (2). Among those studied for African cassava mosaic virus (ACMV), a whitefly transmitted geminivirus, there are: the site and the date of planting, the clone used, and the situation in the field.

Factors influencing disease spread. The information below indicates that disease development with time is very variable.

	Site[a]			Position in field[b]			Clone[c]			Date of planting[d]		
	1	2	3	1	2	3	1	2	3	1	2	3
% Disease incidence	2	39	62	18	34	89	18	34	89	12	44	99

[a]Contrasting epidemics can develop in different sites, even among sites very close to one another. In site 1 (Toumodi, 200 km north of Abidjan) the level of contamination of healthy fields is much lower than at Tontonou (site 2) (a few km from Toumodi) and than at Adiopodoumé (20 km west of Abidjan, site 3) ("Development of ACMV at the regional level," same issue).

[b]Within a field, the disease spread varies according to the position in the field. In the center of a field (Position 1) and near the down-wind borders (Position 2) the infection is much lower than on the up-wind borders (Position 3) ("Spatial spread of ACMV", same issue).

[c]Clones showed a wide range of "field resistance" - a very low disease incidence was observed in clone 1(hybrid of *M. esculenta* and *M. glaziovii*) whereas high incidence was noticed in clones 2 and 3 (local clones) ("Multicomponent resistance of cassava to ACMV," same issue).

[d]Within a site, with a similar exposure and the same clone, ACMV spread is very dependent on the date of planting; it is low in October (1st), high in April (3rd) and moderate December (2nd).

Annual fluctuation of the inoculum pressure. From 1981 to 1986, an area of 0.1 ha of cassava was planted each month. Surveys were carried out each week, the disease incidence assessed, and the infected cassava uprooted. Inoculum pressure index was computed from the increase of disease incidence in cassava plots from the second to the third month. Whitefly populations were evaluated by weekly sampling and cassava foliage growth followed through leaf area index (LAI) between 60 and 90 days after planting. Detailed climatic data are available for the whole

period. Progress curves of ACMV contamination are different from one month to another and simple adjustments to the mathematical treatments available cannot be applied for each disease curve as a whole. Heavy infection, despite removal of the diseased plants, indicated that there is, over the year, influx of viruliferous whiteflies into the fields. This situation differs from that of Kenya where a low level of infection has been reported (1).

From the results obtained over 5 years there appears to be an annual fluctuation of every variable followed.

- inoculum pressure: high from March to July, low from August to November
- whitefly population: high from February to June, low from July to October
- cassava foliage growth: heavy from February to May, light from June to September
- temperature: highest from February to May, lowest from June to October

We analyzed the relationships between the virus, the vector, the plant and the climatic conditions of the environment (Fig. 1).

The close relationships between climatic conditions and infections allow predictions of the spread: 1) on the yearly scale, a rough prediction of high and low contamination periods ($r = 0.77$); 2) on a 2-month scale, a more accurate prediction based on the climatic area ($r = 0.98$).

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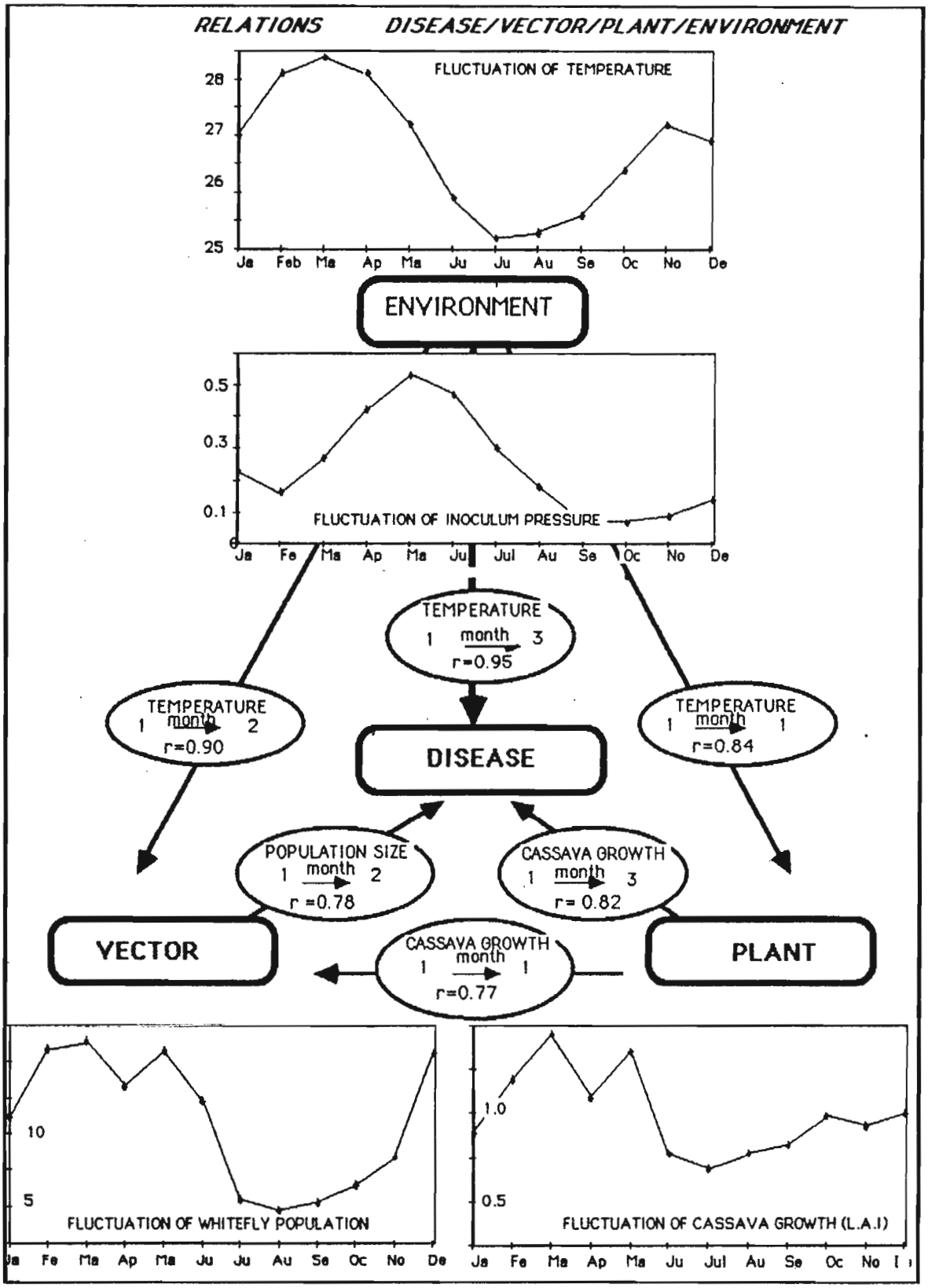

Fig. 1. Relationships between the annual fluctuations of the environmental conditions (temperature °C), the vector (number of whiteflies per plant), the plant growth (increase of the leaf area index), and the inoculation pressure (% of plants which became diseased). Coefficients of correlation and the optimal delay are indicated: for example, 1 ---> 2 indicates that the correlation is based on values of a month (1st) for the first variable with those of the following month (2nd) for the second variable.

MULTICOMPONENT RESISTANCE OF CASSAVA TO AFRICAN CASSAVA MOSAIC VIRUS

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Storey conducted in East Africa, in 1938, the first program of selection of cassava (Manihot esculenta) against the African cassava mosiac virus (ACMV) (5). Intra-specific hybrids were initially done, using the African clones and a javanese one (F279), creating the hybrid 37244E. Then, he accomplished inter-specific hybrids and particularly the hybrid, Manihot esculenta x M. glaziovii, followed by three back-crosses with M. esculenta, selecting in this manner a resistant clone, the 46106/27. The same source of resistance was then used by Jennings in 1951 (4) who selected the hybrid 5318/34. Ekandem in 1958, working in Nigeria with seeds coming from this selected resistant hybrid, produced the clone number 58308 (1). The latter was the source of resistance to ACMV, used in the selection program of IITA (2). Hahn concluded (3) that the ACMV resistance of cassava 1) is polygenic and recessive, 2) is resistant to inoculation and to movement of the virus in the plant, and 3) there is no resistance to the vector itself.

In order to test the resistance of the selected clones in comparison to local clones in the Ivorian conditions and to determine the different levels of resistance, we have studied the different resistance components to ACMV. According to Russell (6) we have distinguished six different types of resistance: RC field resistance, R1 resistance to the vector, R2 resistance to inoculation, R3 resistance to the virus multiplication, R4 resistance to symptoms, and R5 resistance to movement of virus.

MATERIALS AND METHODS

Collection of clones. The clones are of nine different origins: Ivory Coast, Togo, Nigeria, Central Africa, Zaire, Kenya, Malagasy, India and South America. We conducted an experiment in 1984 with 28 clones including the East African resistant clones and another experiment in 1985 comprising the East African and the Nigerian resistant clones.

Experimental trials. The experimental trial consisted of four repetitions of 15-m wide plots facing the prevailing wind ("Spatial pattern of ACMV spread," same issue). Each plot was composed of a random series of tested clones of 20 plants, surrounded by two lines of the CB clone considered as susceptible.

Evaluation technique. The study is based on two principles: first, the variables are registered without any a priori classification; second, each of them is measured, if possible, a great number of times (1 to 25) to minimize climatic, agronomic and experimental effects. Curves representing the evolution in time of these variables are reduced by transformation to one characteristic number. The six different types of

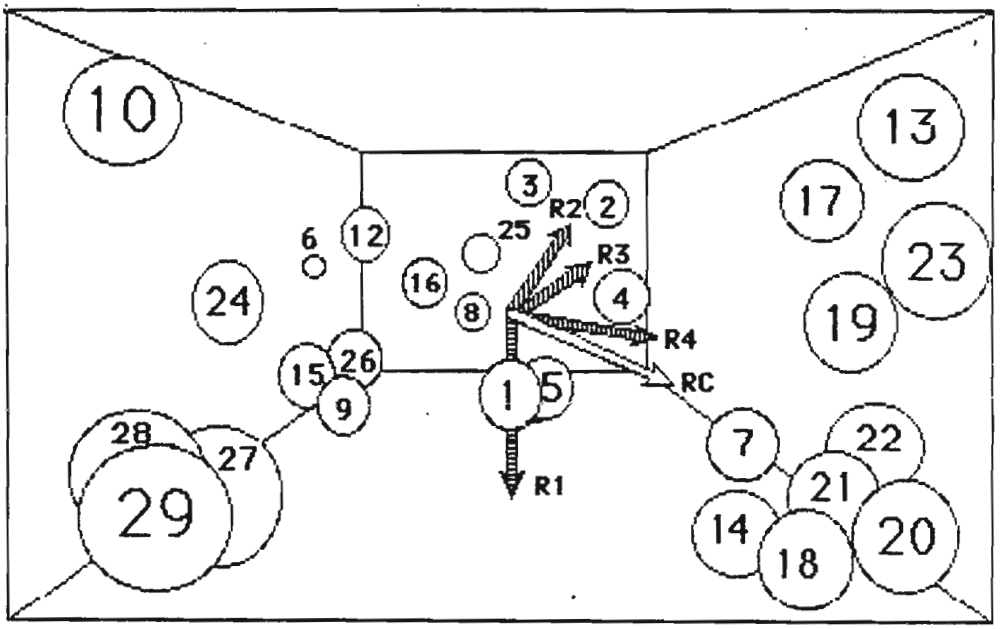
resistance are represented by: -RC, an approximation of the curve surface of the cumulative percentage of contamination in time; -R1, the cumulative number of counted whiteflies on the plants; -R2, the regression of the change of the ratio of the cumulative number of whiteflies on the cumulative percentage of contamination; -R3, the virus content of the diseased plants (only one measure in 1984); -R4, the intensity of the symptoms (mean of three different counts); R5, the regression of the time change on the intensity of the symptoms (only in 1985).

Data analysis. We analyzed the correlations between the variables, then we performed principal components analysis and hierarchical classifications and finally multiple regressions.

RESULTS AND DISCUSSION

A correlation matrix of these resistance components was established, showing that the field resistance (RC) is significantly correlated with all the others ($r = 0.48$ to 0.80). The most independent type of resistance is the vector resistance (R1). The R2, R3 and R4 were also significantly correlated.

The principal component analysis aims at describing the five different resistance components of the cassava clones to ACMV.



The figure above is a three-dimensional diagram representing 93% of the total variability and the correlation coefficient for each resistance type; the three axes vary between 0.75 and 0.95. Axis 1 is mostly represented by the RC and the R4, while axis 2 is only the R1, and axis 3 is more correlated with R2 and R3. The same analysis performed in 1985 with another cassava collection leads to a similar diagram.

A hierarchical classification of the cassava clones according to the different types of resistance divides them into several groups ranging from the most susceptible to the most resistant one. The resistance groups contain not only all the hybrids from East Africa and Nigeria but also the local clones from Kenya, two clones from India and Aipin Valenca, which was the most widely used clone in the selection schemes.

Using multiple regressions, it is possible to connect field resistance (RC) to the other resistance types with a high level of correlation ($r = 0.85$); consequently field resistance (RC) is a good criterion of the general cassava resistance to ACMV.

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COMPARISON OF THE FLYING STRATEGIES OF ALEYRODIDS AND APHIDS

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During recent investigations we have learned that the aerial distribution of migrating aleyrodids (whiteflies) is different from that of aphids (2). Using cylindrical sticky traps in agricultural settings, we captured more than 80% of our populations of both sweet potato whitefly, Bemisia tabaci, and banded-winged whitefly, Trialeurodes abutilonea, in traps placed at ground level. Approximately 11% were captured in traps placed at 50 cm and only 7% of our populations were captured in traps placed at 100 cm. This is quite different from the trapping patterns commonly reported for aphids. As an example, Broadbent (1) captured 14% of his populations of black bean aphids, Aphis fabae, in traps placed at heights of 5 to 36 cm, 34% in traps placed at 81 to 118 cm, and 52% in traps placed at 157 to 188 cm. The height at which insects travel during immigration flight is important because: 1) it influences distributional patterns and disease epidemiology in situations where the animals are potential vectors of plant pathogens, and 2) it indicates that other aspects of the strategies employed during flight may be different, even for two closely aligned groups.

With these facts in mind, we elected to investigate other aspects of aleyrodid locomotor activity in order to draw comparisons with those of aphids. We were also interested in how what we learned about the flight mechanics of these small insects compared to what is known about insect flight mechanisms in general. We specifically examined wing morphology and the relationship between wing loading and wingbeat frequency in five aleyrodid species and five aphid species.

Wing surface areas were determined using a microfiche reader and acetate templates. Weights of wing templates were compared to weights of templates of known dimensions using regression equations. Fresh animal weights were obtained using microbalances. Wingbeat frequencies were determined using an optical tachometer and a digital oscilloscope.

Morphometrics of our aleyrodids and aphids are shown in Table 1. Also shown is the ranking of our measured animals in relation to the values for the same measurements abstracted from the literature.

As a general statement, the five aphid species weighed significantly more and had significantly larger wings than did the five aleyrodid species ($P < 0.05$). Additionally, aphids had significantly lower wingbeat frequencies than did aleyrodids: range; 81.1 to 123.4 Hz for aphids, 165.6 to 224.2 Hz for aleyrodids ($P < 0.01$). Considering the calculated wing loadings, those for aphids were all larger than those for aleyrodids. The ranges were 0.006347 to 0.014116 g/cm^2 for aphids and 0.001741 to 0.005232 for aleyrodids.

To put in perspective aleyrodid and aphid morphometrics, we considered them in relation to similar values for 148 insects found in the

literature. These rankings are shown parenthetically in Table 1. When the species are sorted by ascending values by weight, the range was from 0.000033 for Bemisia tabaci to 2.809 g for Oryba achemenides, a sphingid moth. The weights for the 11 homopterous insects were below any of those previously published. Our homopterans also had the smallest wing loading values. Conversely, the wingbeat frequencies of our animals were among the highest recorded values, overall range 8 to 480 Hz.

