Dossier

Maize streak, maize stripe and maize mosaic virus diseases in the tropics (Africa and islands in the Indian Ocean)

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Photos D. Debert:
Bagging maize inflorescences.
Maize fields in Burkina Faso.
Photo B. Reynaud: *Peregrinus maidis*.

- Impact of maize virus diseases and current research
- Vectors and epidemiology
- Maize virus diagnosis and maize streak virus variability
- Maize resistance and breeding

Impact of maize virus diseases and current research

Regions

The three main tropical maize viruses are: maize streak virus (MSV), maize stripe virus (MStpV) and maize mosaic virus (MMV). Their impacts vary markedly between countries (Figures 1 & 2).

MSV was first detected in South Africa at the beginning of the century, and similar virus diseases have been described in sugarcane and in many wild Poaceae species. It is transmitted by homopteran insects of the genus *Cicadulina (Homoptera, Cicadellidae).* MSV infections have been noted in many different countries, with varied incidence. It is endemic to all East African countries, Madagascar, Réunion, and central Africa. The disease is also sometimes found in West Africa, particularly in Mali, Togo, Côte d'Ivoire and Senegal. In West Africa, there was a widespread outbreak of MSV in 1983-1984, sometimes completely destroying all maize crops. This disease also occurs in India in wheat and millet, but it has not been detected in the South American tropics.

MStpV is not as common. *Peregrinus maidis* Ashmead *(Homoptera, Delphacidae),* the insect vector, is present in many African countries (Côte d'Ivoire, Zaire, Nigeria, Burkina Faso and Cameroon). Since 1936, this insect has been reported in East Africa, Tanzania, Kenya, Mauritius and Réunion, but MStpV has caused very little damage. The virus was recently identified in West Africa (Côte d'Ivoire, Togo, Nigeria, Burkina Faso and Cameroon). However, it seems to have had a greater impact in Latin America, particularly Venezuela and the West Indies. It also occurs in the Philippines and Australia.

MMV, first discovered in 1921 in Hawaii, is also transmitted by *P. maidis.* It was later detected in Cuba, Guadeloupe, Surinam, Venezuela, Guyana, Puerto Rico, Burkina Faso, Côte d'Ivoire, Nigeria, Mozambique, Tanzania, Kenya, Réunion, Mauritius and India.

Evolution of host plant/vector/virus complexes

P. maidis and the viruses it transmits evolved together in a few plant species. Maize is the favoured host of this vector/virus complex; the original host was teosinte or sorghum. The close host plant/vector relationship perpetuates the complex with the virus, despite the narrow range of host plants and susceptible species.

The evolution of MSV, transmitted to maize by *Cicadulina mbila* Naude, is different: the virus was revealed in maize crops, but also infects wild grasses through the leafhopper vector, which is common in Africa and has a wide host range.

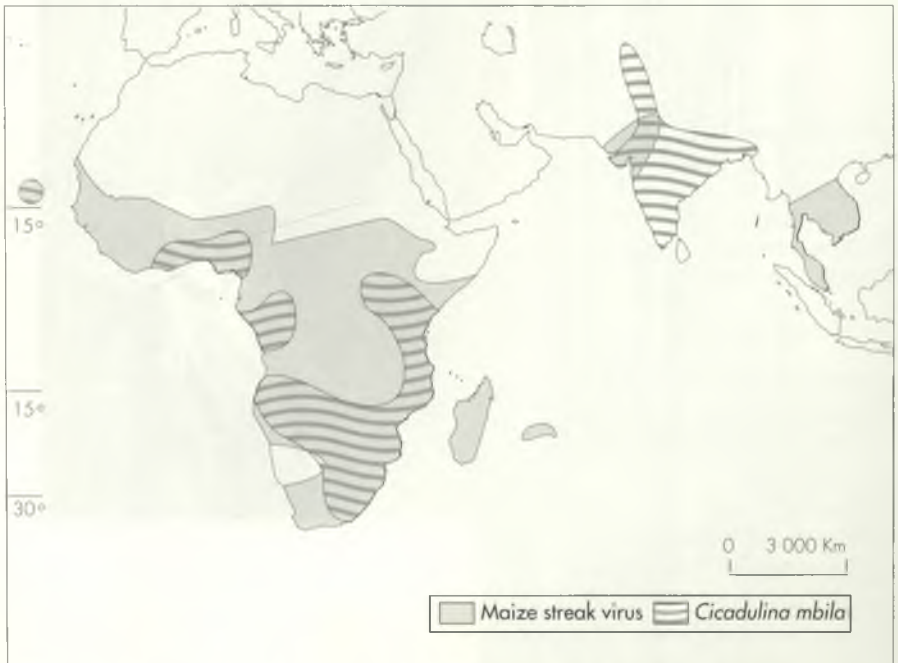


Figure 1. Geographical distribution of maize streak virus and its main vector *Cicadulina mbila* in tropical regions.

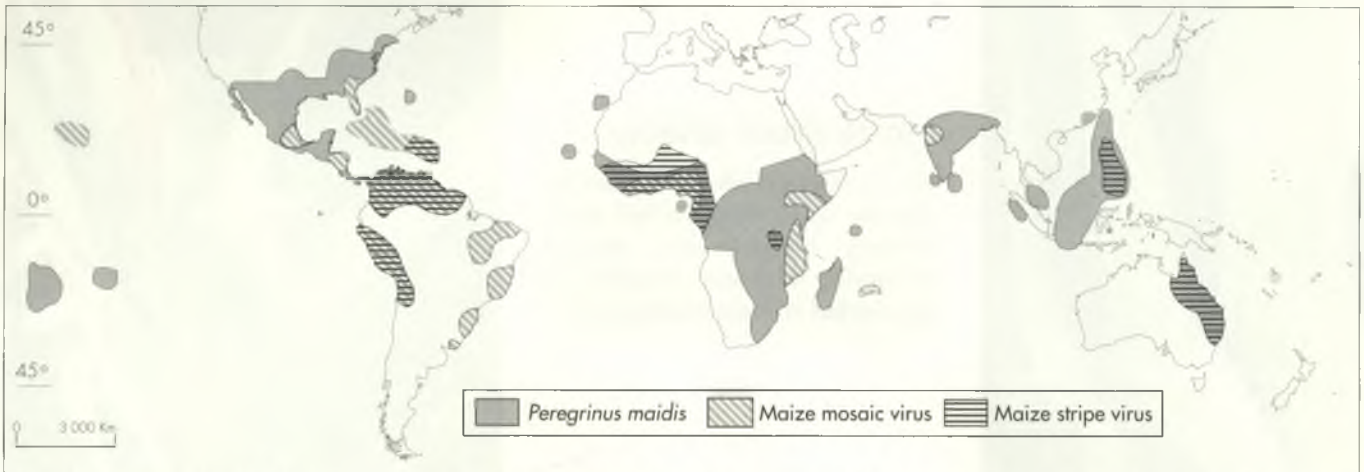


Figure 2. Geographical distribution of maize mosaic virus and maize stripe virus and their vector *Peregrinus maidis* in tropical regions.

