Diagnosing Maize Diseases in Latin America

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Cover pictures: Pictures taken during the field visits and the diagnostics training workshop in Brazil by ISAAA (K.V. Raman).

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Introduction and Overview

Diagnosing Maize Diseases with Proprietary Biotechnology Applications Transferred from Pioneer Hi-Bred International to Brazil and Latin America

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In the early 1990's, scientists in Brazil became concerned about the growing spread of unidentified maize diseases in the crop's major production areas. Researchers at the Brazilian National Maize and Sorghum Research Center (CNPMS) at Sete Lagoas had previously seen virus-like symptoms in several maize varieties, but were unable to identify them. They had little information on the development of the diseases and were in need of reliable methods of detection. At risk was the country's nearly 15 million hectare crop. Its annual production of 30 million tons

is valued at about US$700 million. Although total production meets today's Brazilian demand, the average maize yield at 1.8 tons per hectare is very low. This reflects the lack of high-yielding varieties, use of better soils for other crops and lack of technology.

At the same time, there were reports of new viruses spreading into Brazil from neighboring countries and from other areas of Latin America. Scientists from the International Maize and Wheat Improvement Center (CIMMYT) in Mexico had concluded that Brazil's

[1] Krattiger, A.F. Kulisek, E.S. and Casela, C. 1998. Introduction and Overview: Diagnosing Maize Diseases with Proprietary Biotechnology Applications Transferred from Pioneer Hi-Bred International to Brazil and Latin America. *In* Diagnosing Maize Diseases in Latin America (Eds. C. Casela, R. Renfro and A.F. Krattiger). *ISAAA Briefs No. 9*. ISAAA: NY. Pp. 1-4.

problem was a higher prevalence of the corn stunt virus complex, probably the result of an increase in year round cropping of maize. Because of the growing importance of the crop in Brazil and rapid spread of the diseases, a national virus detection program was given a high priority designation by the country's Agricultural Ministry. CNPMS officials had earmarked funding for staff training and project expenses.

The immediate need was a simple test to quickly identify the diseases under field conditions. Once that was done, scientists could better understand the spread of the diseases, undertake control programs and breed maize varieties with resistance to the diseases. Although some diseases can be visually diagnosed, many require laboratory testing that can take days and weeks to complete. As a result of advances in biotechnology, new products and techniques are now available that can replace time-consuming and sometimes inaccurate laboratory procedures.

Above:Alejandro Ferreira inspecting maize diseases.
Below: Alejandro Ferreira and Dr. Carlos Casela (right).

Early in 1993, Brazilian officials contacted the International Service for the Acquisition of Agri-biotech Applications (ISAAA) for assistance. Following an intensive survey by ISAAA and discussions with several potential donors of the technology, Pioneer Hi-Bred International of Johnston, Iowa (a corporate sponsor of ISAAA since 1992), was selected as the partner.

The project, brokered by ISAAA to assist Brazil, involved the development and donation by Pioneer of its proprietary ELISA technology for detection of diseases in addition to training CNPMS scientists and technicians in laboratory and field techniques. Pioneer also agreed to organize and co-sponsor a three-week training program in Iowa, for a Brazilian scientist, on the development and application of ELISA diagnostic kits.

Of the three major diseases infecting Brazilian maize varieties, two diseases, Corn Stunt Spiroplasma (CSS; a bacterial disease) and Rayado Fino (RF; a virus), were selected by Brazil and Pioneer for initial study because of their prevalence in Brazil and many other countries in Latin America.

The production of enzyme-linked immunosorbent assays (ELISA) is a diagnostic kit in detecting viral and bacterial diseases. These diagnostics are based on a method that uses antibodies to detect disease causing organisms of plants.