It is commonly stated that insects compensate for having high wing loading values by increasing their wingbeat frequency. Among our homopterous insects, we found that no such relationship existed, that is animals with higher wing loading values did not necessarily have higher wingbeat frequencies. An examination of all data revealed that when weight is taken into consideration, we find that these relationships do not hold true for small insects (Table 2). Two factors are likely operating among insects whose body weight does not exceed 0.03 grams. Calculating Reynolds numbers, we find that animals smaller than that weight are likely relying on drag rather than lift to accomplish flight. Also, it is entirely possible that in migrational flights aphids and aleyrodids are so light that they are subject almost entirely to the vagaries of wind, and move about in the manner similar to inert particles, rather than as flying machines.

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Table 1. Morphometrics of aleyrodids and aphid species.

Species	Wing Surface Area (cm^2)	Body Weight (g)	Wing Loading (g/cm^2)	Wingbeat frequency (Hz)
Acyrthosiphon kondoi	0.1106 (14)[a/]	0.000702 (11)	0.006347 (10)	81.10 (89)
Aleurothrixus floccosus	0.0194 (5)	0.000065 (5)	0.003359 (5)	165.60 (133)
Aphis fabae	0.0526 (10)	0.000411 (9)	0.007807 (13)	104.74 (101)
Aphis gossypii	0.0103 (2)	0.000114 (7)	0.011059 (18)	123.39 (110)
Aphis nerii	0.0663 (13)	0.000467 (10)	0.007047 (12)	118.14 (104)
Bemisia tabaci	0.0134 (3)	0.000033 (1)	0.002451 (3)	168.55 (134)
Dialeurodes citri female	0.0264 (8)	0.000080 (6)	0.003033 (4)	175.60 (138)
Dialeurodes citri male	0.0207 (6)	0.000036 (3)	0.001741 (1)	
Myzus persicae	0.0237 (7)	0.000334 (8)	0.014116 (22)	90.87 (94)
Trialeurodes abutilonea	0.0096 (1)	0.000050 (4)	0.005232 (7)	224.16 (153)
Trialeurodes vaporariorum	0.0165 (4)	0.000035 (2)	0.002120 (2)	180.04 (140)

a/ ranking among referenced insects

Table 2. Coefficients for the regression equation y = a + bx where wing loading is the dependent variable for all insects.

Weight (g)	n	Intercept	Slope	Multiple R^2
0.000033 to 0.030	27	0.0178	0.0001	.103
0.030 to 0.104	27	-0.0056	0.0007	.778
0.107 to 0.201	27	0.0010	0.0011	.890
0.226 to 0.399	27	-0.0120	0.0017	.903
0.425 to 0.702	27	0.0180	0.0003	.748
0.720 to 2.809	23	0.0762	0.0027	.602
All insects .000033 to 2.809	159	0.0773	.0003	.055

THE EPIDEMIOLOGY OF A WHITEFLY-BORNE VIRUS IN ISRAEL

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Four whitefly-transmitted diseases have been described in Israel. Among them only two are of economic importance: (i) the tomato yellow leaf curl virus (TYLCV) - A Gemini type, persistent virus. This virus causes severe damage to tomatoes grown in summer and autumn in all regions, particularly in the Jordan Valley and the northern Negev. (ii) The cucumber vein yellowing virus (CVYV) - a semipersistent elongated virus. This virus is similarly widespread and causes damage to several cucurbits. Preliminary data on the epidemiology of TYLCV in Israel are presented herein.

Whiteflies were caught on sticky yellow traps in order to evaluate the population size. Tomato bait plants transplanted weekly in the field were used to estimate the proportion of TYLCV-carrying whiteflies in the population. Preliminary results show a trend toward a positive correlation between the size of the Bemisia tabaci population and TYLCV spread.

Since 1960, TYLCV epidemics have been occurring in the Jordan Valley. Therefore, our main efforts were concentrated on the search for the natural host of TYLCV in this region. Several tens of types of plants commonly growing in the Jordan Valley were artificially inoculated in the laboratory to test whether they can serve as potential hosts of the virus. So far it was possible to infect Cynanchum acutum L. (Asclepiadaceae) and Hyoscyamus desertorum (Asch.) Eig (Solanaceae). However, only C. acutum was found to be a natural host of the virus, and TYLCV was recovered from more than 50% of the C. acutum samples collected along the Jordan River.

In studies of the flight behavior of B. tabaci, attempts were made to mark the insects in the field using fluorescent dust. In these experiments cotton or naturally growing C. acutum plants were dusted with "Fire Orange" (Day Glo®) dust, which has been found to be suitable for marking B. tabaci. It persisted on the whiteflies for more than a week, with no effect on the life span of the insects. Preliminary results pointed to a short distance active flight while the long distance distribution is probably passive. However, whiteflies that had been marked on C. acutum plants growing along the bank of the Jordan River were trapped at the main tomato production area located at a distance of about 7 km.

Additional factors which may be of importance in studies of the distribution of B. tabaci were investigated: the flight hours of B. tabaci and the combined effect of temperature and relative humidity (RH) on the survival of the insects. Most of the whiteflies were trapped during the morning (before noon). The survival of the whiteflies was reduced by increasing temperature, and by decreasing RH when the temperatures ranged between 30 and 35°C. At lower (25°C) or higher (41°C) temperatures, RH had little influence. At 41°C survival was very low already after 2 h of exposure, regardless of the RH.

The possibility cannot, however, be excluded that the findings on the limited hours of flight activity per day are a result of the death of those whiteflies which did not land early enough before being killed by the high temperatures prevailing at midday during the summer in Israel.

UNUSUAL SOURCES AND METHODS OF DISPERSAL OF PLANT VIRUSES

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The occurrence of plant viruses in unusual environments and dissemination of them by atypical methods have been examined only recently. The significance of these discoveries to the epidemiology of plant virus diseases is somewhat speculative at this time. Potentially, these uncommon sources and methods of spread of viruses may provide explanations for unexpected virus introduction into healthy crops.

Plant Viruses in Lakes and Streams. Koenig and Leseman (3), Tomlinson et al. (4), and Tosic and Tosic (5) have demonstrated the presence of some well-known plant viruses as well as some previously unidentified isolates belonging to the Tombus, Potex and Tobamovirus groups in various rivers and lakes in England, Germany and Yugoslavia. These included carnation mottle virus, tomato bushy stunt virus, and tobacco mosaic virus. These viruses were isolated from as little as 200-300 ml of lake or river water by ultracentrifugation.

Speculation of sources of this contamination of waters was dumps of virus-infected vegetables and ornamental plants or plant composts. Since several plant viruses have been shown to pass through the alimentary tracts of both humans and wild and domestic animals, sewage could also account for contamination of natural waters with these viruses. Several viruses have been shown to be released from undisturbed roots of plants into soil water which could be transported into rivers and lakes. The infection of healthy plants with viruses via roots with or without vectors has been demonstrated. Therefore water taken from lakes and rivers for crop irrigation could be a source of dissemination of some important viruses and could have a significant role in the epidemiology of diseases caused by them.

Airborne Plant Virus Dispersal. The movement and pathogenicity of plant pathogenic bacteria via aerosols are well documented. The dissemination of certain mechanically transmissible plant viruses via this mechanism is also possible. For example tobacco mosaic virus (TMV) develops 2.0 mg/g titers in Nicotiana tabacum, and its leaf surfaces have abundant trichomes whose cells contain copious TMV inclusions. The trichomes may be easily broken in driving rain, wind and abrasion among plants and the TMV particles could be dispersed into aerosols. Aerosols up to 0.5 μm and 20.0 μm size in a 4.8 KPH wind can be carried over 600 KM and 0.3 KM, respectively. After wind-borne aerosols are deposited on plants, infection could occur via abrasion among closely-spaced plants or by injury from machinery.

To test the hypothesis that certain mechanically transmissible plant viruses may form airborne contagion, aerosols of purified TMV and potato virus X (PVX) were generated using Environmental Research Company spinning disc and fluid atomizing aerosol generators in a wind tunnel.

These aerosols were collected with an Andersen 6-stage aerosol sampler and the collections produced local lesions on Nicotiana glutinosa and Gomphrena globosa. Aerosols of TMV and PVX were also produced by air-blast and water spray of injured infected plants in a chamber, which were collected with the Andersen 6-stage aerosol sampler and produced local lesions on the above respective indicator species. To test the possibility of naturally formed aerosols in the field, assays were made in 0.25 hectacre plots of N. tabacum, in two seasons. TMV aerosols were collected using a Sierra Model 235 High Volume Aerosol Sampler, in 14 of 30 sampling periods ranging from 2 to 24 hr. These collections inoculated on N. glutinosa caused local lesions. A summation of weather conditions during periods when virus was collected indicated that wind speeds of at least 25 km/hr with or without precipitation or overhead irrigation favored collection of TMV aerosols. Also, no TMV was collected until the tobacco plants were 1.5 m tall. Similar assays were made to find out if PVX also produced aerosols in the field. However no PVX aerosols were detected in 20 assay periods of 24 hr duration in a 40 hectacre field of PVX-infected potatoes. These experiments indicated that at least TMV can be disseminated via aerosols (1).

Transmission of Plant Viruses by Birds. Broadbent (2) demonstrated that house sparrows, could transmit TMV from infected to healthy tomato plants. Presumably virus may be carried on their feathers when they fly among plants, on their feet when they perch or on their beaks when they peck fruits.

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VIRUSES AFFECTING CULTIVATED AND WILD MEMBERS OF THE COMMELINACEAE

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Members of the Commelinaceae include at least 50 genera and 700 species and are widely distributed around the world. Several species are used in the ornamental industry, many are maintained in botanical collections, and three are important weeds. Because these plants are used as ornamentals and most are easily propagated by cuttings, some species have become naturalized outside their native habitat. For example, Tradescantia fluminensis, which is not native to New Zealand, has become a major threat to the regeneration of native forest species in New Zealand (3).

Eleven viruses from seven different virus groups have been reported to infect members of the Commelinaceae. We attempted to determine the occurrence of these viruses in weed, ornamentals and botanical collections of this family. Because antisera were not readily available for all 11 viruses, special emphasis was placed on host range and the light microscopic techniques developed by Christie & Edwardson (2). Results of these techniques were supported by serological and/or electron microscopic techniques when possible.

Five viruses were found in this study (1). Four were identified as viruses previously reported to infect members of this family. These included commelina mosaic virus (potyvirus), cucumber mosaic virus (cucumovirus), tobacco mosaic virus (tobamovirus) and tradescantia virus (potyvirus). The fifth virus found appears to be an unreported potyvirus infecting the Commelinaceae.

Commelina mosaic virus was found in 5 of 25 samples of the common weed, Commelina diffusa. All five showed mosaic symptoms like those described by Morales and Zettler (5). Cuttings of C. diffusa were collected from Florida, the Dominican Republic and two botanical collections. This virus was found in samples from three of the seven Florida counties surveyed. Commelina mosaic virus was also found in two plants of the ornamental Rhoeo discolor maintained at the Plant Pathology greenhouses in Gainesville, FL, but not in the 10 other samples of this species.

Cucumber mosaic virus was found in 9 of the 25 samples of C. diffusa. All showed the typical chlorotic ringspots and mosaic as described in the literature (5). It was found in samples from the Dominican Republic and four counties in Florida. In inoculation studies it also infected the weeds C. communis and Murdannia nudiflora.

Tobacco mosaic virus was found in 3 of 12 specimens of R. discolor. All three showed strong mosaic symptoms. Based on serology, the strain detected in these plants was the U-2 strain. This virus also infected

C. communis and the ornamental Zebrina pendula following manual inoculation.

Tradescantia virus (4) caused leaf distortion and stunting in 2 of 3 samples of Tradescantia albiflora and 3 of 5 samples of T. fluminensis. Tradescantia virus was found in one specimen of C. diffusa and in 6 of 12 samples of R. discolor exhibiting leaf distortion and a mild mosaic. Tradescantia virus was also found in 9 of 20 samples of Z. pendula; however only one plant showed the reported symptoms of leaf distortion.

Tradescantia virus was the only virus found in weeds, houseplants, landscape ornamentals, and botanical collections. It also was the only virus found in commercial ornamentals at both the wholesale and retail levels. At the wholesale level, and in botanical collections from Czechoslovakia and Mexico, tradescantia virus was found in symptomless plants of Z. pendula.

The fifth virus was detected in only one botanical collection. The virus caused mosaic symptoms in 13 of 15 species from this collection including three species of Commelina, five species of Aneilema, and two species of Rhopalephora. The majority of plants in this collection were of African or Asian origin. However, this virus infected manually inoculated plants of C. diffusa, C. communis, C. erecta, M. nudiflora and Tinantia erecta. Except for T. erecta, all are common weeds in the southeastern United States.

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CHAYA (CNIDOSCOLUS ACONITIFOLIUS), A NATURAL HOST OF CASSAVA COMMON MOSAIC VIRUS IN THE YUCATAN

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Chaya (Cnidoscolus aconitifolius (Miller) I. M. Johnston subsp. aconitifolius cv. Chayamansa) is an edible member of the Euphorbiaceae indigenous to Mexico. It has been grown since pre-Columbian times and today is found in groups of 2-5 plants in home gardens throughout the Yucatan where it is cultivated as a leafy vegetable (1,2). Imported chaya plants growing in Florida were shown to be infected with a strain of cassava common mosaic virus (CCMV) which infects cassava, Manihot esculenta Crantz, but is serologically distinct from cassava isolates described elsewhere (3). We report here that the incidence of CCMV in chaya is high in Yucatan and that the chaya viral isolates collected there are antigenically similar to the one previously described from Florida.

Surveys for CCMV infections of chaya and cassava were made 20-22 August, 1985 within a 70 Km radius of Homún, Yucatan.

Viral symptoms were not obvious in most of the 33 chaya samples collected. Although inconspicuous mosaic symptoms were occasionally seen in some of the specimens, nutritional disorders and insect damage often made diagnosis difficult. The cassava plants generally were in much better horticultural condition than chaya, but mosaic symptoms were not detected in any of them.

The CCMV-Ch antiserum described by Zettler and Elliott (1986) was used in immunodiffusion tests of all samples collected. Some of the samples were also compared in immunodiffusion tests with antiserum to a cassava CCMV isolate (-BPL) provided by B. L. Nolt (CIAT, Cali, Colombia). The Clark and Adams (1977) direct double antibody sandwich method of enzyme-linked immunosorbent assay (DAS-ELISA) as performed by Zettler and Elliott (1986) was also used to test the samples.

CCMV was detected serologically in 23 of the chaya plants collected but not in any of the 25 cassava samples. DAS-ELISA and SDS immunodiffusion results were in agreement. In SDS immunodiffusion tests, fused precipitin lines without spur formation were noted between CCMv-Ch antiserum and all of the infected chaya samples; in contrast, none of the infected samples reacted with CCMV-BPL antiserum. Precipitin lines were not observed in SDS immunodiffusion tests with extracts of cassava and CCMV-Ch antiserum.

ELISA A_{405} values of 0.114->2.000 were noted for the chaya samples which reacted positively in SDS immunodiffusion tests. Chaya samples that did not react in immunodiffusion tests yielded ELISA values of only 0.000-0.066. Healthy chaya extracts used as controls gave values of 0.006-0.020 in comparison to infected chaya controls which gave a

maximum A_{405} value of 0.207. A_{405} values of only 0.000-0.038 were noted for nine cassava samples selected and tested by DAS-ELISA, whereas an average value of 0.783 was noted for a CCMV-Ch infected cassava sample used as a control.