Organizations involved

CIMMYT, Centro Internacional de Mejoramiento de Maíz y Trigo, Mexico

CIRAD, Centre de coopération internationale en recherche agronomique pour le développement, France

CORAF, Conférence des responsables de la recherche agronomique africains

IRA, Institut de la recherche agronomique, Cameroon

INCV, Institut national des cultures vivrières, Togo

INERA, Institut national d'études rurales et agricoles, Burkina Faso

IITA, International Institute of Tropical Agriculture, Nigeria

John Innes Institute, Norwich, UK

The incidence of MMV is generally low in all of these countries. Nevertheless, serious MMV epidemics have been reported in some regions of maize growing countries such as USA (Hawaii, Florida), Brazil and Mexico.

Control

Virus-resistant varieties of maize are of considerable economic interest due to the extent of damage caused by maize viruses in the tropics (Africa, South America) and the lack of efficient agronomical and chemical control techniques. Higher and more regular maize yields could be obtained with such varieties.

Maize breeding studies began in the 1930s in East and South Africa with the aim of obtaining MSV resistance. Moreover, the 1972 MSV epidemic that occurred in West Africa prompted certain research organizations (IITA and CIMMYT) to conduct similar studies.

During the 1970s, CIRAD (in collaboration with African and European partners) focused research on this topic in order to deal with the serious maize virus epidemic problems — the three main maize viruses and their vectors are present including local resistant varieties.

The research and who is involved

Since the 1980s, CIRAD and partners have been focusing on the following topics:

– insect vectors and their epidemiology;

– maize virus diagnosis;

– breeding and investigating the genetic factors determining resistances, and transferring them into susceptible maize genotypes.

These investigations revealed the vectors and factors involved in triggering and spreading viral epidemics in maize crops and led to the development of specific maize virus diagnosis techniques. Resistant ecotypes in Réunion have been used in resistance transfers to create maize varieties that will be more efficient under cropping conditions found in Africa and throughout the Indian Ocean region. The developed diagnostic techniques can be applied at a variety of sites, without special equipment, to identify viruses and assess varietal susceptibility.

CORAF, during the general assembly meeting of its maize network in Yaoundé (Cameroon) in 1987, decided to launch an MSV research project because of the urgency of the situation. For resistance transfers, national CORAF correspondents chose a number of varieties from among already (or soon to be) widely grown maize varieties.

This multidisciplinary project brings together virologists, entomologists and geneticist/breeders:

– virus studies by John Innes Institute, Norwich (UK) and CIRAD, Montpellier (France);

– interactions between viral isolates and resistant genotypes in Réunion (CIRAD) and Burkina Faso (INERA);

– insect vectors in Réunion (CIRAD), Burkina Faso (INERA) and Cameroon (IRA);

– maize virus dynamics in host plants in the tropical phytovirology laboratory (CIRAD);

– resistance genetics in Réunion (MSV, MStpV, MMV) (CIRAD) and in Togo (MSV) (INCV);

– creation of resistant varieties in Réunion, Togo and Cameroon. ■

Symptoms

MSV leaf symptoms.

Maize streak disease

Maize streak is characterized by chlorotic spots along the leaf veins, forming discontinuous streaks of varying thickness. Dwarfism is very marked in young infected plants.

Dwarfed young plant.

Continuous chlorotic stripes caused by MMV.

Maize mosaic disease

Maize mosaic is characterized by regular continuous chlorotic lines running along the whole length of the leaf, parallel to the veins. The symptoms vary according to the thickness of these lines, their spacing and discontinuous appearance.

Limited growth of infected plants.

Chlorotic stripes on maize leaves due to MStpV.

Maize stripe disease

Maize stripe occurs in two forms. The first, simply called stripe, is characterized by chlorotic stripes of various widths along the leaves, and the apex is typically curved. The second form, called chlorotic stripe, is characterized by the formation of chlorotic stripes, and the leaves eventually become totally chlorotic, with the reappearance of thick green discontinuous stripes. Young infected plants present severe dwarfism, and then dry up and die.

Infected plants are dwarfed and dry up.

Photos P. Baudin and B. Reynaud

Vectors and epidemiology

As early as 1973, severe epidemics of several maize viruses were noted in Réunion. In 1985, three viruses were identified by serological techniques: MSV, MStpV and MMV. Insect vectors are required for their transmission. MSV is transmitted by at least eight different *Cicadulina* species. MStpV and MMV are transmitted by *Peregrinus maidis*. Research carried out to date has mainly focused on the Sudano-Sahelian region (particularly Burkina Faso) and Réunion.

Insect vectors

In Réunion, *Cicadulina mbila* is the main MSV vector even though this insect occurs in relatively small quantities on maize, the final host of the disease. It is a very active and efficient Poaceae-specific vector. *C. storeyi* is the next important MSV vector in Réunion.

In Burkina Faso, five *Cicadulina* species have been identified in all ecological zones: *C. mbila, C. similis, C. arachidis, C. triangula* and, less commonly, *C. hartmansi*. The virus transmission potential varies in the different leafhopper species, with *C. mbila* being the most efficient and *C. arachidis* the least.

P. maidis is a sedentary vector that is specific to maize and sorghum which it colonizes in relatively high densities. Its maize virus transmission efficiency is intrinsically low.

MSV host plants and vector epidemiology in the Sudano-Sahelian region

A detailed study, representative of Sudano-Sahelian regions, was set up in Burkina Faso in 1984.

Highly MSV-sensitive maize cultivars (Safita II and Jaune flint de Saria) were used for all tests of virus transmission with the leafhopper *C. triangula*. The serological ELISA (enzyme-linked immunosorbent assay) technique was used to detect MSV in collected samples.

Virus reservoir plants

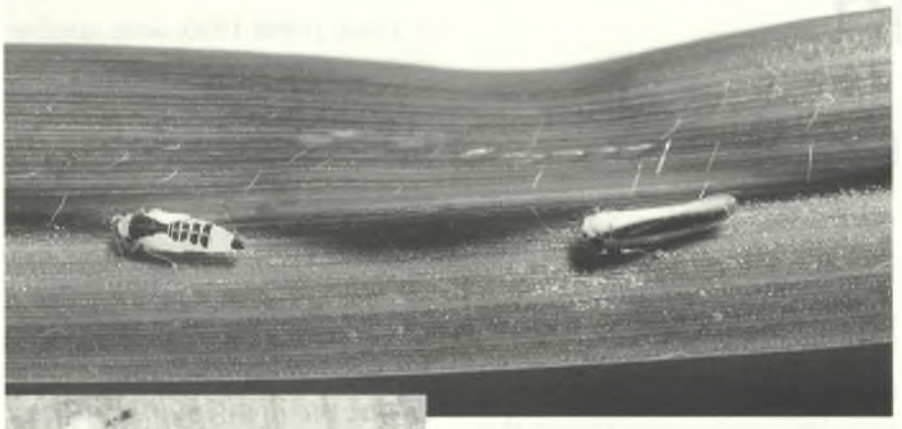
A 3-year (1988-1990) wide ranging survey of herbaceous plants was conducted in Burkina Faso, resulting in the detection of 41 Poaceae species with MSV symptoms. ELISA tests revealed the presence of the virus in 25 species.