Extracts of six samples each collected from different locations in Yucatan infected manually inoculated Nicotiana benthamiana seedlings. The foliar mosaic and distortion symptoms in these plants were like those described for CCMv-Ch (3). When tested in SDS immunodiffusion tests against CCMV-Ch antiserum, leaf extracts of N. benthamiana plants formed precipitin lines that fused with one another and CCMV-Ch antigen without spur formation. Precipitin reaction lines noted for N. benthamiana leaf extracts were much stronger than those of the same isolates in chaya. Precipitin lines between CCMV-Ch antiserum and N. benthamiana plants infected either with CCMV-Ch or any of the six Yucatan chaya isolates spurred over those of the cassava CCMV isolates provided by A. S. Costa and E. W. Kitajima. In reciprocal tests using CCMV-BPL antiserum no reactions were observed for the chaya isolates tested.

This study shows CCMV to be widely distributed in cultivated chaya, which is not surprising considering the widespread popularity of this plant and that it can only be vegetatively propagated. Futhermore, CCMV, like most potexviruses, is readily transmitted manually. The similarity of all the chaya isolates from Yucatan suggests they are from a common origin. Cassava, however, is not likely to be the source of CCMV inoculum for chaya in the Yucatan, considering the absence of CCMV in any of the cassava samples tested in this study and serological differences noted between chaya isolates and cassava isolates from South America and Taiwan (3). Although currently grown experimentally at Uxmal, cassava is not widely grown in Yucatan, where maize is the primary starch source.

The CCMV-Ch infected chaya plants studied in Florida (3) were from stock collected in Yucatan and subsequently grown in Mayagüez, Puerto Rico. It is possible that this isolate, like its host, originated from Yucatan on cultivated chaya plants.

Although it is widespread in Yucatan, the significance of CCMV as a pathogen of chaya is not known. However, the indiscriminate exchange of chaya germplasm from Yucatan could pose a threat to cassava plantings elsewhere. Because chaya isolates are significantly different serologically from those previously reported from cassava (3), they could be overlooked easily in programs which rely on relatively strain specific indexing methods, such as DAS-ELISA. In this study, CCMV-BPL antiserum failed to react in immunodiffusion tests against any of the CCMV isolates found in the Yucatan.

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THE NATURE OF RESISTANCE IN UK BARLEY VARIETIES TO BARLEY YELLOW MOSAIC VIRUS AND ITS FUNGAL VECTOR

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Barley yellow mosaic virus (BaYMV) was first reported from Japan in 1940 (2). Following the first UK record (1) it is now causing serious concern to intensive cereal growers throughout the country. BaYMV is a filamentous virus with particles c. 275 nm and 550 nm in length. It is one of a group of cereal mosaic viruses vectored by Polymyxa graminis Ledingham. P. graminis is an obligate root parasite with resting spores that survive in the soil for many years. On germination these produce zoospores which penetrate the root to produce zoosporangia from which more zoospores are produced (Fig. 1).

Field experiments suggested that varieties vary greatly in the amount of infection when planted in infested soils. Seedling inoculation using viruliferous zoospores produced in sand culture showed varietal differences similar to those expressed in the field.

Zoospore production was measured from a number of varieties but no differences were found. However, zoospores produced on the resistant variety Athene rarely transmitted BaYMV to test seedlings of the susceptible variety Maris Otter. Several other varieties behaved similarly to Athene and ISEM and ELISA tests showed that little virus multiplication had occurred in their roots.

A mechanical inoculation demonstrated similar varietal differences and gave results more quickly than using the vector. Because of its convenience this test would be suitable for routine screening by breeders.

Results showed that all varieties tested were equally susceptible to the vector but that little virus multiplication occurred in resistant varieties. New varieties of winter barley with resistance to BaYMV are being introduced in the UK.

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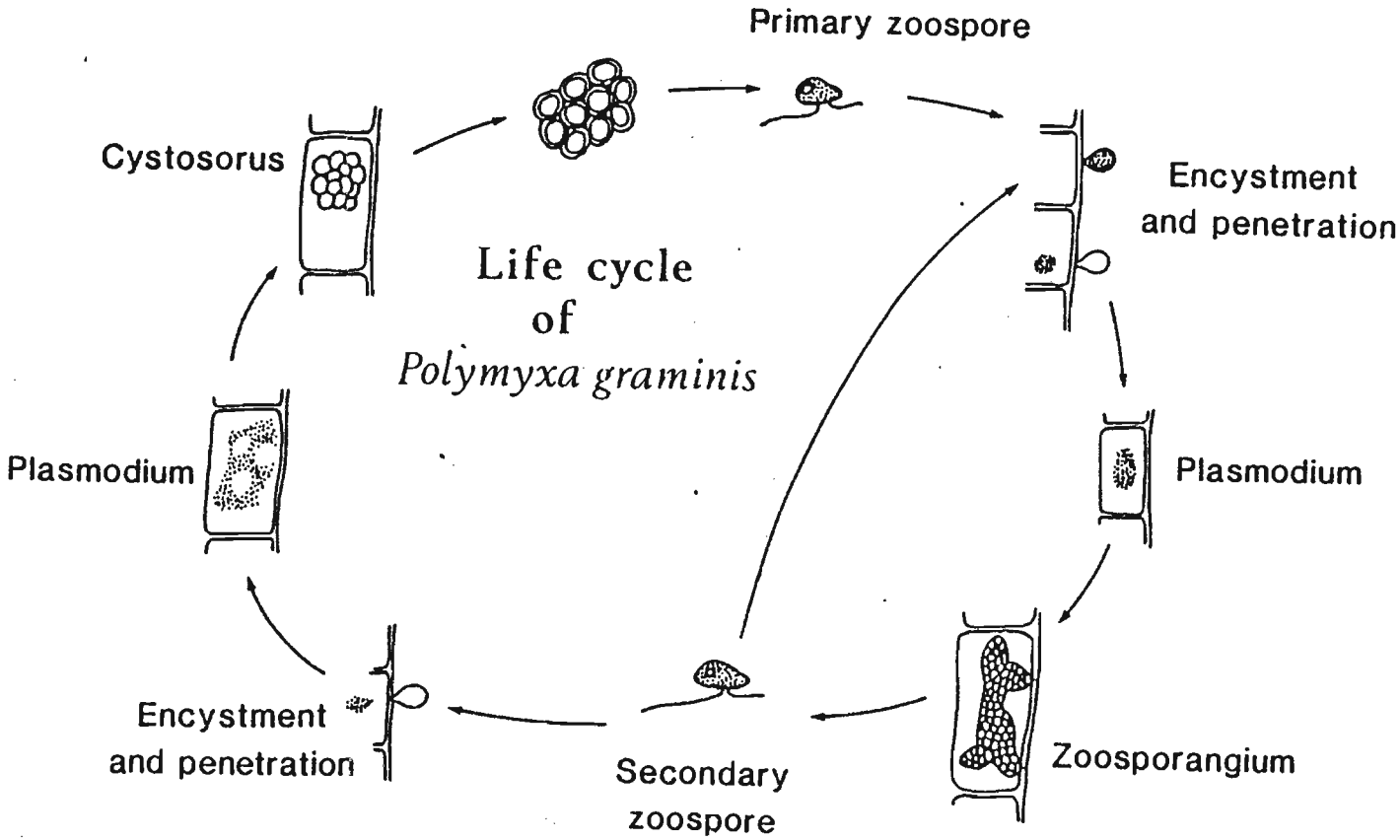
Primary zoospore
Cystosorus
Encystment
and penetration
Life cycle
of
Polymyxa graminis
Plasmodium
Plasmodium
Encystment
and penetration
Secondary
zoospore
Zoosporangium

POSSIBLE VECTOR OF SANDAL SPIKE

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Sandal spike disease in India, which is prevalent in the southern states, is an age-old problem. The disease causes heavy losses of both sandalwood and oil. Currently more than $75,000,000 worth is exported to various countries and, therefore, the disease is responsible for reduction of revenue in terms of foreign exchange.

The disease is of the "yellows type" and was earlier thought to be caused by a virus. It was bark-transmitted by Coleman in 1923. However, no virus particles have been seen by electron microscopy. In 1968 three independent groups of scientists from India, The Netherlands, and the U.K. reported the presence of mycoplasma-like organisms (MLOs) in sieve tubes of diseased sandalwood trees. Remission of disease symptoms by tetracycline antibiotics was reported by Hull et al. in 1969 and by our group in 1972. The experimental work on chemotherapy was extended to two forests in Bangalore and two forests in Mysore.

Earlier, useful work was done on this disease, especially on the observation of insects feeding on sandalwood trees. Possible insect vectors like Moonia albimaculata, Coelidia indica (Jassus indicus), Nephotettix virescens and Radarator bimaculatus have been reported from time to time. However, none of these four insect species has yet been confirmed as the vector of sandal spike by any other group of workers. Further, the vector ecology needs to be investigated thoroughly since it has a direct impact on disease incidence.

The disease agent has several hosts which include some weeds. The sandalwood tree grows in Indonesia and other areas; however, the best heart wood formation is observed in India where the spike disease is so common.

The symptoms of the disease have been described by various scientists and attempts have been made to transmit the disease by parasitic dodder from sandalwood to periwinkle and back with success. Similar "yellows type" symptoms have been noticed in several plants in the forests where sandlawood trees are grown. Transmission of the disease to healthy sandalwood trees through haustoria should be investigated in detail.

In efforts to control the disease by chemotherapy, tetracycline antibiotics as well as the systemic fungicide benomyl have been found to be effective, although the suppression of symptoms is temporary.

If and when the vector is confirmed, the vector-relationship may be studied by insect tissue culture as well. A good deal of work has yet to be done for proper understanding of vector transmission of sandal spike. It is also necessary to confirm the vector of the pathogen.

EPIDEMIOLOGICAL ASPECTS OF RIZOMANIA

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Rizomania, a serious virus disease on sugar and fodder beets, occurs in all parts of southern Europe, Japan, China and the USA. It is caused by the beet necrotic yellow vein virus (BNYVV), which is transmitted by the soil-inhabiting fungus Polymyxa betae Keskin. Various aspects of this disease have been studied in the Federal Republic of Germany since 1976, resulting in the Ph.D. theses of Horak (1980), Hess (1984) and Hillman (1984). From these investigations, a few points of epidemiological interest will be presented.

Primary infection. In 1979-83, spring sown sugar beet seedlings were tested at weekly intervals after emergence for the presence of BNYVV (3). Under favorable climatic conditions primary infection took place in the first week after emergence. Once the seedlings had reached the four-leaf stage, the virus was readily demonstrated with ELISA, which means that the primary infection had occurred 10-15 days earlier. This process is apparently governed by the temperature in 5 cm soil depth. A period of several consecutive days with temperatures above 15 C for several hr/day appears to be essential for infection and virus synthesis. There was no difference in the earliest possible virus detection between susceptible and tolerant sugar beet cultivars.

Seed transmission. The comparatively rapid spread of Rizomania in sugar beet growing areas lead to a speculation about seed transmission of BNYVV, the ubiquitous fungal vector being present in moist soils. Employing ELISA, the possibility of seed transmission was tested with sugar beet seeds and the processing residues (2). Furthermore, leaves, inflorescences and seeds from non-systemically diseased bolters and stecklings were checked. The virus was detected only once, when sugar beets were planted as bait into the dust fraction, largely consisting of soil adhering to unprocessed seeds. Altogether, it is highly improbable that BNYVV will be spread with processed seeds from non-systemically diseased seed-bearers into hitherto disease-free areas. The question whether virus transmission can occur with seeds from systemically diseased seed-bearers is still unsettled.

Weeds as alternate hosts. There are reports and observations that the BNYVV was present in soils which had not been cultivated with sugar beets in the last 10 years. This survival could either be due to a carry-over of the virus by resting spores of P. betae in the absence of appropriate host plants or to weeds as alternate hosts, maintaining the inoculum at an effective level. Investigations of field-grown weeds as well as weeds sown into highly contaminated soil with ELISA yielded only negative results, while sugar beets as corresponding checks had a high virus titer. Surprisingly, weeds belonging to the Chenopodiaceae were free of the virus, despite their close relationship to the sugar beet.

Until now there is only one weed - Gomphrena globosa (Hillmann, 1984) - which appears to be an alternate host under natural conditions in the USA (1). Susceptibility to the BNYVV seems to be restricted to the various forms of Beta vulgaris as well as Beta macrocarpa and Spinacia oleracea.

Spread through manure. Fodder beets are a common feed for cattle also in areas with rizomania. Parts of infected tap and feeder roots drop into the liquid manure, which will then be distributed in the fields. In our tests, inoculum consisting of virus and vector survived a 3-wk submersion in fresh liquid cattle manure without significant reduction in infectivity. This means that a spread through this type of manure is possible.

After harvest, fields are often grazed by sheep. Can inoculum survive a passage through their intestines and be infectious in the droppings? In a feeding experiment, three sheep obtained tap and feeder roots of highly infected sugar and fodder beets as a substantial part of their daily ration. Sugar beet plants grown in a mixture of sterile soil and collected droppings had resting spores of P. betae and Olpidium brassicae in various amounts. The BNYVV was detectable in only 10% of the bait plants. Although this percentage is not high, it means that the virus together with its vector can be spread by sheep droppings to hitherto disease-free fields, if only to set a primary focus.

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OCCURRENCE OF PLANT VIRUSES IN WATER SAMPLES IN APULIA

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In Apulia, (Southern Italy), five plant viruses have been isolated so far from rivers, channels and pits routinely used for irrigation. Two of these viruses, both isolated from rivers, were identified as cucumber mosaic virus (CMV) and tobacco mosaic virus (TMV). By extending water sample collection to channels and pits, three other viruses were found, a preliminary characterization of which is given in this study. Samples consisting of 1 liter of water were collected and centrifuged at 10,000 rpm for 20 min and their resulting sediment suspended in 5 ml of 100 mM neutral phosphate buffer. It was then inoculated mechanically on the following: Nicotiana benthamiana, N. clevelandii, N. glutinosa, Gomphrena globosa, Chenopodium quinoa and C. amaranticolor. A polyethyleneglycol (PEG)-treatment was also given to the supernantant obtained from low-speed centrifugation and the precipitate tested for infectivity. As estimated by the number of local lesions induced in herbaceous hosts by the same water sample, infectivity "associated" with sediments obtained from low-speed centrifugation was much higher than that found with PEG precipitates. By this procedure, the following plant viruses were isolated.

1) AMCV-like tombusvirus: The virus was isolated from the river Bradano. it caused a severe sytemic mosaic in N. benthamiana and N. clevelandii followed by death of the whole plant within a week after inoculation. It was serologically indistinguishable from artichoke mottled crinkle virus (AMCV), a tombusvirus responsible of losses of economic importance in Apulia, and from another tombusvirus isolated from naturally infected N. glauca plants in Greece the characterization of which is still in progress.

2) Tobamovirus: A virus with rod-shaped particles closely resembling those of tobamoviruses was isolated from the river Ofanto and from an irrigation canal. It induced typical necrotic local lesions in N. glutinosa 2-3 days after inoculation and chlorotic/necrotic local lesions in C. quinoa. It was tested serologically against antisera to ten isolates of TMV, five of which were obtained from different countries, and to the following tobamoviruses: cucumber green mottle (CGMV), odontoglossum ringspot (ORSV), wheat soilborne mosaic (SWMV) and peanut clump (PCV). With immune electron microscopy (IEM), virus particles in purified preparations were "decorated" by an antiserum to ORSV and by antisera to the following strains of TMV: TMV-Al; TMV-Pll; TMV-P8 and TMV-WU1. It is worth mentioning in this respect that this virus was not serologically related to any of the local TMV isolates, nor to a TMV isolate from the Ivory Coast. A very close serological relationship (SDI=1) was obtained with an antiserum raised against a no better characterized tombusvirus isolated from the German river Aller (1). Size and number of nucleic acid species and protein subunits as

well as the cytopathological alterations viewed in infected tissues were those elicited by other definitive tobamoviruses.