Analysis of the spatiotemporal distribution of virus reservoirs (host plants) highlighted three main points: reservoirs were greater in maize crop zones; the incidence of MSV in maize fields was proportional to the number of reservoirs; and there were very few infectious reservoirs in May, with an increase 3-4 weeks after the onset of the rains to reach a peak in August. In Burkina Faso, the incidence of MSV is generally 6% or less in maize plants presenting virus symptoms; it increases to 30-40% during severe epidemics.
In Sudano-Sahelian regions, the risk of early infection (often severe) could be minimized by early sowing (mid-May to mid-June), during a period when the inoculum source is minimal. However, this recommendation cannot always be met on account of the cotton and rice cropping calendar.

The importance of climatic factors

In Burkina Faso, 10 years of agroclimatic studies demonstrated the effects of climate on MSV incidence, in order of importance: relative humidity in February; relative humidity in January; and temperatures in May.

In January, at the beginning of the dry season, insect densities are high and they are obliged to migrate to refuge areas (sites with permanent water supplies, irrigated crops) because of the lack of host plants. The relative air humidity decreases through January and February, which can affect insect breeding and survival and lead to high mortality. The number of MSV vectors available in the following rainy season is determined by the size of



Cicadulina mbila nymph and adult.

Peregrinus maidis adult.

Photos B. Reynaud

the residual insect population, which in turn determines the extent of MSV development. May precipitation was found to be very important in the MSV epidemics of 1983-84. In Zimbabwe, March-June precipitation is also a factor that determines *Cicadulina* spp. population densities from July to September, and thus MSV infection rates. Moreover, in Zimbabwe and Réunion, the rising temperatures during the southern hot season promotes development of vector populations.

Impact of three maize virus diseases in Réunion

In Réunion, the population dynamics of maize virus insect vectors were studied by sowing maize on a bimonthly basis from 1983 to 1985, and on a weekly basis from 1985 to 1988.

The virus symptom observations and vector trapping results for lowland areas in Réunion indicated that epidemics of the three virus diseases differ in terms of their locations, extents and seasonal patterns. At low elevations, MSV causes very high to total maize losses. It is the most common maize virus disease during the hot season, when temperatures are optimal for *C. mbila* and precipitation favours development of Poaceae hosts. With hot temperatures, there is a higher risk of epidemics of MSV than MStpV, with a reversal of the situation at the end of the dry season. The cooler weather is more suitable for *P. maidis* than *C. mbila*.

P. maidis can survive at more moderate temperatures, which means that epidemics can occur at high elevations. Since *P. maidis* generally infests maize, areas where this crop is grown intensively are seriously affected by MStpV.

The MMV infection rate of maize is not very high in irrigated lowland zones. However, it should be noted that the symptom-based technique for evaluating viral infection levels in maize plants often underestimates MMV incidence; this is particularly true when there are secondary MSV and MStpV infections, since these two virus diseases mask MMV symptoms.

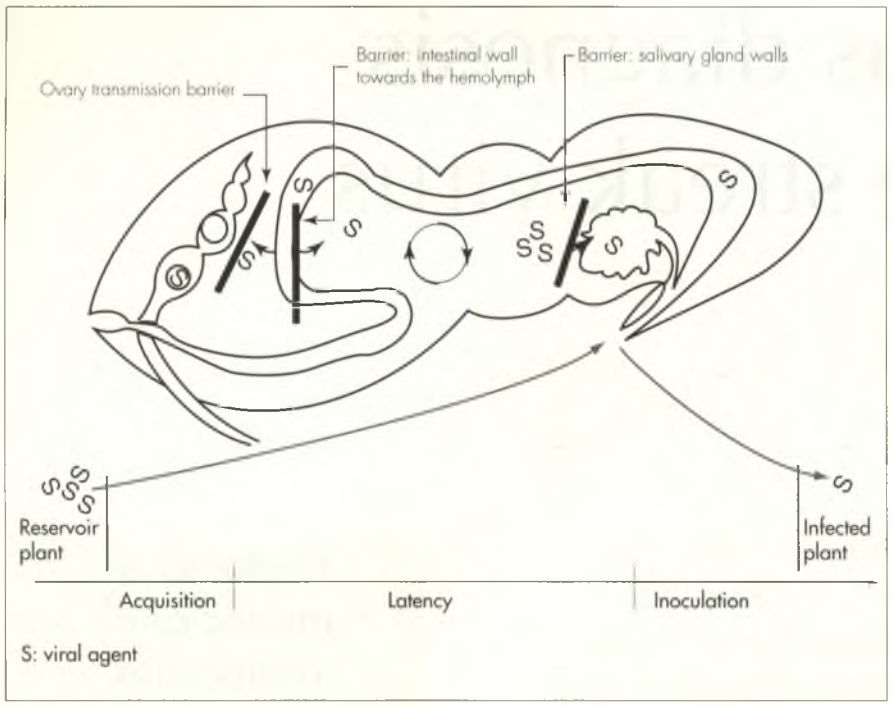
Efficiency of virus transmission to the insect vector

The intrinsic transmission ability expresses the specificity of the infectious agent/vector relationship.

With persistent viruses, the cycle of virus transmission to the insect occurs in the following steps:
– acquisition access period, when the insect acquires the virus after feeding on an infected plant;
– latent period in the insect, or time required for the insect to become infective;
– inoculation access period, when the plant is contaminated through an infective insect puncture.

Moreover, there is a virus retention period in the insect, during which the vector remains infective.

The cycle of MSV transmission to the vector is short, i.e. 24 h or less; those of MStpV and MMV are about 15 days.



Virus acquisition can be followed by a latency period and the inoculation. Depending on the virus and insect vector, various transmission barriers have been identified. In different insect organs, some cell membranes can act as barriers or as pathways linked to a specific virus recognition mechanism.

Figure 3. Diagram of the infection process in the insect vector (from REYNAUD, 1988).

Transmission studies revealed that MSV, MStpV and MMV have characteristic circulative-type virus patterns, with a latency period in the insect, maintenance of the infective potential during moulting, presence of the virus in the insect and inoculation of the plant with insect saliva (Figure 3). Very little is known about the latency period in the insect — it includes the transit time of the virus through the insect's body and its uptake in the salivary glands to a specific concentration threshold. Percentages of infective insects in a population are determined to assess vector transmission efficiencies. Captures and tests of different leafhopper populations showed that 50% (on average) of *C. mbila* insects in a population are MSV vectors, while 20% of *P. maidis* insects are MStpV and MMV vectors.

MSV transmission by *C. mbila*

In Réunion, *C. mbila* is an excellent MSV vector with a high intrinsic transmission capacity (above 50%), which corresponds to levels noted in populations studied in South Africa and Burkina Faso.

No MSV multiplication in the insect was noted since, after a short acquisition access period, there was an overall reduction in transmission rates and viral concentrations. This insect vector is efficient because of its rapid uptake of the virus during acquisition and the low viral concentrations required during inoculation. The virus takes a long time to disappear in the insect, thus explaining its excellent long-standing infective potential.