3) PZSV-like virus: A virus with quasi-isometric particles and distantly serologically related to pelargonium zonate spot virus (PZSV) was isolated from an irrigation pit near Ordona (Apulia, Southern Italy). It induced necrotic local lesions in N. benthamiana and G. globosa and chlorotic/necrotic ringspots in N. glutinosa. A systemic vein clearing was also induced by the virus in Cucurbita pepo and in N. benthamiana but both hosts recovered about 4-5 days after symptom appearance.

Preliminary observations with an electron microscope using the dip method showed that the concentration of virus particles in infected tissues was very low. In serological tests it reacted with an antiserum to PZSV forming a spur at the junction of precipitin lines but failed to react with antisera to the following viruses with isometric particles: cucumber mosaic, cucumber fruit streak, cucumber soilborne, cucumber leaf spot, melon necrotic leaf spot, carnation mottle, raspberry bushy dwarf, tobacco streak, olive latent-1 and to alfalfa mosaic and olive latent-2. It is worth mentioning that in 1985, in the same area, a necrotic isolate of PZSV caused a severe disease in tomato (2).

The present investigation provides further evidence that the water used for irrigation may play an important role in the epidemiology of plant viruses. Although it is not clear how virus particles can retain their integrity and their infectivity in water, there is no doubt that irrigation water can be an important factor in the dissemination of plant virus diseases. This could be of greater relevance for those countries and regions, like Apulia, in which crop yields are strictly dependent on irrigation water.

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EPIDEMIOLOGY OF VIRUSES TRANSMITTED PERSISTENTLY BY APHIDS

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The plant viruses transmitted persistently by aphids comprise four groups, namely the monotypic pea enation mosaic virus (PEMV), some rhabdoviruses, the luteoviruses, and a disparate group that have not yet been characterized and classified. These viruses cause major losses in plant production worldwide.

PEMV occasionally causes serious losses to susceptible pea cultivars in North America (28) and may reduce the yield of faba beans by 50% (29). The virus has a latent period in its vectors. The pea aphid Acyrthosiphon pisum is the most important vector and forage legumes are important reservoirs of infection (29, 42). Some aphid species that do not infest legumes also transmit PEMV (43). A dominant gene for resistance has been used by some pea breeders (27, 55). Locating susceptible crops away from lucerne stands may help control diseases caused by PEMV.

Nine rhabdoviruses are known that are transmitted by aphids (46). The virus vector relationships are highly specific and host ranges are narrow. Some have only one vector species. Where studied, these viruses were found to multiply in their vectors. They have long temperature-dependent incubation periods in their vectors and nymphs born on infected plants may not become infective until they are almost adult(3). They are transmitted more efficiently when the feeding times are long. Rhabdoviruses infecting berry fruits frequently occur as complexes with other aphid-transmitted viruses. The components of these complexes may be separated by serial transfers of aphids on healthy plants (47). They are controlled through the implementation of clean stock schemes after sources of healthy material have been identified or produced (18).

Lettuce necrotic yellows virus (LNYV) causes an important disease of lettuce in Australasia and is interesting epidemiologically (40). There is only one source of infection of any consequence, the common sowthistle, only one vector species, the sowthistle aphid (Hyperomyzus lactucae), and only one target crop plant is affected, lettuce. Good control was obtained by destroying sowthistles nearby (56). H. lactucae is of palaearctic origin and established in Australia without its natural parasites. A specific parasite (Aphidius sonchi) was recently introduced and now has become established (6).

The luteoviruses are phloem-limited and are only transmitted by aphids. Most members are serologically related. On the basis of comparisons to date, the definitive members form seven fairly distinct sero-groups represented by barley yellow dwarf-MAV, barley yellow dwarf-RPV, bean leaf roll, beet western yellows, carrot red leaf, potato leaf roll and soybean dwarf (61). There may be additional sero-groups

amongst tentative members of the group such as banana bunchy top (9), groundnut rosette assistor (7),Indonesian soybean dwarf (31) and strawberry mild yellow edge (SMYEV) (41).

The serological properties of viruses causing barley yellow dwarf (BYD) in North America are correlated with their vector specificities (52). The external surfaces of the capsid proteins that confer serological specificity apparently determine the specificity of virus movement through the gut wall and salivary glands of aphids (20, 21, 22). These relationships between vector specificity and serological specificity have been useful in epidemiological studies, especially now that the viruses can be detected by ELISA (39). For example, identification of an isolate as RPV by ELISA provides tacit evidence for it being vectored specifically by Rhopalosiphum padi. Similarly, the abundance of different serotypes in a region reflects the activity of vectors there. Thus in eastern Australia, RMV and RPV occur in south-east Queensland (23), PAV in New South Wales (60), MAV, PAV and RPV in Victoria (57; R. J. Sward, personal communication, 1985) and PAV and RPV in Tasmania (26). The distribution of these viruses reflects the abundance of their vectors and hosts in eastern Australia. The vectors found are Metopolophium dirhodum, Rhopalosiphum maidis, R. padi, Sitobion gragariae nd S. miscanthi (5, 26, 30). Schizaphus graminum and S. avenae do not occur in Australia.

Inferences on vector specificity and vector activity as a result of serological tests must be made with caution. Likewise, assumptions as to the serological tests must be made with caution. Likewise, assumptions as to the serological specificity on the basis of vector specificity tests may be wrong. For example, not all clones of some species, such as S. fragariae and S. miscanthi transmit efficiently (26; R. J. Sward, personal communication, 1985) and, therefore, care must be taken not to use non-transmitting clones of such aphid species.

Changes in cropping patterns may alter the abundance of vector species and thus the types of BYD virus that are found. In eastern Washington PAV types now predominate with cyclical changes in populations of R. padi and S. avenae, and alternation of virus between irrigated maize and winter wheat plus other small grains (4). In New York State, PAV types slowly displaced MAV when winter grain crops became more important than grasses as sources of infection for spring-sown cereal crops (51,54).

Species of Poaceae vary in their susceptibility to different BYD viruses and thus influence the prevalence of these viruses in a region. A survey of Tasmanian Poaceae indicated that PAV was most common in species of the Pooideae subfamily while RPV was most common in the Arundinoideae and Panicoideae (26). Occurrence of PAV and RPV in the bambusoideae and Chloridoideae was intermediate. A notable exception to this generalization was that two species of Pooideae, cocksfoot and Kentucky bluegrass, were infected almost exclusively with RPV. This agreed with North American studies on Kentucky bluegrass (16, 44). A number of sampling sites in our survey contained mixes of Panicoideae infected only with RPV and Pooideae infected almost exclusively with PAV. At other sites we found many plants of some species of Pooideae

with PAV alongside Panicoids and other species of Pooideae (e.g., English couch, annual Poa) that were healthy. These data suggested sources of resistance or immunity for breeding. The occurrence of PAV and RPV within the Poaceae hierarchy is a further criterion for considering PAV and RPV distinct viruses.

PAV and RPV were found together as mixed infections far more frequently than would have occurred by chance. This suggested a selective advantage for the spread of mixtures. Mixed infections were also common in North America (53). Transcapsidation permits the dependent transmission of virus genomes from mixed infections (48). Dependent transmission has been reported for eight distinct combinations of BYD viruses. The phenomenon permits transmission by non-vector species and the exploitation of plant species as hosts that might otherwise not become infected (49, 50).

Luteoviruses probably occur together as mixtures more frequently than has often been recognized. Beet western yellows virus (BWYV) was common in leaf roll-affected potatoes from California (12) and was also associated with leaf roll-affected potatoes in Tasmania (13). One of the first commercial kits to test potatoes for PLRV BY ELISA contained BWYV antigens and antibodies mixed with those of potato leaf roll virus (PLRV) (J. E. Duffus and R. Casper, unpublished information, 1982). BWYV from potatoes in California infected virus-free potato cultivars and induced primary leaf roll symptoms (Duffus, 1981) but Tasmanian isolates of BWYV from potatoes that had been passaged through crucifers other than shepherd's purse would not infect virus-free potatoes cv. Kennebec. We believe our BWYV isolates may infect potato when present in source plants together the PLRV.

BWYV and soybean dwarf virus (SDV) (=subterranean clover red leaf virus) frequently occur together in Tasmania, particularly in legumes (35). White clover is an important source of these mixtures for spread by Aulacorthum solani, but not by Myzus persicae, to annual legumes (2). Luteovirus mixtures in legumes in California are even more complex as bean leaf roll virus (BLRV) (=legume yellows virus) occurs together with, and in addition to, BWYV and SDV (Johnstone, Liu and Duffus, unpublished information, 1983).

Mixed luteoviruses infections often prove difficult to study, especially when reference virus isolates and antisera are unavailable. The presence of several components in a field isolate may easily go unrecognized and often only becomes apparent after sequential transfers with aphids through a range of plant species which leads to the loss of a component from the mixture (35, 59). It is surprising that there are so few reports on mixed luteovirus infections. Perhaps some viruses currently considered to be single entities may prove to be mixtures. Extensive comparisons of a PAV-like isolate from Tasmania (OA6) and its antiserum with the PAV-New York isolate and antiserum indicated that they were similar (25). However we found that extracts of grasses from Japan reacted strongly in ELISA tests with PAV-NY antiserum but failed to react with OA6 antiserum. The application of affinity chromatography using monoclonal antibodies (10) to obtain "pure" luteovirus isolates could help resolve luteovirus mixtures.

Luteoviruses also help the dependent aphid transmission of some sap-transmissible viruses (14). Six such disease complexes are known, namely bean yellow vein-banding (BYVB), carrot mottle, groundnut rosette, lettuce speckles, tobacco yellow vein and tobacco mottle. It appears the dependent viruses are transmitted by aphids when their genomes are encapsidated in helper coat protein. BYVB virus is exceptional in that both BLRV and PEMV can help (8). A few luteoviruses helped the dependent transmission of viruses with which they were not normally associated (1, 15, 62). Some of these experimental combinations changed the vector specificity and indicated the potential for diversification with respect to vector specificity and host range.

Host range and vector specificity studies of luteoviruses need careful planning because the experimental conditions may lead to changes in virus properties. For example, SMYEV was no longer transmissible by Chaetosiphon fragaefolii after four passages through wild strawberry by grafting (41), the type culture of BWYV maintained in ground cherry will no longer infect sugar beet (J.E. Duffus, personal communication, 1983), the original isolates of LYV from lucerne (11) kept in faba bean no longer infect lucerne (34) and BWYV isolates that we apparently separated from SDV in Tasmanian legumes by passage through shepherd's purse "reverted" to SDV-like isolates after passage through subterranean clover.

Similar changes also occur in the field. Up to 1980 we found all our SDV-like isolates were transmitted only by A. solani (32, 35, 37). A. pisum was introduced to Tasmania in 1980 and has displaced A. solani as a major component of the aphid fauna. Now, most SDV-like isolates recovered from the field are vectored specifically by A. pisum. These pea aphid isolates were serologically similar to those transmitted by A. solani and to the pea aphid specific isolates from California where A. pisum is common and A. solani is rare (36).

The causal agents of some diseases transmitted persistently by aphids have not been identified. These include filaree red leaf (17) and subterranean clover stunt (SCS) (24). These diseases are similar and the causal agents may be unrelated to other virus groups. The transmission cycle of SCS is completed in one hour but the vector remains infective for life (24). There may be distinct components present in plants with SCS.

Deltamethrin is a pyrethroid that hinders the inoculation of plants with viruses by aphids (19). It controlled primary infections of SDV in faba beans while an organophosphate, demeton-S-methyl, did not (33). Demeton-S-methyl controlled secondary spread (38). These materials differentiate primary and secondary virus spread. Some new synthetic pyrethroids, e.g. PP321, are highly aphicidal at low concentrations (45) and can give economic control of persistent virus diseases.

New biotechnologies developed recently promise to help resolve intractable problems posed by virus diseases transmitted persistently by aphids but only if they are married with "old-fashioned" biological approaches to studies on epidemiology and control.

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VECTORS OF BARLEY YELLOW DWARF VIRUS IN MEXICO AND THE SCREENING OF SMALL GRAIN CEREALS FOR RESISTANCE TO THE VIRUS

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Barley yellow dwarf (BYD) should be considered a second generation disease because its presence is often masked by other diseases such as the rusts. Once resistances have been developed to these diseases the effects of BYD become more obvious.

Control of BYD can be partially effected by adjusting planting date, the judicial use of insecticides for aphid control, or biological control of the aphid vectors. The most efficacious control, however, is the use of plant resistance. Any combination of the above can be integrated.

Aphid Vectors and Virus Isolates of Mexico. Preliminary work has been completed on the identification of the aphid vectors in Mexico and the virus isolates they are transmitting. The main aphid species so far identified in Mexico that feed on small grains include Rhopalosiphum padi, R. maidis, Sitobion avenae, Shizaphis graminum, Metopolophium dirhodum and Diuraphis noxia.

The main isolate of barley yellow dwarf virus present in Mexico is PAV-like (transmitted by R. padi and S. avenae) with both R. padi and S. avenae being efficient vectors. There are also MAV- and RPV-like isolates present in Mexico. Help with ELISA testing of these isolates of BYD has been obtained from several laboratories. To date we do not have conclusive proof that D. noxia is an efficient vector of BYDV in Mexico.

Germplasm Screening. Currently we are beginning the third cycle of screening for BYD resistant germplasm at our Toluca research station in Mexico. This site is generally good for screening in both summer and winter with BYDV infection being provided by natural epidemics due to viruliferous aphids.

This year we are screening approximately 6000 lines. These include materials from all of our breeding programs: bread wheat, durum wheat, triticale, barley and wide crosses, plus lines that have appeared BYDV resistant in previous tests at Toluca. In addition, we are screening selections from the University of California, Davis and from Sainte-Foy, Quebec. Materials for testing are sown as spaced plants (15-20 cm apart) either as one 2-meter row or two 1-meter rows. Two repetitions of all entries are seeded. Observations on symptoms are taken using a 0-9 scale where 0 is resistant and 9 is fully susceptible (1). We retest material that scores 5 or below.

The plots are sprayed every two weeks with fungicides to eliminate other foliar disease, to simplify note taking.

Materials appearing resistant to BYD in our tests are selected and maintained by the program for retesting and BYD resistant screening nurseries for the four main crops composed of this material are distributed to cooperators for their testing and use. Entries in the preliminary BYD screening nursery that were resistant at Sainte-Foy, Quebec, Canada; Palmerston North, New Zealand and Davis, California, U.S.A. are the bread wheat lines FLN/ACC//ANZA, PRL"S"(2 sisters) JUP/EMU"S"//GJO"S" (3 sisters), DODO"S" and ERA/MN 69146//PUM"S". Last year, from 228 lines of bread wheat selected for resistance the previous year in Toluca, 50 lines appeared resistant at both Davis, California and Toluca, Mexico.

Lines of winter wheat that have shown resistance at Toluca are NS974/NB69565, PYN, OK77164, SDY, NR 72.837, F4472 (all for 4 years) and ANZA/SUT//CTK (2 sisters for 2 years).

Few durum wheats appear to have resistance to BYDV and so far we have selected only 27. Lines of durum that were selected in Toluca in 1984-1985 for BYD resistance and that have also exhibited a level of resistance in Sainte-Foy, Quebec, Canada, are as follows:

CR"S"/GS"S"//PG"S", CIT 71, MOA"S", AFN"S"/IBIS"S"//COO"S"/3/GOO"S", CARC"S", GS"S"/CR"S"//SBA81/3/HO/4/MEXI"S"/5/MEMO"S", SCO"S"/RABI"S"/MEXI75, MO"S", GEDIZ"S"/CIT71, YAV"S"/SNIPE"S"/3/MEXI"S"/P66.270//GTA"S"(2 sisters), PI 178083/FRIG"S"//GOO"S", QFN/MEMO"S" /3/OYCA"S"//RUFF"S"/FG"S" and ZUD.

This year we will be looking at F2's and F3's from crosses of this material.

We have a group of approximately 120 barleys that exhibit resistance. Most of these probably have the Yd gene for resistance. Lines that exhibit excellent resistance to BYD in Toluca are PROMESA, SUTTER, BEN 4D, 79W41762, LIGNEE 640, Atlas 68, TERAN 75, DUCHICELA, DORADA, and PI2325/MAG102//COSSACK. Many of these lines have exhibited resistance at other testing sites but there are some reversals. The ICARDA-CIMMYT breeding program based in Mexico is aiming to combine the Yd with resistance available from the winter materials from Missouri.

We have over 100 triticales that appear to have good BYD resistance. Some Elymus giganteus wheat crosses appear to have a degree of resistance. This year we are testing Aegilops squarrosa and Triticum dicocoides accessions. This season for the first time we will have the capability to infest materials with greenhouse reared viruliferous aphids.

CIMMYT has developed strong links with programs that are screening germplasm for BYDV. From the preliminary data it appears that screening at different sites is very important as there appears to be variation between sites. Current data is preliminary and further testing will be required to determine the extent and significance of this variation. The real strength of the CIMMYT BYD program will depend on the feedback

from its cooperators who are growing and evaluating the BYD screening nurseries.

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TRANSMISSION AND DETECTION OF SOYBEAN DWARF VIRUS, A PERSISTENTLY TRANSMITTED LEGUME VIRUS

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Crop losses from virus diseases of legumes have been observed by growers and scientists since the early part of the 20th century. Representatives of at least 15 currently recognized plant virus groups are known to infect legumes and of those, the mechanically transmitted viruses have received the most attention. Luteoviruses, persistently transmitted by aphids, have been described on a variety of economically important field and forage crops. Although cultivars of some crops exhibit striking symptoms following infection, many hosts appear to be suffering from nutritional deficiencies or environmental insults. Furthermore, the ca. 25 nm icosahedral particles are: 1) confined to the phloem, 2) in very low concentration in the plant hosts, and 3) typically difficult to extract for purification. These characteristics, in turn, have limited characterization studies, the development of sensitive assays for detection and identification, and surveys to accurately determine distribution.

Soybean dwarf virus, a member of the luteovirus group, was first recognized as a distinct disease of soybeans [Glycine max (L.) Merr.] in the southern area of Hokkaido, Japan in 1952 (4). Yield losses in soybeans can be modest to almost total depending on the cultivar, virus strain, aphid population, plant age, and environment (4).

Other diseases of soybeans with symptoms similar to soybean dwarf have been reported from New Zealand, Tasmania, central California, Indonesia, and Nigeria. The New Zealand virus, subterranean clover red leaf virus (SCRLV-NZ), is serologically indistinguishable from the Tasmanian virus (SCRLV-T) and the yellowing strain of SDV. All three viruses are vectored by Aulacorthum solani. The California virus is related serologically to SCRLV-T and is vectored only by Acyrthosiphon pisum.

SDV has not been reported to occur in the United States but the demonstrated potential for damage, the importance of soybeans and forage legumes in North America and the ubiquitous nature of A. solani led us to study SDV and related diseases in the containment facilities at Frederick, MD. Our mission objectives are 1) to determine transmission and other biological characteristics that will help assess the potential for the establishment of SDV in the U.S., 2) to develop sensitive biological, serological, and molecular techniques for detection and identification of SDV and other luteoviruses, and 3) to study relationships among the luteoviruses, especially those infecting legumes.

Four populations of A. solani have been compared as vectors of two strains of SDV and SCRLV-NZ. The Japanese population (J) transmits both SDV-D and SDV-Y more efficiently than do the populations from California

(CA), New Zealand (NZ), and New Brunswick (NB), Canada. The NZ population transmits SDV-Y as efficiently as the J population. The similarity in transmission characteristics of SDV-Y and SCRLV-NZ by the four aphid populations is not surprising since Ashby and Kyriakou (1) found that SCRLV-NZ and SDV-Y could not be differentiated with polyclonal antisera. We confirmed their results and observed that SDV-D is serologically indistinguishable from either SDV-Y or SCRLV-NZ. SDV-D and SDV-Y do differ in transmission efficiency and other biological and physiochemical characteristics (2,3).

Our efforts to develop serological assays have been concentrated on the enzyme-linked immunosorbent assay (ELISA) because it is a simple, rapid, sensitive, quantitative assay which conserves antisera and requires minute amounts of antigen. All antisera are cross-absorbed against healthy host tissue and ammonium sulphate precipitated. Antisera for relationship studies are further purified by affinity chromatography. Extraction of virus from infected tissue remains the most important aspect of successful ELISA tests with any luteovirus. With paired samples, a variety of methods and extraction devices were tested in an attempt to optimize extraction. All methods worked well but the most efficient method for processing a large number of samples was the following: freeze tissue samples in liquid nitrogen and shatter with a Teflon rod before the samples thaw, add buffer (1:3 or 1:5 w:v), and grind in a tissue homogenizer (Tissumizer) for 30 sec to 1 min.

ELISA assays with polyclonal antisera are useful for detection but cannot be used to differentiate among SDV-D, SDV-Y, and SCRLV. The symptoms of the two SDV strains are easily differentiated on Wayne or other soybeans (4; Damsteegt, unpublished data) and symptoms of a mixed infection are distinct from a single infection of either strain. However, symptoms of SCRLV and SDV-Y are difficult, if not impossible, to separate on any host that we have tested. Double-stranded RNA (dsRNA) assays clearly differentiate among the three viruses. Two major bands of about 3.8 and 2.0 x 10^6 M_r, and several minor dsRNAs have been observed. When mixtures of any two of the three viruses are analyzed in the same lane and individual dsRNA preparations of each virus are placed in other lanes on the same gel, one or both of the two major dsRNA bands of each virus migrate differentially, clearly indicating that two different dsRNA preparations are present (Hewings, unpublished data). Luteoviruses, like other virus groups, have a dsRNA banding pattern that appears to be typical of the group. Distinguishing between two known strains can usually be accomplished by dsRNA analysis but whether the technique can be used for diagnosis is questionable. ELISA and dsRNA analysis together are powerful tools for detection and identification of luteoviruses.

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AERIAL SUCTION TRAPS TO MONITOR GRAIN APHIDS

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Southern Idaho is frequently subject to epidemics of barley yellow dwarf (BYD), an aphid-borne virus disease of grain and grasses. In epidemic years when viruliferous aphids are abundant at the time fall planted wheat and barley emerge, growers can lose 20-70% of their crop (2). Losses can be mitigated by applying a systemic insecticide with the seed or by delaying planting until aphid flights have subsided.

In the fall of 1984 plants in pairs of fields in Arbon Valley with different planting dates were indexed for BYD. Early planted fields had more BYD than the neighboring late planted fields (Table 1) (1). The objective of our research was to determine whether aerial suction traps could be used in Idaho to monitor grain aphid flights and provide reliable information to advise growers of safe planting dates.

Suction traps were installed at the following locations: Parma, Wilder, Moscow, Kimberly, Aberdeen, Arbon Valley, Rockland Valley, Shelley, and Preston. A disproportionate number of the traps were installed in southeastern Idaho because of the frequency of BYD epidemics there and because little is known about aphid movement in mountainous areas. Traps in Moscow, Rockland Valley and Preston were installed too late to be of much value during the first season.

Though several traps (at Aberdeen, Parma, Shelley and Wilder) collected high numbers of Rhopalosiphum padi (L.), these flights were overshadowed by the vast flights of Schizaphis graminum (Rondani) in southeast Idaho. Except in the high mountain valleys (Arbon and Rockland), flights had subsided by the time crops are normally planted. In Aberdeen, the peak flight of S. graminum occurred the last week of July but few were collected by the first week in September when grain is usually planted there. In Arbon Valley, on the other hand, the peak flight occurred a week later, and aphids were still too numerous at planting time (around 15 August) (Fig. 1). These S. graminum collections were used in a decision to advise growers to delay planting winter wheat in Arbon and Rockland Valleys until after 1 September. One untreated field planted on 15 August had at least 38% BYDV infection by 25 October.

Of interest in terms of prediction of S. graminum outbreaks is the increasingly later date of flight peaks from west to east across southern Idaho (Fig. 2). We suspect that this progression reflects retarded insect development rates at increasingly higher elevations rather than aphid migration, but this question needs further study.

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Table 1. Indexing results for plants collected in neighboring fields in Arbon Valley, Idaho, 1984.[a]

		% Infected indicators		
		Vector		
Field pair	Planting date	*S. graminum*[b,c]	*R. padi*[c]	Total
1	10 Sept	0	0	0
	15 Aug	8	2	10
2	10-15 Sept	2	2	4
	10-15 Aug	20	4	24

[a]Table from Bishop, Forster and Sandvol (in prep.)

[b]*S. graminum* transmitted a laboratory stock culture of SGV to 15/32 indicators.

[c]No aphids taken directly from stock culture transmitted BYD.

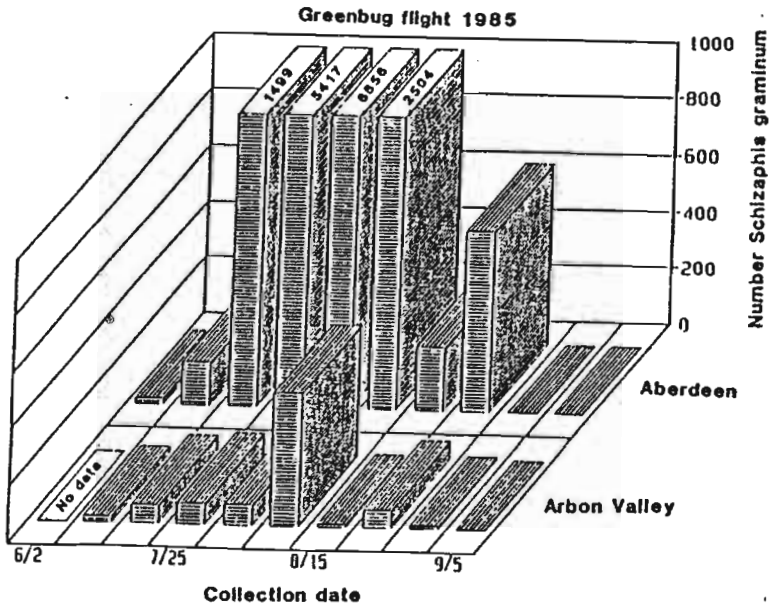

Figure 1.

GREENBUG FLIGHT PEAKS AT SELECTED LOCATIONS. 1985

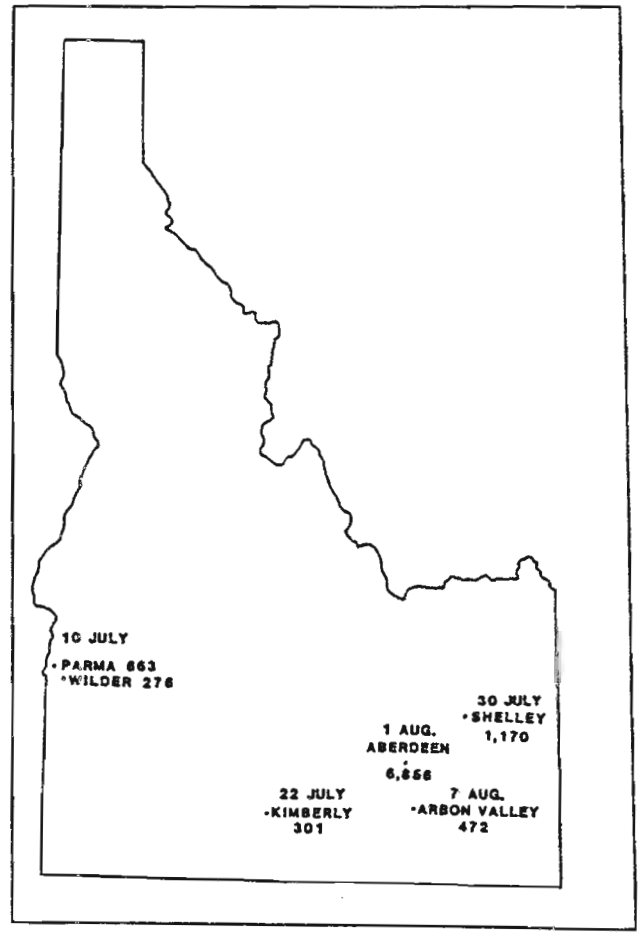

Figure 2.

APHID TRANSMISSION OF THE VIRUSES CAUSING CHLOROTIC ROSETTE AND GREEN ROSETTE DISEASES OF GROUNDNUT

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Chlorotic rosette and green rosette diseases of groundnut (peanut) occur commonly in northern Nigeria. In nature each disease is believed to be caused by two viruses. The first one, groundnut rosette virus, is probably responsible for the disease symptoms, but it is dependent on the second virus, groundnut rosette assistor virus, for aphid transmission. The black cowpea aphid, Aphis craccivora, is the vector of the viruses which are transmitted in a persistent manner. Comparisons of aphid transmission of the chlorotic rosette viruses (CR-V) and green rosette viruses (GR-V) were made in these studies.

Acquisition of CR-V and GR-V occurred within 4 hr (30 min in one test) and 8 hr, respectively. The median latent period (LP_{50}) was 24 hr for CR-V and 36 hr for GR-V. After the latent period, both CR-V and GR-V could be transmitted within 10 min (inoculation access period), but longer periods increased the number of successful transmissions. The maximum retention period in the vector was 14 days for both CR-V and GR-V. The acquisition access period affected the retention period similarly for both CR-V and GR-V; disease agents were retained for 11 and 14 days when the acquisition period was 48 and 60 hr, respectively, and 6 days or less for shorter acquisition periods. Aphids on groundnut test plants began to die at 16 days and all were dead by 17 days.

Serial transfers to healthy groundnut seedlings demonstrated that individual aphids could transmit CR-V more frequently (71%) to multiple plants than GR-V (45%). Virus retention and aphid survival tests showed that transmission efficiency was highest during the earliest stages of sequential transfers, particularly with CR-V. During the sequential transfer tests, transmission was not continuous to each test plant for either CR-V or GR-V.

Transmission efficiency by single aphids was greater for CR-V (38%) than GR-V (25%) in two tests and the same in one test. When groups of two and five viruliferous aphids/plant were used, transmission efficiency increased for both CR-V and GR-V. Transmission efficiency to resistant groundnut cultivars was similarly low (3-4%) for both CR-V and GR-V, and multiple (five) viruliferous aphids/plant increased transmission.

When 14 plant species in the Leguminosae and Solanaceae families were aphid-inoculated with CR-V and GR-V, only Canavalia ensiformis developed virus-like symptoms, and then only after transmission attempts

with CR-V. However, neither CR-V nor GR-V was recovered from any plants of the 14 species by back inoculation by aphids to groundnut.

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EPIDEMIOLOGY AND ELECTRON MICROSCOPY STUDIES OF BARLEY YELLOW DWARF VIRUS

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Barley yellow dwarf virus (BYDV) has been reported in maize by some authors in different countries. Maize also has been suspected to play a role in the epidemiology of BYD (1). In Italy, the disease is reported to be a practical problem only for maize inbred lines, but it has been demonstrated by ELISA and by experimental aphid transmission that both maize hybrids and inbred lines either showing symptoms of BYD or symptomless can be a host of the virus (2,3). The virus has been detected in sap (4) and in ultrathin sections (5) of maize plants.

The aim of our research was to determine the possible role played by maize in northern Italy in the epidemiology of BYD and to study any possible alterations induced by BYDV in maize-infected cells.

Maize as host of aphid vectors of BYDV. Field surveys have shown that maize is a poor host of the aphid vectors of BYDV during the first growth stage when the alatae visit the maize plants but very seldom colonize them. On the contrary, in summer and in autumn maize becomes a good host. Sometimes large and continuous aphid colonies mostly of Rhopalosiphum padi L., followed by Methopolophium dirhodum Walk, Sitobion avenae Fabr and R. maidis Fitch, are present. Even in October large colonies of R. padi are still present on partially green maize plants, i.e., soon before barley sowing.