MSV could be considered as a circulative non-propagative virus.

MStpV transmission by *P. maidis*

P. maidis populations have a relatively low MStpV transmission capacity, i.e. about 20% in Réunion, which is quite close to levels noted in other regions.

MStpV transmission by *P. maidis* is relatively inefficient. Transmission barriers have been detected: the first involves hindering penetration of the virus through the intestinal wall during acquisition feeding, and secondly through the salivary gland walls. Some females can transmit the virus to their offspring through their ovaries. The virus can multiply in most of these insects from the egg stage onwards.

For artificial infestations (in varietal screening tests for resistance), it is now possible to select for the alimentary and ovarian acquisition ability. Populations of vectors with a high intrinsic transmission ability can thus be obtained within a few generations.

MMV transmission by *P. maidis*

In Réunion, *P. maidis* has a low MMV intrinsic transmission capacity, i.e. about 20% infectious insects. Two transmission barriers, similar to those described for MStpV transmission, were found. ■

Maize virus diagnosis and maize streak virus variability

Symptomatic diagnosis of virus diseases is often imprecise and should be confirmed with various serological tests or electronic microscopy. This is especially true for early stages of infection when diagnoses could be faulty if not confirmed by reliable serological techniques. ELISA (enzyme-linked immunosorbent assay) techniques were used to obtain clear diagnoses of maize virus diseases present in maize selection plots in Réunion. MSV, MStpV and MMV symptoms can be characteristic of each of the three viruses at relatively advanced stages of the disease, but the appearances sometimes vary, especially for MMV. Secondary infections of several viruses can also occur.

Virus purification and diagnostic techniques

The three maize viruses (MSV, MStpV and MMV) have been purified. The extracts were injected into rabbits to produce specific immunoglobulins.

ELISA tests were developed for maize virus diagnosis. These quick simple tests are suitable for routine virus disease diagnosis.

These tests helped to pinpoint MSV in eight African countries. The presence of MStpV and MMV has rarely been proven in Africa, but they were recently detected in Côte d'Ivoire, Mali and Burkina Faso. Conventional ELISA tests can only be carried out in the laboratory. Nevertheless, CIRAD (Montpellier, France) has developed a modified ELISA test for detections of all three viruses on nitrocellulose membranes, without requiring any laboratory equipment. This new technique will facilitate studies on geographical distributions of these viruses and help in determining specific requirements for breeding resistant varieties (Figure 4).

MSV variability

Virus isolates that induce streak leaf symptoms have been described in several grasses, including maize. Initially, on the basis of biological tests, serological techniques (with monoclonal antibodies) and electronic microscopy, the isolates were all considered to be MSV strains. Later, nucleotide sequence analyses of the viral genomes revealed that some of these strains are actually distinct viruses.

In 1986 at CIRAD, serological tests on streak viruses of grasses led to the characterization of three different serotypes.

Features of monoclonal antibodies

Antiserum prepared against a given virus will naturally contain a set of antibodies that recognize several capsid protein sites in the viral particle. In contrast, a monoclonal antibody only recognizes one capsid protein site, thus enabling detection of slight variations in such proteins. Monoclonal antibodies are produced through fusion of lymphocyte cells and myeloma cells. These hybrid cells (hybridoma) have the perpetual traits of myeloma cells along with the ability of lymphocyte cells to produce single antibodies. CIRAD, in collaboration with the Institut de biologie moléculaire et cellulaire (Strasbourg, France), have developed five monoclonal antibodies that recognize MSV.

Virus diagnosis kit

A kit was developed for serological field diagnosis of viruses using nitrocellulose membranes. It can now be purchased (from CIRAD) by anyone wanting to serologically confirm a visual diagnosis.

This diagnosis kit is very easy to use:

– the test kit contains everything required for the analysis;

– it is very compact;

– the test can be stopped after plant extracts are deposited on the membrane, the membrane just has to be dried. In this form, the test can be completed within the next 20 days;

– these dried untested membranes can be sent by mail in a simple envelope and tested later.

For instance, someone not wanting to spend the time required to perform the serological tests can simply deposit plant extracts on the membranes and send them to a correspondent for analysis.

If, on the other hand, the user also wants to conduct the analysis, everything required for the testing and interpretation is supplied in the kits, i.e. unrevealed membranes and positive/negative controls.

Samples from Zimbabwe and Réunion were tested in various different ways to check the validity of kit for field analyses:

– tests performed completely at the sampling site;

– plant extracts deposited at the sampling sites and membranes analysed elsewhere;

– tests performed completely at CIRAD (Montpellier, France) with leaves sent from Réunion and Zimbabwe.

The results showed that this virus diagnosis kit is highly reliable and valid.

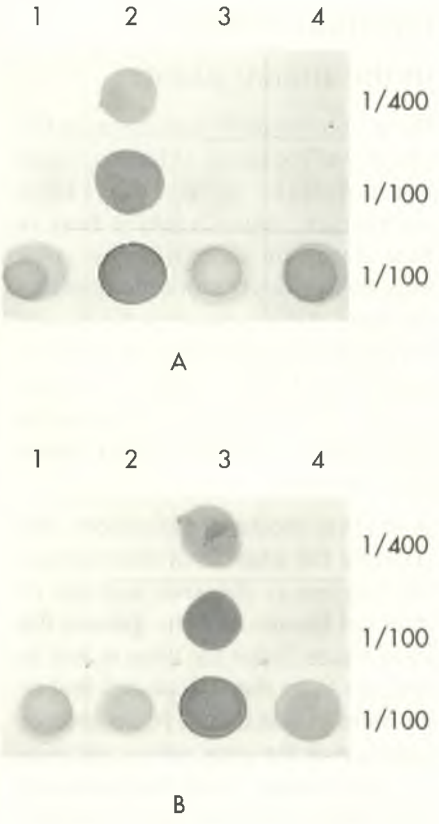


Figure 4. Immunoenzyme detection of MMV (A) and MSptV (B) using ELISA from four crude extracts of maize from Réunion, infected by MSV (1), MMV (2), MStpV (3) and uninfected (4). Each plant extract was tested at dilutions of 1/10, 1/100 and 1/400 (leaf weight/buffer volume).
Photo M. Peterschmitt

Sugarcane, *Digitaria* sp. and *Setaria* sp. isolates could thus be distinguished from various maize and Poaceae isolates belonging to the same serotype.

The gene sequences also confirmed these distinctions with respect to other isolates — even indicating that sugarcane and *Digitaria* sp. isolates are distinct streak viruses that have been called sugarcane streak virus (SSV) and *Digitaria* streak virus (DSV), respectively. Similarly, a *Panicum* sp. isolate was reclassified, i.e. after it was initially identified as an MSV isolate, it is now considered as a distinct virus called *Panicum* streak virus. Further viruses could thus be distinguished in the future with other isolates. *Setaria* sp. isolates have not yet been analysed in detail, only serological tests have been carried out. In summary, the serological techniques used in Réunion did not enable serotype differentiation in MSV isolates, or in other maize isolates even though they originated from 11 different countries, or in wild Poaceae isolates — some of which showed different virulence when tested on maize. The serological technique was found to be unsuitable in several locations, particularly Réunion, for distinguishing isolates with different pathogenicities.