Maize as natural source of inoculum for aphid transmission. Aphid transmissions were carried out by using apterous R. padi of the healthy colony. For the acquisition the aphids were applied to one plant of seven differing maize hybrids and to one of six differing inbred lines, all showing BYD symptoms in the field. The infected aphids were then transferred to Avena byzantina K. Koch (3-5 test plants per source) and to maize inbred 33-16 (5-10 test plants per source). Each A. byzantina plant was infested with 3 infective aphids and 10 aphids were used in the case of maize 33-16. The transmission procedures adopted were as previously described (2). ELISA was applied to all the test plants and sources of inoculum using an antiserum against BYDV/PAV strain. The results are reported in Table 1.

The natural infectivity level of R. padi on maize in autumn. In October, 900 apterous R. padi were collected from 300 maize plants (commercial hybrids) taken by chance in five different areas of the Friuli Venezia Giulia Region. The inoculations were carried out in the greenhouse using three aphids per A. byzantina test plant. The results are reported in Table 1.

Maize as recipient plant of late natural transmissions. At the beginning of October, at the 3-5 leaf growth stage, 13 inbred lines and 3 hybrids were transferred to an infected area (five plants per genotype in single pots). Thirty plants each of A. byzantina and of barley cv. Astrix were also used. The aphid colonization (R. padi and S. avenae) started immediately on barley and A. byzantina but was very low and scarce on maize plants. After 30 days, the mean number of aphids per plant was about 80 for A. byzantina and barley but only 1-4 for maize (half of them being alatae of the species R. padi and S. avenae).

ELISA was applied to four plants of each genotype both 15 and 30 days after the beginning of the exposure. At the time of the 15-day analysis, all the A. byzantina and barley plants checked, as well as 2 maize hybrids and 5 maize inbred lines, gave a positive reaction. At the 30-day analysis, 4 additional maize inbred lines proved to be infected. Obvious BYD symptoms were shown only on barley and A. byzantina.

Electron microscopy. Leaf samples for electron microscopy (EM) studies were cut from infected inbred lines or hybrids and from control plants. At the time of sampling (1 month after inoculation) the infected leaves showed a dark red coloration. The tissues were fixed and embedded as previously described (5) and the ultrathin sections were examined with a Hitachi 300 EM.

DISCUSSION

The results achieved demonstrate that maize can be both a natural source of inoculum and a host plant for BYDV. Maize is also a host for differing aphid species mainly in autumn. During its first growth stage maize is tendentially avoided by aphids; this behavior doesn't depend on the season. The rather low infectivity level demonstrated in autumn by aphids present on maize can be of significant importance because of the size and frequency of the colonies. The most important strain of BYDV in maize seems to be the non-specific (PAV). These results would confirm that maize is an important species in the epidemiology of BYD, mainly for the transmissions in autumn. The EM results seem to confirm the previous ones (5); i.e., the substantial accumulation of starch in the chloroplasts of the bundle sheath cells seem to be a common alteration to all maize plants tested; on the contrary other alterations are peculiar of each line.

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Table 1. Aphid transmissions from naturally infected maize to A. byzantina and maize 33.16.

Source of inoculum	% Test plants infected	
	A. byzantina	Maize 33.16
N.o 7 hybrids*	80	56
N.o 6 inbred-lines*	79	87
N.o 300 plants of commercial hybrids from five crop fields	0.5-7	-

*For all the sources of inoculum positive transmissions have been achieved.

IMPACT OF BIOTECHNOLOGICAL TECHNIQUES ON PLANT VIRUS EPIDEMIOLOGY

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Modern biotechnological procedures have attracted great interest in recent years and have found many applications in plant virus research. They have been used extensively in research on the structure and function of viral genomes but increasingly they are also being used in the more applied forms of virology. Their impact on plant virus epidemiology is of two main kinds. First, they are providing new sorts of diagnostic reagents, complementary DNA (cDNA) probes and monoclonal antibodies (McAbs), which are extremely specific and have great potential sensitivity. Second, they offer the possibility of influencing the course of virus epidemics in new ways, such as by enhancing the virus resistance of host plants by genetic engineering. Selected examples of these approaches are given below.

Identification of infected plants and discrimination between virus strains. Epidemiological studies require a means of ascertaining which plants are infected. The most widely used methods of detecting and identifying plant viruses are serological, such as immunodiffusion or immunoprecipitin tests, or enzyme-linked immunosorbent assay (ELISA). Such methods have been applied to more than 300 plant viruses with the aid of conventional antisera obtained by immunizing animals, usually rabbits, with purified preparations of virus particles. However, McAbs of mouse or rat origin (1) have now been prepared to more than 30 viruses, and their usefulness is being evaluated. McAbs have some advantages and some disadvantages as diagnostic reagents. The advantages include 1) lack of reactivity with host antigens, b) specificity for one viral epitope and hence suitability for distinguishing different strains of the same virus, c) high titer in ascites fluid, which commonly is diluted 10^5- to 10^6-fold for use, and d) ability to be produced in large amounts with uniform quality. The disadvantages include a) the considerable labor and expense needed to make them in the first instance, b) their specificity, which may be too great for them to react with all, or even most, strains of a virus, c) failure of some McAbs to give precipitin reactions, and d) the need of some McAbs for special conditions to react optimally.

McAbs to viral particle protein are particularly valuable for distinguishing between antigenic variants of a virus. This gives them the potential for use in investigative work to trace the sources of individual virus isolates found in crops, and in work to study the fate of variants produced during an epidemic or a series of epidemics. However, there are considerable differences between viruses in the degree to which isolates vary antigenically. For example, nine main variants of potato virus X were distinguished among 33 isolates by a panel of 10 McAbs (2) whereas in comparable work with over 40 field isolates of potato leafroll virus from Britain and Australia only three variants were found, a common British strain, a rare British strain and a common Australian strain (3). Similarly, tests with a panel of 23

McAbs to African cassava mosaic virus, a whitefly-transmitted geminivirus, showed that among isolates from five countries there were only three main strains, which had different geographical distributions, and one minor variant (4). But despite the antigenic variation that occurs among potato virus X isolates, one McAb was able to detect all strains, and another two proved to be suitable for identifying a rare resistance-breaking strain (2). These have obvious practical uses. Clearly, it is vital to take account of the purposes of the work and of the preferred kind of serological test when selecting the McAb(s) to be used.

Virus detection and strain differentiation can also be approached with the aid of cDNA probes, which have the advantage of being able to react with any or all parts of the viral genome, whereas most serological tests detect only one or a few viral gene products. Although most plant viruses have RNA genomes, cDNA probes can be made either directly by reverse transcription of virus RNA (5,6) or by first cloning the cDNA molecules so produced and then nick-translating these clones (7). cDNA probes for more than 50 viruses have been prepared. They can be used for virus detection by a variety of tests including Southern and Northern blotting, hybridization in liquid, and spot hybridization (8), which is the simplest method suitable for routine use. The advantages of tests using cDNA probes are a) the probe can be specific for either the whole or a known part of the virus genome, b) viroids (9), satellite nucleic acids (10), and virus isolates that do not produce virus particle proteins (11), can be detected, and c) the probes can be prepared in large amounts with reproducible reactivity. Disadvantages include a) the loss of detection sensitivity in spot hybridization tests that is caused by normal components of plant sap, b) the need to label probes radioactively until satisfactory non-radioactive procedures can be devised, and c) the considerable labor and expense needed to make cloned probes in the first instance.

Experience with cDNA probes for potato spindle tuber viroid (9) and for potato X, Y and leafroll viruses (7) has shown that spot hybridization tests provide a reliable routine method for detecting these agents. With tobacco rattle virus, cDNA probes have proved to be particularly suitable for detecting virus isolates that do not produce virus particle protein (11), and also for detecting antigenically diverse virus strains that are not detected reliably by conventional serological methods (12). With African cassava mosaic virus, which has a bipartite ssDNA genome, particle-deficient virus strains can again be detected (13). In addition, other strains of the virus with divergent nucleotide sequences in one part of the genome can be distinguished from the type strain and, when less stringent washing conditions are used, the probes can be used to detect several other whitefly-transmitted geminiviruses (14).

McAbs and cDNA probes therefore both promise to be increasingly important aids to routine virus detection and identification, with the method of choice depending on the virus, and the facilities and reagents that are available.

Detection of viruses in vectors. For most plant viruses, any analysis of the course of an epidemic or any attempt to predict the timing and extent of an epidemic will demand information on the numbers

and activity of one or more species of virus vector, and on the proportion of individuals of the vector species that are viruliferous. Many viruses that multiply in their insect vectors can be detected readily in individual insects by serological tests. Among viruses that do not multiply in their vectors, however, some can be detected in these vectors by serological tests and others not reliably or not at all. Because of their potential reactivity, McAbs may be helpful for increasing the reliability of detection of viruses that occur in vectors in amounts at the limit of sensitivity of tests using conventional antisera. For similar reasons cDNA probes show promise for detecting agents such as maize streak virus in vector leafhoppers by the 'squash-blot' test (15). In such work, it is important to remember that the presence of a virus in a possible vector does not necessarily imply an ability to transmit it. Poor vectors, or perhaps even non-vectors, may acquire as much virus from an infected plant as good vectors.

Genetic engineering and virus resistance. Methods of genetic engineering have now advanced to the point where several novel approaches to improving the virus resistance of plants are being tested. One approach is based on the cross-protection phenomenon - the fact that plants infected with one strain (even a mild strain) of a virus do not develop additional symptoms when inoculated with a second strain (16). It is hypothesized that this effect is attributable to a specific part of the genome of the protecting strain and that if a DNA copy of this sequence could be incorporated in the plant genome, disease resistance might be enhanced. A second approach is to insert into the plant genome a DNA sequence which can be transcribed into RNA that is complementary to a key part of the virus genome. The presence of this anti-sense RNA is considered likely to interfere with gene expression (17) and so to disrupt virus replication in the transformed plants. A third approach is based on the discovery in virus cultures of satellite nucleic acids that depend on a specific virus for replication, may interfere with virus replication and may either ameliorate or intensify disease symptoms (18). Hence plants transformed with DNA copies of a benign satellite RNA might develop only mild symptoms when inoculated with a satellite-free culture of the virus and/or might be resistant to infection.

Few results are yet published from experiments of these kinds. However it has been found that tobacco plants transformed with DNA copies of the coat protein gene of tobacco mosaic virus produce the coat protein (19) and are slower to develop systemic symptoms than control plants (20). The use of such plants would therefore be expected to slow down the progress of an epidemic.

Progress has also been made in work with the satellite RNA of cucumber mosaic virus (CMV) (10). Tobacco plants transformed with DNA copies of 1.3-unit length molecules of satellite RNA produced RNA transcripts corresponding to this size in small amounts, and appeared to grow and develop normally. When they were inoculated with a satellite-free isolate of the CMV, however, large amounts of unit length satellite RNA were produced in inoculated and systemically infected leaves, and the amount of CMV genome RNA in the systemically infected leaves was decreased. When sap from these leaves was inoculated to Nicotiana

clevelandii, satellite RNA was again produced and was packaged in CMV particles, and the plants developed milder symptoms than plants inoculated with satellite-free CMV. The virus cultures grown in the transformed plants had therefore acquired genetic material from the host genome and become less virulent as a result. Again it seems probable that the use of these transformed plants would slow the progress of epidemics.

Finally, although little progress has yet been made in transferring host genes for virus resistance from one plant species to another by genetic engineering, this should become possible once the nucleotide sequences that comprise these genes are identified. It is worth noting that in all these approaches the objective is not to produce radically altered plants but instead to correct faults (e.g. virus susceptibility) in otherwise satisfactory cultivars. Genetic engineering is therefore a complement to, not a substitute for, conventional methods of breeding virus-resistant crop plants as a strategy for preventing epidemics.

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ULTRASTRUCTURAL INVESTIGATIONS ON PLANT VIRUSES FROM SPRUCE, BEECH AND BIRCH ASSOCIATED WITH FOREST DECLINE (WALDSTERBEN) IN THE FEDERAL REPUBLIC OF GERMANY

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Waldsterben, a general decline of forests in Central Europe, is a disease syndrome probably caused by a complex of several predisposing and stress-inducing factors. Atmospheric deposition of air pollutants or pollutant-related toxic, acidifying or growth altering substances are among the primary causal factors (3). The biotic hypothesis is advocated especially by Nienhaus (2) and Kandler (1) concerning the possible causal relation of Rickettsia-like bacteria (RLB), mycoplasma-like organisms (MLO) and plant viruses as predisposing factors of Waldsterben. Presented in this poster-demonstration is the abstract result of the virus investigation in beech (Fagus sylvatica L.), birch (Betula pendula Roth), and Norway spruce (Picea abies Karst.) referring to the biotic hypothesis. In a cooperative investigation with Professor F. Nienhaus from the University of Bonn, we observed smaller sized leaves followed by necrosis and early leaf fall, long and short shoots and abnormal location of leaves within the crown of the diseased beech trees in the provinces of Rhineland and in Westfalia. Several different viruses in beech trees were identified as poty-, potex-, tobamo-, and nepoviruses according to their mechanical transmission to herbaceous plants and their morphological characteristics (Figs. 1 to 4). In young birch with mosaic and leaf patterns, changes in size of leaves were observed in the mountain area of Vordereifel (province Rhineland). According to the mode of transmission and electron microscopical studies a rod-shaped virus and pinwheel inclusions from potyvirus-groups could be demonstrated (Figs. 5 & 6). Young and older spruce with yellowing syndrome from the Bavarian Forest and Harz Mountains (Niedersachsen) were investigated. A spheric virus-like particle was isolated from the current year's, and older spruce needles with a normal diameter of 30 nm (Fig. 7). Mechanical transmission of the spheric virus-like particles on herbaceous plants did not succeed. In the fine structure of some damaged spruce with yellowing syndrome, we found very small spheric particles with a diameter of 5-7 nm (Fig. 8 arrow). They showed resistance after treating the thin sections with ribonuclease enzyme but the ribosomes themselves were digested. However, conclusions about the causal relations of viruses to the forest decline (Waldsterben) are not yet possible.

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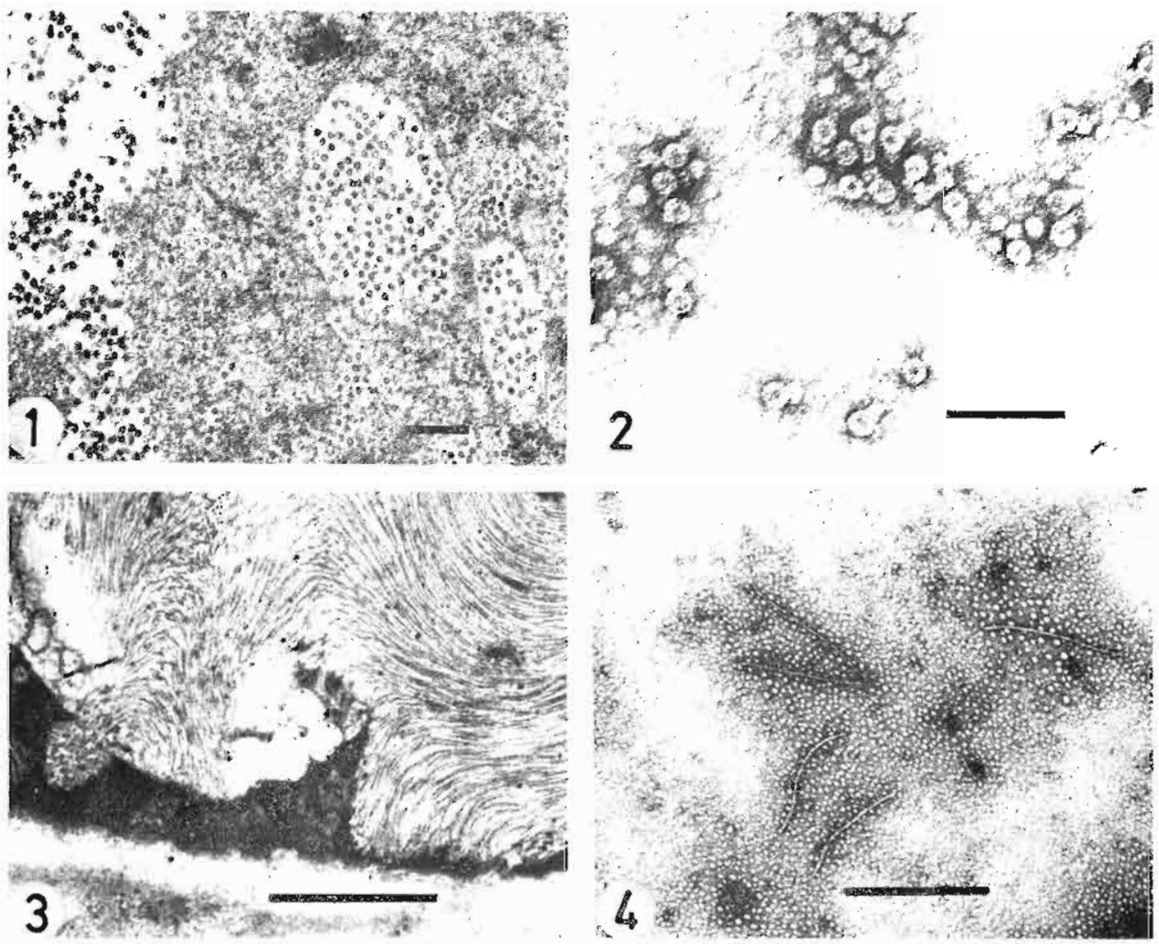

Fig. 1. A large aggregate of virus particles in a section of a degenerate cell of Chenopodium quinoa inoculated with the isolate of isometric virus from beech trees. Bar representes 100 nm.