Studies carried out in the Sudano-Sahelian zone confirmed the presence of one dominant serotype in maize crops. In more than 300 maize isolates analysed, 99% showed a characteristic serological maize profile (which was previously determined by CIRAD). However, half of about 100 isolates tested were found to contain both the maize serotype and another serotype that had been identified in wild Poaceae species. This modifies previous conclusions, indicating that major serotype in maize can occur in the association with other strains whose serological profiles and pathogenicities might be masked by the maize serotype. As in other locations, it is likely that in the Sudano-Sahelian region, where the maize serotype and two others have been identified, molecular analyses will reveal the presence of streak viruses of grasses that differ from MSV.

MSV resistance mechanisms in maize

Various mechanisms can be involved in a plant's virus resistance. Such mechanisms were compared in the resistant maize variety IRAT 297 and the susceptible variety INRA 508.

Location of MSV in the maize plant

Virus colonization processes in the plant (MSV capsid antigens) have been defined using the ELISA technique. After a plant leaf is inoculated by *C. mbila*, the virus migrates through phloem canals to the stem. MSV colonizes the leaves through young dividing cells in which viral DNA replication can occur. Virus spread can only be detected in tissues that form after infection.

With this mode of infection, the younger the plant is at inoculation, the higher is the percentage of infected tissues and the greater the yield losses. Since the virus is able to migrate from the inoculated leaf to the stem in less than 2 h, no prepropagation of the virus seems necessary. Leafhopper virus transmission tests showed that the virus can only be acquired from leaves showing symptoms.

Resistance mechanisms

Analysis of virus distributions in maize plants indicated identical distributions in the susceptible variety (INRA 508) and in a resistant variety (IRAT 297). On the other hand, a viral concentration difference between these varieties was detected by measuring virus nucleoproteins using the ELISA technique, i.e. these nucleoproteins were 10- to 90-fold more concentrated in INRA 508 than in IRAT 297. These results indicated that virus resistance in cv IRAT 297 involves mechanisms that hinder viral propagation rather than blocking migration of the virus.

The lower viral propagation in IRAT 297 is thus responsible for reduced severity of the initial infection; this is shown by less severe symptoms and a longer period before symptoms appear as compared to the susceptible variety (in comparative tests, 5 days *versus* 3 days, respectively). Secondary infections are less severe in resistant plants since they are a poor inoculum source. ■

Maize resistance and breeding

The main aim of the research carried out in Réunion is to transfer the resistance of some local maize varieties to three maize viruses (MSV, MStpV and MMV) into other susceptible varieties of high agronomic interest. MSV-resistance transfers are also under way in Togo, and very recently in Cameroon. This breeding work with varieties that are already being grown by farmers, or promising ones, interests many African CORAF-member countries, and also countries in Latin America and the West Indies.

Resistance sources found in Réunion

In Réunion, resistance was discovered in local maize varieties, especially in coastal varieties. This adaptation was acquired through natural selection. In 1974, the Réunion variety "Revolution" was found to be MSV resistant in Benin. This was confirmed in several countries, i.e. in Kenya in 1976, USA in 1983 and Nigeria in 1982.

About 100 ecotypes were collected in a 1979-80 survey conducted in Réunion and Rodrigues. They were tested under natural infestation conditions of MSV, MStpV and MMV, and 41 of them were found to be resistant to maize viruses. This resistance was later confirmed under artificial infestation conditions.

These ecotypes were polycrossed to produce the "Composite viroses résistant" (CVR), which was further improved through three recurrent breeding cycles, and then used as a resistance donor in transfers.

Overall, more than 800 maize varieties from tropical and temperate regions have been screened in Réunion under natural virus infection conditions. None of them are sufficiently resistant for cultivation in Réunion, except for ecotypes from Rodrigues which are genetically similar to those found in Réunion. One long-term objective is to improve yields and agronomic qualities in local maize varieties. The main problem is that these varieties have low yield potentials and they are all early producers. They have a poor improvement potential because of their very narrow genetic bases. Hence, resistance transfers would be quicker and more efficient, and enable creation of a wide range of resistant varieties from already improved plant material of high agronomic value.

Preliminary studies

The following four conditions had to be met to be able to transfer resistance to these three viruses

through backcrossing:
– pinpoint resistance in germplasm of the Indian Ocean islands, and select the most resistant populations to polycross them in a "Composite virose résistant" (CVR);
– carry out mass rearing of insect vectors to be used for inoculations. *C. mbila*, present in Réunion, is considered to be the main MSV vector and was thus chosen as vector for the varietal improvement programme. *P. maidis* is the only known vector of MStpV and MMV, which is also present in Réunion;
– develop a system to screen plants for virus resistance, through artificial infestation techniques using *C. mbila* (currently being perfected for *P. maidis*). A resistance scoring scale is established;
– serological tests were developed to check, when necessary, whether the transmitted virus is the right one.

Preliminary studies demonstrated that resistance to MSV from Réunion ecotypes was high in many countries, and that the Réunion MSV strain was especially virulent.

MSV resistance research

C. mbila is easy to breed and artificial infestation can be carried out without difficulty. Research on MSV is therefore more advanced than on MMV and MStpV in Réunion.

This virus is also mainly found in Africa, thus explaining the development of MSV resistance transfer programmes in Togo, and more recently in Cameroon.

CVR improvement

Since preliminary tests revealed high internal variability in resistance, recurrent selection work was undertaken to improve CVR, first under natural viral MSV pressure (cycle 1, CVR-C1), then under artificial infestation (cycles 2 and 3, CVR-C3). This markedly increased the level of MSV resistance in CVR.

Rating

Virus symptoms are graded visually on a semi-quantitative 0-5 scale, while taking the seriousness of the symptoms into account.
Plant symptom scoring:
0 - the plant shows no symptoms;
1 - a few chlorotic spots are detected on the plant during a detailed inspection;
2 - slight streaks are clearly visible on the plant;
3 - moderate streaks are visible;
4 - the plant presents serious streaking with dwarfism;
5 - the plant presents very serious virus infection with highly marked dwarfism.
The symptoms do not change after 35 days postinfection. Symptoms are thus scored and plants for resistance transfers are chosen between this stage and flowering. Overall scores can be attributed to families or varieties on the basis of these individual plant scores. For instance, in tests carried out in Réunion, it is considered that plants with scores of 2 or less, contrary to those scored 3-5, will not produce lower yields because of the presence of the virus. The overall scores take this threshold into account, e.g. a variety with no plants scored 3 or higher is attributed an overall score of 0 and considered as a resistant variety. In contrast, a variety with all plants scored 5 is very susceptible and is allotted an overall score of 500. The overall score is calculated as follows:

$100 \times (3 \times N_3 + 4\ N_4 + 5 \times N_5) / \Sigma\ N_i$ (0 to 5)

where N_i = number of plants scored i (1, 2, 3, 4, 5).