Fig. 2. Virus particles from a leaf dip preparation from Chenopodium quinoa inoculated with the isolate of isometric virus from beech trees. Bar represents 100 nm.

Fig. 3. Parallel aggregate of virus particles in a section of Chenopodium quinoa infected with crude sap from naturally infected beech trees. Bar represents 500 nm.

Fig. 4. Thread-like virus particles from a leaf dip preparation from tobacco inoculated with the isolate of rod-shaped virus from naturally infected beech trees. Bar represents 500 nm.

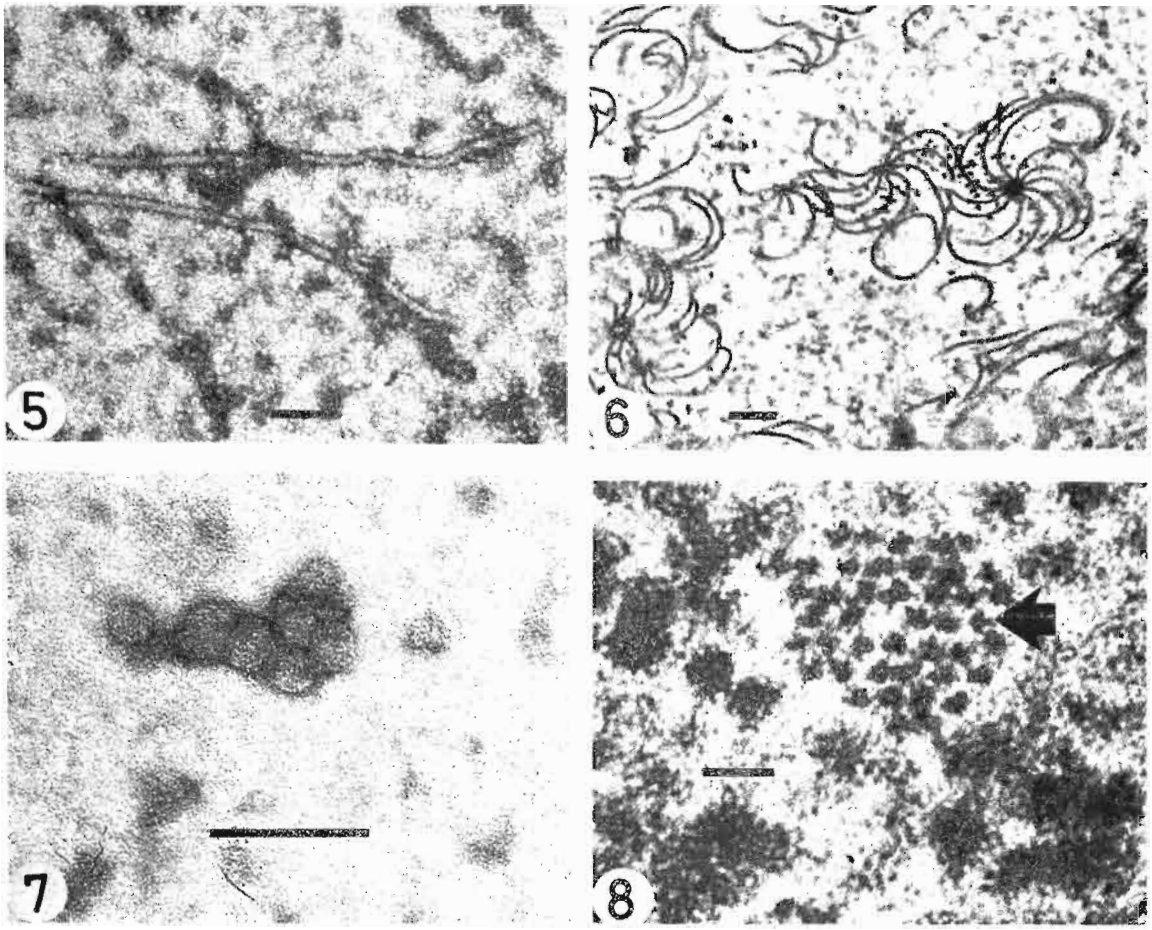

Fig. 5. Virus particles from a leaf dip preparation from tobacco inoculated with crude sap from a naturally infected birch tree. Bar represents 100 nm.

Fig. 6. Section of pinwheel inclusions induced in tobacco infected with crude sap from a naturally infected birch tree. Bar represents 100 nm.

Fig. 7. Virus particles from a partially purified preparation from the current year's needles with yellowing of affected young Norway spruce (15 yrs old). Bar represents 100 nm.

Fig. 8. A crystalline array of virus-like particles (5-7 nm) in a matrix of ribosomes in the phloem cell of a fine root from affected old Norway spruce (90 yrs old). Bar represents 25 nm.

GEOGRAPHICAL VARIATION IN AFRICAN CASSAVA MOSAIC VIRUS

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Mosaic disease occurs in cassava in many parts of Africa, on several Indian Ocean Islands and in India. It is caused by a whitefly-transmitted geminivirus usually known as African cassava mosaic virus (ACMV) (3), but also referred to as cassava latent virus. The virus genome consists of circular ssDNA (5) and is in two separate molecules, DNA-1 (2779 nt) and DNA-2 (2724 nt), which have different nucleotide sequences except for a shared region of about 200 nt, known as the common region (9). The virus particles contain one main protein species of molecular weight c. 30,000 that is encoded by DNA-1. Relationships between virus isolates transmitted to Nicotiana spp. by mechanical inoculation with sap from mosaic-affected cassava from different regions have been assessed by serological tests with polyclonal antibodies to the type strain of ACMV from West Kenya (ACMV-T) (1,8) and by nucleic acid hybridization tests with probes containing cloned DNA of ACMV-T (7). The results of this work, of further tests with DNA probes, and of experiments with monoclonal antibodies, show that the virus isolates fall into three main groups.

Group A includes ACMV-T, together with isolates from Angola (ACMV-A) and Nigeria (ACMV-N). Preliminary tests suggest that an isolate from Ghana is similar. In Nicotiana clevelandii and N. benthamiana, the particles of these isolates often reached concentrations exceeding 100 ng/mg leaf tissue. ACMV-T, ACMV-A and ACMV-N were indistinguishable in gel-diffusion precipitin tests and they all three reacted strongly in ELISA with polyclonal antibody to ACMV-T. Of the 23 monoclonal antibodies to ACMV-T that were tested, all reacted with ACMV-A but two failed to react, or reacted only weakly, with ACMV-N. Sequencing studies too have indicated that ACMV-N differs slightly from ACMV-T and that its particle protein has 11 amino acid replacements (10). The DNA of ACMV-A and ACMV-N reacted strongly in spot hybridization tests with probes for ACMV DNA-1 (nt 1-2779 but lacking nt 805-1410) and DNA-2 (nt 1-2724).

Group B comprises isolates from the Kenyan coast (ACMV-C). These were less readily transmitted to N. benthamiana, in which they reached only low concentrations and had a higher optimum temperature for multiplication than Group A isolates, and they were transmitted to N. clevelandii with difficulty, if at all (1,2,7). Their particles were antigenically distinguishable from those of ACMV-T. A spur formed when ACMV-C and ACMV-T were compared in gel-diffusion precipitin tests with ACMV-T antiserum, and only 11 of the 23 monoclonal antibodies to ACMV-T reacted strongly with two ACMV-C isolates. In nucleic acid hybridization tests with the ACMV-T probes, ACMV-C DNA reacted strongly with DNA-1 probes but only weakly with a probe for the complete DNA-2 sequence. However, ACMV-C DNA also reacted with a 266 nt probe for the part of ACMV-T DNA-1 that includes the common region.

Group C includes isolates from India (ACMV-I). These seemed to occur in cassava in lower concentrations than other isolates and transmission to N. benthamiana was difficult. They reacted in ELISA with polyclonal antibody to ACMV-T but with only 2 of the 23 monoclonal antibodies. They hybridized with ACMV-T DNA-1 probes, but not strongly, and they did not hybridize perceptibly with the probe for the complete DNA-2 of ACMV-T.

These results indicate, firstly, that variation among the geminivirus isolates found in mosaic-affected cassava parallels the geographical origin of the samples: either (a) West Africa or western Kenya, (b) eastern Kenya, or (c) India. Secondly, differences in antigenic specificity between the particle proteins of isolates in the different groups, a property determined by DNA-1, are accompanied by sequence differences in DNA-2 which, moreover, are greater than the sequence differences in DNA-1. Thirdly, the observed antigenic differences suggest that Group C isolates may be as different from Group B isolates as either are from Group A isolates.

This pattern of variation should be seen against the background of that found among geminiviruses in general (6). The geminivirus group can be divided into two sub-groups. Members of one sub-group have monopartite genomes, each has a different leafhopper vector, and most members are serologically unrelated to other members. In contrast, members of the other sub-group, which includes ACMV, have bipartite genomes, are all transmitted by the same whitefly species, Bemisia tabaci, and are serologically related to one another. Indeed, the particle protein gene is particularly strongly conserved in different whitefly-transmitted geminiviruses, perhaps because it plays a crucial role in vector transmission. Also, whereas considerable sequence similarities are found in the DNA-1 of different whitefly-transmitted geminiviruses, DNA-2 sequences are much less strongly conserved. However, the genome sequences of different whitefly-transmitted geminiviruses from America seem more closely related to one another than to those of comparable viruses from Africa or Asia, some of which likewise show strong sequence similarities to one another. This suggests that the American viruses have in more recent times evolved separately from, and in parallel to, the African/Asian ones (4).

Cassava is of American origin but seems not to be infected by ACMV or other geminiviruses in the Americas. The species is thought to have reached Africa by two routes, (a) across the Atlantic to West Africa, from which it spread eastward across the continent, and (b) across the Pacific, via intermediate countries, to the Indian Ocean islands and East African coast. Possibly a branch from this second route led to India. ACMV variation is consistent with the hypothesis that, when introduced to each of these three areas, cassava became infected with an indigenous geminivirus that was already established in another species. Indeed it is debatable whether the geminivirus isolates from cassava in different regions are best considered strains of one virus or whether they have already evolved to the point where they would more correctly be regarded as different geminiviruses.

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DETECTION OF POTATO LEAF ROLL AND BEET WESTERN YELLOWS VIRUSES IN APHIDS

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Monoclonal antibodies have been used to detect potato leaf roll virus (PLRV) and beet western yellows virus (BWYV) in individual aphids, Myzus persicae, and composites of one viruliferous aphid homogenized with up to 49 virus-free aphids. The PLRV specific and BWYV specific monoclonal antibodies react with homologous antigens but not with the heterologous antigens in ELISA. A two animal ELISA with rabbit polyclonal antisera as a trapping antibody, mouse monoclonal antibodies as the second antibody and a commercially available rabbit antimouse phosphatase conjugate (Jackson Immuno Research Laboratories, Inc., Avondale, PA) was used. The coating antibody was added in carbonate buffer at pH 9.6 at 1 µg/ml. The ELISA plates were then blocked with PBS-Tween containing 2% fetal calf serum (PBST-FCS). PBST-FCS was used as the sample grinding buffer, second antibody buffer, and the conjugate buffer. Aphids were homogenized in 200 µl of buffer in a 1.5 ml microfuge tube with a stainless steel pestle machined to fit the tube. Monoclonal antibodies were used at a 10^{-4} dilution of crude ascitic fluid. ELISA plates were incubated at 37 C for 1 hr at each step of the procedure. The A_{405} values were recorded after 16 hr incubation at room temperature.

In previous studies background or nonspecific reactions have limited the use of ELISA to detect plant viruses in aphids. With the use of monoclonal antibodies and FCS as a blocking agent during the ELISA procedure we have successfully detected PLRV and BWYV in aphids (Table 1). Background reactions remained low even with up to 50 aphids per well. The A_{405} values decreased between 5 and 1 viruliferous aphids per well as is expected since the amount of virus in one aphid should be less than the amount of virus in 5 aphids. The signal does not decrease when an increasing number of virus-free aphids were combined with one viruliferous aphid. This is expected since the nonspecific reactions are eliminated and the total amount of virus added per well should be constant.

The detection of PLRV and BWYV in composite aphid samples will enhance the study of the epidemiology and ecology of these viruses. Aphids can be collected in disposable microfuge tubes (50 aphids per tube) and taken back to the laboratory for testing. With this test virus can be detected in winged as well as apterous aphids so that alate aphids can be monitored for the presence of PLRV or BWYV. These tests are being applied currently as part of a project to develop an IPM program for the production of seed potatoes in the Fraser Valley of British Columbia, without the use of unnecessary pesticides.

Table 1. Detection of PLRV and BWYV in single aphids and composite aphid samples[1]

Ratio of V[2] to VF aphids	Viruliferous		Virus-free		
	PLRV	BWYV	No. aphids	PLRV	BWYV
5:0	1.85	1.84	5	0.04	0.03
1:0	0.55	0.33	1	0.02	0.01
1:4	0.48	0.64	5	0.02	0.03
1:9	0.53	-[3]	10	0.02	-
1:24	0.47	0.64	25	0.03	0.06
1:49	0.49	0.65	50	0.02	0.05

[1] A_{405} values shown are average of four individual tests.

[2] V = viruliferous aphids fed on source plants for at least 3 days, VF = virus-free aphids.

[3] - indicates test not done.

USE OF SPOT HYBRIDIZATION WITH A CLONED PROBE FOR SQUASH LEAF CURL VIRUS TO INVESTIGATE VECTOR RELATIONSHIPS

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The sweet potato whitefly, Bemisia tabaci, is the vector of squash leaf curl virus (SLCV) and an intermittent pattern of transmission is common for this and other whitefly transmitted geminiviruses. To further understand the nature of this phenomenon and other aspects of the virus-vector relationship, physical detection of the virus in single whiteflies was monitored under continuous or discontinuous access to infected plants and compared to detection in a non-vector, the greenhouse whitefly Trialeurodes vaporariorum. Detection of viral nucleic acid in extracts from individual insects was done by spot hybridization with a cloned DNA probe made from SLCV.