Artificial infestation

Artificial infestations are essential for plant screening since the proportions of the three maize viruses can vary during the year: natural viral pressure is not homogeneous or constant enough for precise screening. These artificial infestations require highly productive mass rearing and active vectors to limit the number of insects that have to be produced. The percentage of infective insects is increased by lengthening the virus acquisition period on diseased plants and by breeding insects to enhance their transmission ability. Insects used for transfers of MSV resistance are obtained from a selected *C. mbila* population in which all insects are able to transmit the virus. These are placed on plants presenting very severe symptoms and left for 3 days for virus acquisition. Carbon dioxide anesthesized insects are set in the whorl of each plant. A plant's susceptibility decreases with age, which means that very early infestation is necessary. Infestations are carried out 10 days after sowing for practical reasons (at this point the plants have grown to a sufficient size for depositing the vectors). The infestations must be efficiently conducted to certify that all plants are infected. Experience has shown that complete infestation can be obtained by depositing three viruliferous leafhoppers on each plant.

Artificial field infestation with *C. mbila*.
Photo B. Reynaud

In 1986, variety CVR-C1 was registered under the name IRAT 297 with the Crop Science Society of America. Its MSV-resistance value was verified through comparisons of susceptible varieties with IRAT 297 top-crosses.

Genetic factors determining MSV resistance

Based on the improved CVR-C3 form, 500 S1 families were produced by selfing, and tested through artificial MSV infestation. The very low percentage of MSV symptom-free plants, the variability in the symptom scores in each family and a gauss distribution curve plotted for all families indicated that factors determining MSV resistance are complex and likely controlled by a polygenic system.

Nevertheless, a few S5 lines with very high levels of resistance (even complete resistance in one line) were obtained after four more selfing cycles. Genetic analysis of this material revealed a major system of very heritable complete resistance, with positive incomplete dominance, involving at least three different genes. Moreover, the presence of partial resistance was indicated during the breeding of some lines — but this cannot yet be attributed to a separate (probably polygenic) system or to partial expression of a major system.

Resistance transfers

MSV resistance transfers are performed through a series of conventional backcrossings.

In Réunion, for each screening, 5 000 F1 plants are sown and infected 10 days later by depositing three preinfected leafhoppers in the whorl of each plant. Resistant plants are chosen before flowering through the elimination of susceptible plants. The chosen plants (only 250-300) are intercrossed. The F1 and F2 generations of each breeding cycle are thus screened.

The same procedures are used in the research programme carried out in Togo, but the artificial infestation technique differs. Maize plants are sown in pots. Once the maize plant emerges, the pots are placed in cages containing infected leafhoppers. The plants are then planted out in the field. The chosen plants are selfed and then crossed again. The resistance screening is very efficient. Resistance is at least partially maintained after the first backcrossing (Figures 5 & 6). It is useful to begin screening at the F1 generation in order to discard as many recessive genes as possible from the outset.

The resistance transfer procedure is limited to a single initial cross with the donor and then two backcrossing cycles. The final variety therefore contains the resistance trait and 90% of the genes of the receiving variety.

Selfing is necessary to recover the colour and grain-type of the receiving variety — when they differ from those of CVR. This was done for each screening in the Togo trials. In Réunion, selfing was carried out after the second backcrossing cycle.

Current results

Presently, five varieties from the backcrossing programme in Réunion, i.e. CN7, Tiémantié, Pool 16 SR, IRAT 171 and Suwan 8331, are considered to be modified after two backcrossing cycles and three selfing cycles. In Réunion, they have been found to possess excellent resistance. Most plants showed no symptoms under severe artificial infestation conditions with a highly virulent virus isolate.

Breeding techniques

The three main maize breeding techniques are based on certain features in this plant's floral biology, i.e. its cross-pollination and selfing potential.

Pedigree selection. Pedigree selection involves selfing a certain number of plants, thus producing S1 families. Families of interest are chosen according to various criteria and then selected plants selfed within these families. This produces S2 families, with subsequent selfing and choosing to produce S3 and S4 families, etc. The breeding process is generally carried out up to S6-S8, thus producing homozygous lines with low vigour, but which can be used to create hybrids through heterosis.

Recurrent selection. Recurrent selection involves choosing some plants of a variety or composite. Seeds of cobs from these plants can be sown in "ear to rows" for instance with each cob producing a seed line. Choices are then made according to one or several criteria and chosen families are crossed to produce a new variety. The variety is progressively improved through several choosing and crossing cycles.

Backcrossing. Backcrossing is used to transfer a trait to an interesting variety that does not already have it. The F1 progeny of the receiving variety and the trait donor are screened for the trait. Plants with the trait are backcrossed with the receiving variety. After several backcrossing cycles, varieties are produced that somewhat resemble the initial variety but contain the sought-after trait.

MSV resistance research conducted by IITA in Africa

In 1972, MSV resistance was detected by IITA in the cultivar Tropical Zea Yellow (TZY). This variety was then bred and resistance transferred to other higher-yielding varieties. The improved line IB 32 (obtained in 1979) was found to have high partial resistance. It was used as a resistance donor. A selection programme was carried out but completely symptom-free plants were rejected; many varieties have been produced and distributed throughout Africa.

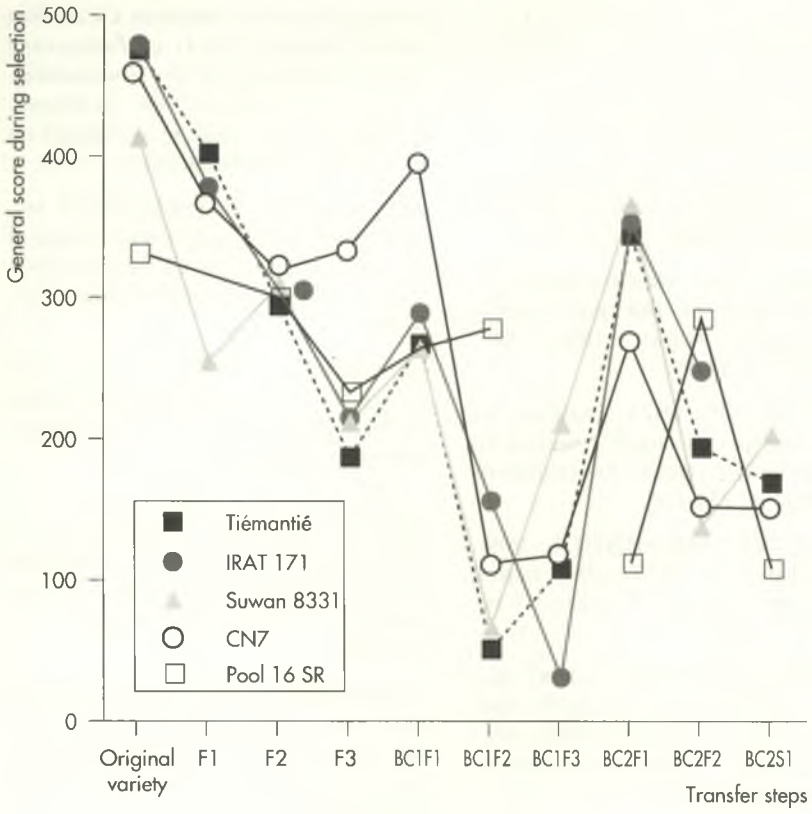


Figure 5. Efficiency of MSV resistance transfers: variations in the overall susceptibility scores during different transfer steps.