Effect of acquisition feeding period on detection was studied using B. tabaci and T. vaporariorum. In one experiment 600 B. tabaci adults were placed on infected bean plants. Sixty whiteflies were removed at 24-hr intervals. Forty whiteflies were macerated and extracts from individuals were analyzed by spot hybridization. The remaining whiteflies were placed individually on 20 healthy Top Crop bean plants. The plants were observed for 25 days for symptom expression. This experiment was repeated using infected squash as the source of inoculum but with no concurrent transmission attempts.

In another experiment 600 newly enclosed B. tabaci adults were caged on infected bean plants. Samples of 40 whiteflies were withdrawn at 24-hr intervals. In addition a similar sample was removed 12 hr after caging the whiteflies on the infected plants. In an experiment using the greenhouse whitefly, T. vaporariorum, 600 whiteflies were placed on infected squash plants. Forty whiteflies were removed at 24-hr intervals and analyzed by spot hybridization.

Retention of the virus in B. tabaci was investigated in a series of experiments. About 600 B. tabaci were allowed to feed on infected bean plants for 5, 8, 12 or 24 hr. All whiteflies were moved to cotton (a non-host for SLCV) after the predetermined acquisition access period. At various time intervals, 40 whiteflies were removed, and analyzed by spot hybridization.

Nymphal transmission was studied by placing 600 B. tabaci adults on infected Top Crop bean plant for 7 days. Fourteen to 17 days later the diseased leaves were detached and pieces of leaves with a known number of 4th nymphal instars were air dried for 2 days before they were stuck to the inside wall of transmission cages or kept in petri dishes. Emerging whiteflies were transferred to transmission cages at the rate of 5-16, 10-30, 30-40 or 40-50 individuals per plant.

Detection of viral nucleic acid in immature stages was carried out by allowing 600 whiteflies to deposit eggs on infected bean plants. Immature instars were detached from the infected plant, macerated individually or in groups and analyzed by spot hybridization. In addition, newly enclosed whiteflies emerging from the 4th instar of this experiment, which were denied access to the infected plant by drying the infected leaves prior to their emergence, were analyzed.

Effect of acquisition access period on detection levels of viral nucleic acid in B. tabaci and T. vaporariorum. Detection in 100% of the population of B. tabaci was not achieved in three experiments despite a lengthy acquisition feeding period. The detection level fluctuated and reached its maximum (42% to 70%) after an acquisition feeding period of 144-168 hr for three experiments. A drastic drop in detection level at 168 hr was associated with a high mortality rate of whiteflies at that time. The detection level at 24 hr was already between 67% and 99% of the highest level detected in the three experiments.

The highest transmission rate was obtained between 72 and 120 hr for whiteflies from infected beans. This corresponded with the highest level of physical detection. However, the level of physical detection remained high at 144 and 168 hr whereas transmission dropped drastically at that time. In contrast to B. tabaci, 100% detection was possible in T. vaporariorum (non-vector), even after a relatively short acquisition feeding period.

These results suggest that an agent may be destroying viral nucleic acid in B. tabaci but not in T. vaporariorum. Fluctuating detection levels in B. tabaci may indicate cyclic production of that agent in the vector. Such an agent may be an antiviral factor, which has been reported and considered the main reason for sporadic acquisition of some whitefly transmitted viruses (1).

Retention of detectable viral nucleic acid in B. tabaci after initial acquisition feeding. Virus was detected up to but not beyond 72 hr after an acquisition period of 5-24 hr in 1.6-12.5% of tested whiteflies, and in any given experiment, retention was intermittent. Intermittent transmission has been reported in other retention experiments, especially when short acquisition feeding periods were used (2). Whiteflies failed to retain detectable amounts of virus for their entire lives.

No viral nucleic acid could be detected in eggs deposited by viruliferous whiteflies. This confirms that there is no transovarial passage of the virus to the progeny (3). The viral nucleic acid in nymphal instars was detected at a very low rate, and then only in 2nd and 3rd instars. The low level of physical detection corresponded with a very low rate of transmission by adults emerging from these instars.

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VIRUS DETECTION IN BEECH FAGUS SYLVATICA L. ASSOCIATED WITH FOREST DECLINE

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Regional decline of many different forest trees in West Germany has led to the formulation of different hypotheses concerning its origin. In addition to abiotic factors there are suggestions that biotic factors may be involved in the decline syndrome. Systematic examinations of trees showing symptoms of decline for virus contamination are required before hypotheses can be developed on their role in forest decline.

This paper describes efforts to detect viruses in beech trees affected by forest decline. Besides the loss of foliar biomass and abnormal branching in diseased trees, the following foliar symptoms have been observed: smaller and chlorotic leaf tissue, leaf curling, chlorotic spotting and mottling, vein clearing, premature senescence and casting of leaves.

Crude sap from leaf samples of declining beech trees showing the described symptoms was inoculated onto leaf surfaces of Chenopodium quinoa, Nicotiana benthamiana and Nicotiana tabacum var. Xanthi-nc plants. In these transmission studies six viruses could be detected (2). The viruses were transmitted to different diagnostic species and propagated in suitable host plants.

Electron microscopic examinations of drops of leaf extracts negatively stained with 2% aqueous uranyl acetate showed the presence of threadlike particles. They were tentatively grouped according to their particle morphology into the Potyvirus and Potexvirus groups. In two cases spherical particles could be demonstrated by electron-microscopy. All viruses were propagated and extraction procedures were optimized for virus purification. The viruses of spherical particle morphology (R1 and R5), propagated in Chenopodium quinoa plants were homogenized in 0.5M boric acid buffer (pH 6.7), frozen, thawed and treated with granular ammonium sulphate. Leaves of Nicotiana tabacum var. Xanthi-nc plants systemically infected with the Potexvirus (RX) were homogenized in 0.5M citrate buffer (pH 7.2), blended with 5% (v/v) chloroform and clarified by low speed centrifugation. Latent infected Nicotiana benthamiana plants were used as propagation hosts for the Potyvirus (RF). All procedures used for purification resulted in low virus yields. Finally a modified extraction procedure of Koenig et al. (1) was used.

The clarified plant sap extracts were further purified and concentrated by differential centrifugation. The purification procedure was terminated by density gradient centrifugation through linear saccharose gradients that were subsequently analyzed photometrically and fractionated.

The threadlike particles sedimented as single bands whereas two RNA containing zones could be detected in density gradient profiles of the spherical particles (R1 and R5). The virus containing fractions were pooled, centrifuged to remove sugar and analyzed spectrophotometrically and electronmicroscopically. The partially purified virus preparations served as antigens for immunization procedures.

The immunized rabbits produced antisera which were tested in gel double-diffusion and microprecipitin tests against the homologous virus and against different viral antigens. In reverse tests purified virus was tested against antisera to different viruses. Antiserum against the spherical virus isolate R1 with an antibody titer of 1:512 showed reactions with Cherry leafroll virus (CLRV) and the antiserum against the virus R5 with an antibody titer of 1:1024 reacted with Brome mosaic virus (BMV). The results were confirmed in reverse tests of the purified virus preparations tested against an antiserum to a birch isolate of CLRV (obtained from Dr. M. L. Edwards, Oxford, UK) and an antiserum against BMV produced in our laboratory. The exact determinations of the degree of the serological relationship needed for the identity of the other virus isolates, is still being studied.

The Immunoglobulin G fractions were isolated and conjugated to alkaline phosphatase for ELISA-tests. The extraction buffer of beech leaf extracts was modified to reduce the interfering activity of phenolic compounds.

The double sandwich antibody form of ELISA was used to detect the viruses in swelling buds and leaves of beech trees in forests where the virus samples were first taken. ELISA failed to detect virus in buds, but strong positive reactions were obtained in 3 out of 100 samples examined for R1 (CLRV), in 5 out of 70 samples against R5 (BMV) and in 3 out of 100 samples for the Potexvirus isolate (RX).

Stem cuttings of the beech trees whose leaves showed positive reactions were grafted onto 2-3 year old beech seedlings. The results of back transmission experiments of the virus isolates to young greenhouse grown beech seedlings are still pending.

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ANALYSIS OF PSEUDORECOMBINANTS OF TWO STRAINS OF CUCUMBER MOSAIC VIRUS DIFFERING IN SYMPTOM EXPRESSION AND APHID TRANSMISSIBILITY IN SQUASH

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Two strains of CMV (nominally referred to as "fast" = F or "slow" = S based upon differing rates of virus spread) were originally isolated from muskmelon and were maintained in either muskmelon or summer squash. Previous studies indicated a strong correlation in percent virus transmission for the two strains as measured by the use of single Aphis gossypii transfers and spectrophotometric ELISA readings over a 7-wk period (1). One explanation for the difference in spread observed in the field might be due to a rapid increase in virus titer for CMV-F early in the growth of plants.

In the present study, we wanted to analyze these two strains for potential genetic differences. Our overall objectives were: 1) to prepare pseudorecombinants between the mild and virulent strains; and 2) to analyze the reaction and aphid transmissibility of the recombinants on squash.

Pseudorecombinants of CMV-F and CMV-S were prepared by isolation of genomic RNAs 1, 2, and 3 using a combination of polyacrylamide gel electrophoresis and sucrose density gradient centrifugation. All possible combinations of genomic RNAs for CMV-F and CMV-S were prepared and assayed on Chenopodium quinoa. Twenty single lesions per combination were chosen for subsequent assay to each of three summer squash plants (60 plants total). Symptoms were read 9 days later using the scale of 0 = no visible symptoms to 5 = severe cupping of leaves and strong mosaic with eventual plant death in some cases. These ratings and subsequent selection of representative plant samples from each of the eight plant groups were made without knowledge of the genomic makeup. When the average symptom rating for each group was calculated, it revealed that plants in groups 1, 2, 3 and 4 had overall the most severe symptom ratings (averages ranged from 3.3 to 3.8), while plants in groups 5, 6, 7, and 8 had less severe symptoms (averages ranged from 2.4 to 3.6). Examination of the genomic code for the two groupings revealed that RNA 1 from CMV-F was present in plants of groups 1, 2, 3, and 4, while RNA 1 from CMV-S was present in plants of groups 5, 6, 7, and 8. These preliminary results suggest that RNA 1 conferred more severe symptom expression for the time period plants were observed.

To determine if the initial symptom ratings for the eight groupings were reproducible, six plant tissue samples representing the average range of symptoms for each of the eight groups (6 samples x 8 groups) were tested individually on 20 zucchini summer squash plants in controlled environment chambers (24°C day/18°C night with 14 hr illumination). The zucchini plants were inoculated mechanically in the cotyledonary stage and were examined daily for 10 days for time of symptom occurrence and final symptom ratings. To serve as controls for

each test, eight plants were inoculated with CMV-F and eight with CMV-S. Results are shown in Table 1.

Analysis of pseudorecombinants showed that RNAs 1 and 3 from CMV-F conditioned for consistent severe symptoms which is typical for those caused by CMV-F. On the other hand, RNAs 1 and 3 from CMV-S conditioned for symptoms consistent with those produced by CMV-S.

In order to test the effect various RNA combinations would have on aphid transmission, a single representative sample was selected for groups 2, 3, 6, and 7 (Table 1). Squash source plants were mechanically inoculated in the cotyledonary stage and then assayed for virus at weekly intervals for 3 wk using one A. gossypii per 20 squash test plants. All plants were grown in growth chambers using conditions identical to the previous studies. Percent transmission was greatest for the sample from group 3 (avg. 60%) intermediate for samples from group 2 (20%), and lowest for groups 6 and 7 (5 and 7%, respectively).

It was apparent that RNAs from CMV-F in positions 1 and 3 were required to achieve maximum transmission of the virus (group 3) and intermediate when RNA 1 from CMV-F was present in position 1. Lower transmissibilities occurred for other combinations.

Previous studies using aphid-transmissible and non-aphid-transmissible strains of CMV suggested that coat protein coded by RNA 3 enhanced overall transmissibility (2). Mossop and Francki (3) in earlier studies with two additional strains of CMV also demonstrated that genomic RNA 3, which specified viral coat and one other protein, also determined the ability of CMV to be transmitted by aphids. They also suggested plants could play a role in virus acquisition and transmission. In the present study, aphid transmissibility depends more on the relative levels of virus present in the leaves (controlled by RNA 1) than on inherent aphid transmissibility alone, although RNA 3 can be a contributing factor.

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Table 1. Influence of genomic composition on time and severity of symptom expression for eight RNA groupings.

Group	Genome	Avg. no. days for 100% infection[a]			Avg. symptom rating
		Cotyledon	1st leaf	2nd leaf	
1	1F+2F+3F	5	10	10	4.5
2	1F+2F+3S	10	10+	10+	2.8
3	1F+2S+3F	4	10	10+	4.3
4	1F+2S+3S	10+	10+	10+	2.1
5	1S+2S+3S	10+	10+	10+	1.0
6	1S+2S+3F	10+	10+	10+	2.1
7	1S+2F+3S	10+	10+	10+	0.7
8	1S+2F+3F	6	10+	10+	2.1
CMV-F	1F+2F+3F	4	7	9	4.3
CMV-S	1S+2S+3S	4	10+	10+	2.0

[a]Based upon 20 plants per test with 6 replications for the 8 groups and 8 plants per test for the standards.

SUMMARY: INTEGRATED CONTROL MEASURES

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Plant virus diseases are seldom easy to control and many different approaches have been adopted in attempts to develop effective procedures for restricting or delaying spread or to decrease the damage sustained after infection has occurred. Nevertheless, many diseases remain difficult or expensive to control and in some instances effective measures are not yet available. Particular difficulties have been experienced with non-persistent aphid-borne viruses of high-value horticultural crops, and whitefly-borne-viruses are currently causing serious problems in many tropical and sub-tropical countries.

It is seldom possible to rely on a single control measure, and standard recommendations usually involve a combination of procedures, each of which is of limited effectiveness when adopted alone. This has long been apparent from early work on such diseases as sugarbeet curly top in the southwestern part of the United States and ground-nut rosette in Africa. Similarly with aphid-borne viruses of sugarbeet, potato and brassica crops in Europe.

One important consequence is that special difficulties are encountered in evaluating potential control measures and in developing an appropriate combination. Field experiments and screening tests of varieties or chemicals are frequently carried out under conditions of high 'infection pressure' using large amounts of inoculum or numerous potent sources of infection planted early. However, under such stringent conditions partial resistance or other possible control measures can appear inadequate and may be completely overwhelmed.

Virus incidence is usually recorded on an arithmatic scale as a proportion or percentage of the total stand and yet increasing amounts of inoculum are required to achieve each successive increment in disease incidence. Thus a 10% decrease in disease incidence from 90% to 80% is more difficult to achieve than a decrease from 50% to 40% and far more difficult than a decrease from 20% to 10%. Such consequences of 'multiple infection' are not always appreciated in designing experiments and in evaluating the results obtained.

Another difficulty in field experimentation is that trials become large, complex, expensive and difficult to analyze if they are to consider a range of possible control measures applied singly and in fractional combination. The problems become even greater if economic criteria are to be considered in evaluating the cost/benefit implications of the measures being tried. Few virologists have the expertise to carry out such evaluations and yet they are essential if growers and control agencies are to be given adequate guidance and if they are to be convinced of the effectiveness of the measures being advocated. One consequence is that some of the standard recommendations which appear in the literature and in advisory leaflets are naive and totally

unrealistic, and it is hardly surprising that they are not adopted or soon discarded.

It is equally simplistic to rely unduly on a single control measure even if one is found to be suitably effective. There is abundant evidence that the performance of control measures is influenced by the overall infection pressure encountered and that the effectiveness of resistant varieties and pesticides is increased and their life extended if they are deployed in ways that decrease the risk of spread. This means the use of healthy planting material, the careful selection of sites and orientation, and the adoption of suitable planting date, spacing, and cultural practices. Virologists have for long had to utilize such procedures to achieve at least some degree of control and this is likely to be the situation for many years to come. Hence the continuing importance of holistic ecological studies to provide the epidemiological information or which to base an effective control strategy.