Origin of converted varieties.

Origin	Mali	Burkina Faso	Benin	CIMMYT	CIMMYT	Côte d'Ivoire	IITA-Nigeria
Variety	Tiémantié	IRAT 171	CN7	Suwan 8331	Tuxpeño	CPJ	16 SR Pool

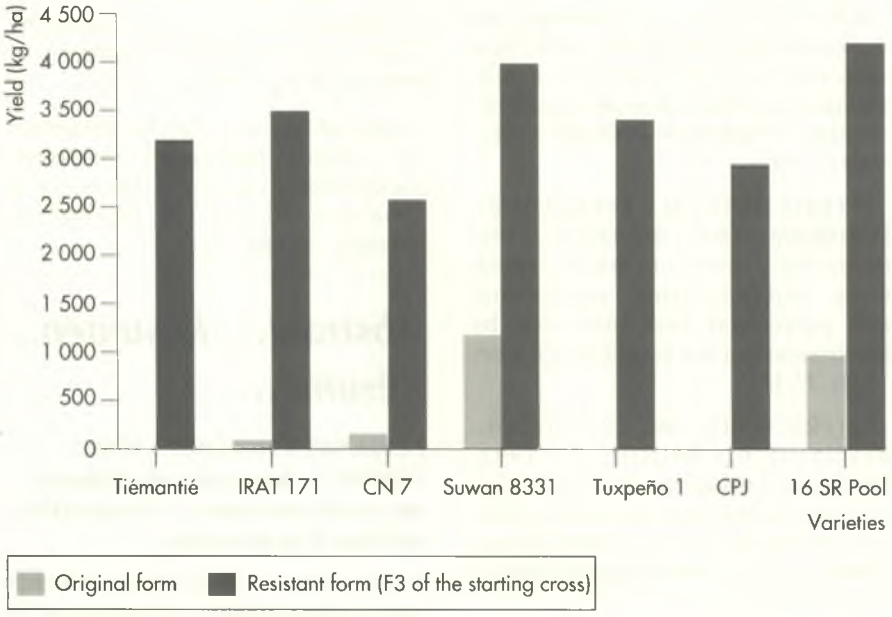


Figure 6. Transfer efficiency. Gram yields of converted varieties compared to those of the original varieties (kg/ha).

Concerning the maize breeding carried out in Togo, five varieties (Violet de Katiola, ZL 2BD, Pool 16 DR, early Blanc 2 and AB 21) are at the second backcrossing stage and are therefore converted. Different breeding procedures are being assessed in terms of MSV resistance and preservation of grain texture and husk coverage of maize cobs, traits that were sought in the selection process.

MStpV and MMV resistance

Comparison of the performance of the IRAT 297 composite with that of the susceptible INRA 508 hybrid under natural conditions highlighted a high level of MStpV and MMV resistance in the local composite, as shown by complete resistance in more than 80% of the plants. The genetic factors that determine resistance to these viruses seem to differ, despite the fact that they occur in the same geographical zones and are transmitted by the same insect vector. IRAT 297 is currently the only known source of MStpV resistance.

MMV resistance is now defined and plant resistance to vector transmission of the virus has also been detected in IRAT 297 hybrid lines. Studies on this latter type of resistance are now under way. These two levels of resistance, i.e. to the virus and to transmission, should be jointly taken into account for developing efficient genetic control techniques.

Prospects

In the short term, it is essential to make between-country comparisons, under artificial MSV infestation, of resistance levels in varieties that were selected for MSV-resistance in Réunion (final forms obtained by crossing S3 lines, with two backcrossing cycles) and Togo. Such tests are being carried out in Cameroon, Togo, Zimbabwe with CIMMYT, and Réunion. The same type of test will then be carried in more countries under natural infestation conditions.

The results should highlight the extent of similarity — in terms of recovery of agronomic traits, grain types, resistance other than to MSV — between resistant and susceptible forms of different varieties.

In the longer term, transfers of MStpV and MMV resistance are planned, especially in open pollination varieties and hybrid varieties; the resulting plants could then be distributed widely throughout the tropics. Research is currently under way on *P. maidis* transmission, resistance transfers into elite maize lines, and on the genetics of resistance to these two viruses. The preliminary results are promising. ■

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Abstract... Resumen... Résumé...

J.-L. MARCHAND, M. PETERSCHMITT, B. REYNAUD, J. DINTINGER — **Maize streak, maize stripe and maize mosaic virus diseases in the tropics (Africa and islands in the Indian Ocean).**

Impact of maize virus disease and current research; vectors and their epidemiology; maize virus diagnosis and variability; maize resistance and breeding. The three virus diseases have been known since the beginning of the

century and are found in the tropics. They were studied in Africa and Reunion. Research in virology, epidemiology and genetics is carried out by CIRAD in collaboration with several African institutions (CORAF, INCV, INERA and IRA), international institutions (CIMMYT and IITA) and the John Innes Institute in Great Britain. Maize streak virus (MSV), maize stripe virus (MStpV) and maize mosaic virus (MMV) are always spread by insects. MSV is spread by members of the genus *Cicadulina* (numerous species have been identified). MStpV and MMV are spread by *Peregrinus maidis*. These insects have been identified in Africa. Streak epidemics cause serious crop damage (in Reunion and in Africa in certain years) but the impact of strike and mosaic is still not well known. The effectiveness of virus transmission to the insect vector was high for MSV and *C. mbila* (50% of insects were infectious) and lower for MStpV and MMV and *P. maidis* (20% of insects were infectious). The viruses were identified by enzyme-linked immunosorbent assay (ELISA). The serological method was the most reliable. A diagnosis kit was developed to test the presence of the three viruses without special equipment. With the new techniques (monoclonal isolates, range of hosts and genome sequencing) used, MSV serotypes were identified. Detection of the virus in plants using ELISA made it possible to understand the mechanism of resistance to MSV. Varietal resistance appeared to be caused by resistance to propagation of the virus. The varietal selection work carried out in Reunion and in Togo was aimed at transferring resistance (found in local varieties) to varieties of clear agronomic interest. A "Composite viroses resistant" was formed from local varieties and then improved. The work on MSV is more advanced than that on MStpV and MMV. Genetic analysis of resistance to MSV revealed partial polygenic resistance and total resistance by virtue of two or three genes. Research has been developped on the three viruses resistance in Reunion, five varieties have been completed. Transfers have been carried out for the MSV resistance in Togo, five varieties have been formed. Trials are in progress in several countries (Cameroon, Togo, Zimbabwe and Reunion) to compare the levels of resistance achieved and the agronomic conformity of the resistant varieties.

Key words: maize, streak, stripe, mosaic, virus, insect, *Cicadulina mbila, Peregrinus maidis,* varieties, resistance, ELISA, tropics, Africa, Reunion.

J.-L. MARCHAND, M. PETERSCHMITT, B. REYNAUD, J. DINTINGER — **Las virosis del estriado, del stripe y del mosaico en el maíz en región tropical (Africa y las islas del Océano Indico).**

El impacto de las virosis y las investigaciones actuales; los vectores y su epidemiología; el diagnóstico de las virosis y la variabilidad del virus MSV; la resistencia del maíz y la selección varietal. Estos tres virosis, conocidas desde comienzos del siglo y difundidas en todas las regiones tropicales, han sido estudiadas más precisamente en Africa y la isla de la Reunión. El CIRAD ha llevado a cabo investigaciones de virologia, epidemiología y genética, en colaboración con varios organismos africanos (CORAF, INCV, INERA, IRA), internacionales (CIMMYT, IITA) y el John Innes Institute de Gran Bretaña. El estriado es transmitido por el *maize streak virus* (MSV), el *stripe* es transmitido por el *maize stripe virus* (MStpV) y el mosaico por el *maize mosaic virus (*MMV). Los tres virus son transmitidos obligatoriamente por insectos, del género *Cicadulina* para el MSV (se han identificado numerosas especies) y por *Peregrinus maidis* para el MStpV y el MMV. Dichos insectos se han identificado en el continente africano. Las epidemias de estriado provocan grandes daños (en la Reunión y, algunos años, en Africa), mientras que el impacto de las virosis provocado por el MstpV y el MMV todavía se conoce mal. La eficacia de las transmiciones virales al insecto vector es elevada para el MSV y *C. mbila* (50% de los insectos infecciosos) y más baja para el MStpV y el MMV y *P. maidis* (20% de los insectos infecciosos). Los síntomas de los tres virus están descritos, y la epidemiología está estudiada en Burkina y en la Reunión. Los virus son identificados mediante pruebas inmunoenzimáticas ELISA (Enzyme-linked immunosorbent assay). El método serológico es más seguro que sólo la descripción de los síntomas. Se ha elaborado un kit de diagnóstico para probar la presencia de estos tres virus sin necesitar un equipo de laboratorio. Otras técnicas utilizadas (gérmenes aislados monoclonales, numerosos huéspedes, sequenciación del genoma), se han distinguido serotipos de MSV. La investigación de virus en la planta mediante la técnica ELISA ha permitido comprender el mecanismo de resistencia al MSV. La resistencia varietal al virus se manifiesta aparentemente por una resistencia a la multiplicación viral. Las investigaciones en selección varietal, tuvieron como objetivo realizar transferencias de las resistencias, presentes en variedades locales, a variedades de buen interés agronómico, en la Reunión y en Togo. A partir de variedades locales resistentes, se constituyó, y mejoró, un "Composite viroses résistant". Los trabajos relativos al MSV están más avanzados que los relativos al MStpV y al MMV. El análisis genético de la resistencia al MSV demuestra que existiría un sistema mayor de resistencia total oligogenica (3 factores geneticos) y tal vez una resistencia parcial poligenica, la qual implica genes minores. Se están realizando transferencias en variedades africanas y efectuando investigaciones en la Reunión y en Togo. Se han efectuado las investigaciones en los tres virus en la Reunión, cinco variedades se han terminado. Se han realizado transferencias para la resistencia al MSV en Togo, y se han constituido cinco variedades. También se están haciendo pruebas en diferentes países (Camerún, Togo, Zimbabwe, la Reunión).

Palabras clave: maíz, estriado, stripe, mosaico, virus, insecto, *Cicadulina mbila, Peregrinus maidis,* variedad, resistencia, técnica ELISA, región tropical, Africa, Reunión.

J.-L. MARCHAND, M. PETERSCHMITT, B. REYNAUD, J. DINTINGER — **Les viroses de la striure, du stripe et de la mosaïque sur le maïs en région tropicale (Afrique et îles de l'océan Indien).**

L'impact des viroses et les recherches actuelles ; les vecteurs et leur épidémiologie ; le diagnostic des viroses et la variabilité du virus de la striure ; la résistance du maïs et la sélection variétale. Ces trois viroses, connues depuis le début du siècle et répandues dans toutes les régions tropicales ont été plus précisément étudiées en Afrique et à la Réunion. Des recherches en virologie, en épidémiologie et en génétique sont conduites par le CIRAD en collaboration avec plusieurs organismes africains (CORAF, INCV, INERA, IRA), internationaux (CIMMYT, IITA) et le John Innes Institute de Grande-Bretagne La striure est causée par le *maize streak virus* (MSV), le *stripe* est provoqué par le *maize stripe virus* (MStpV) et la mosaïque par le *maize mosaic virus* (MMV). Ces virus sont obligatoirement transmis par des insectes, du genre *Cicadulina* pour le MSV (de nombreuses espèces sont identifiées) et par *Peregrinus maidis* pour le MStpV et le MMV. Ces insectes ont été identifiés sur le continent africain. Les épidémies de striure provoquent des dégâts importants (à la Réunion et certaines années en Afrique) ; l'impact des viroses causées par le MStpV et le MMV est encore mal connu. L'efficacité des transmissions virales par l'insecte vecteur est élevée pour le MSV et *C. mbila* (50 % des insectes infectieux), et plus faible pour le MStpV et le MMV par *P. maidis* (20 % d'insectes infectieux). Les symptômes des trois virus sont décrits, et l'épidémiologie est étudiée au Burkina et à la Réunion. Les virus sont identifiés à l'aide des tests immunoenzymatiques ELISA *(Enzyme-linked Immunosorbent assay).* La méthode sérologique est la seule méthode fiable. Un kit de diagnostic a été mis au point pour tester la présence de ces trois virus, sans nécessiter un équipement de laboratoire. D'autres techniques (isolats monoclonaux, gamme d'hôtes, séquençage du génome) ont permis de distinguer des souches différentes de MSV. La recherche du virus dans la plante par la technique ELISA a permis de comprendre le mécanisme de résistance de la plante au MSV, qui se traduirait par une résistance à la multiplication virale. Les sélections variétales, ont eu pour objectif de transférer des résistances dans des variétés d'un bon intérêt agronomique, à la Réunion et au Togo. A partir de variétés locales, un « Composite viroses résistant » a été constitué, puis amélioré. Les travaux sur le MSV sont plus avancés que ceux sur le MStpV et le MMV. L'analyse génétique de la résistance au MSV montre qu'il existerait un système majeur de résistance totale oligogénique (3 facteurs génétiques) et éventuellement une résistance partielle polygénique impliquant des gènes mineurs. Des transferts sont réalisés sur des variétés africaines ou introduites à la Réunion et au Togo. A la Réunion, les travaux de sélection sont conduits sur les trois virus, cinq variétés sont converties. Au Togo, des transferts sont réalisés pour la résistance au MSV et cinq variétés sont converties. Un essai multilocal est en place dans différents pays (Cameroun, Togo, Zimbabwe, la Réunion).

Mots-clés : maïs, striure, stripe, mosaïque, virus, insecte, *Cicadulina mbila, Peregrinus maïdis,* variété, résistance, technique ELISA, région tropicale, Afrique, la Réunion.