In the summer and fall of 1993, Ellen Kulisek of Pioneer, developed and perfected two assays to detect CSS and RF viruses and field tested them in Johnston. The antigens necessary to initiate antibody production were donated by the US Department of Agriculture. The following January, Kulisek trained 14 Brazilian scientists and technicians at the Sete Lagoas research headquarters on both laboratory and field use of the assay procedures for each of the two ELISAs. The assays worked well because they were sensitive enough to detect infected plants that were considered free of disease based on visual observation.

Later that year, Carlos Casela of CNPMS benefited from a three-week ISAAA Biotechnology Fellowship at Pioneer on ELISA development. Meanwhile, CNPMS

named a researcher from the in-country training course, Elizabeth de Oliveira, to head a new diagnostic program at Sete Lagoas. There is a strong potential for diagnostics in Brazil that needs to be encouraged and supported. This requires not only financial support and commitment, but interested members of the scientific community who are committed and willing to donate their time to this. Pioneer had agreed to precisely this. It is also noteworthy that the cost of the project—which was low compared with the value of the diagnostics—was sponsored by CNPMS and Pioneer.

Workshop participants during laboratory work at the CNPMS/ISAAA Maize Disease Manangement *workshop*

Equipped with trained manpower, CNPMS was by then in a position to transfer this technology to others in Brazil, such as farmer cooperatives, seed companies and non-governmental organizations, all of which would strengthen the nation's maize breeding, seed testing, production and extension programs. It also set the stage for the transfer of the kits to other countries in Latin America. Indeed, a Latin American training workshop co-sponsored by CNPMS and ISAAA took place from 20-24 May 1996. It was entitled *Maize Disease Management* and was hosted by CNPMS at Sete Lagos in Brazil. The present *ISAAA Briefs* No. 9 is a result of that workshop.

The objectives of the workshop were two-fold: First, economically important maize diseases in the whole of Latin America were reviewed to share knowledge and experiences about their occurrence, spread and management practices that have been successful. Second, a two-day hands-on seminar enabled participants to learn the ELISA technology developed as part of the collaborative project between Pioneer Hi-Bred International and Brazil. This is 'technology transfer' to the end users in its true sense of the word, be the technology from the private or public sectors, from Brazil or from neighboring countries. Over 150 people participated, ranging from the national programs of Bolivia to Brazil, and Colombia to Costa Rica; some 30 local and national companies from these countries; international agricultural centers, including CIP and CIMMYT; and other private companies ranging from Brasalkalb to Zeneca Seeds (now ADVANTA), and Cargill Seeds to Ciba Seeds (now Novartis Seeds).

From left to right: Drs. Carlos Casela (EMBRAPA), Ellen S. Kulisek (Pioneer Hi-Bred Int.) and Falvio Jader of EMBRAPA inspecting maize fields during a project visit to Sete Laogus, Brazil.

Pioneer's participation in the project was as part of its

commitment with ISAAAand not to create a scientific advantage for Pioneer in Brazil. The company has long standing business interests in Brazil and will always be interested in agricultural efforts and trends there. The company has an established global program for humanitarian assistance. Brazil's view is that there are biotechnology applications, many developed at great cost and owned chiefly by private companies, that developing countries cannot afford, but that are vital to their agricultural development. Through this project, Brazil was able to establish a partnership with Pioneer that is benefiting Brazil and Pioneer, including the farmers, breeders, the environment and, through the workshop, Brazil's neighbors.

It is clear that such pragmatic projects are an effective means of building cooperation and trust between the public and private sector. The success of this project and workshop, the basis of the present *ISAAA Briefs No. 9*, is a result of the commitment by the country and the company to the technology and to the fact that it fulfilled a specific and important need for Brazil.

It is hoped that with such need-driven pilot projects, like the present one between Pioneer Hi-Bred International and Brazil, new mechanisms beyond traditional technology flows are being built, which will open the possibility for larger biotechnology transfers for the benefit of farmers and of the environment.

The debriefing of workshop participants.

The Current Status of Maize Diseases in Brazil

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In 1995 the total maize (*Zea mays*) production in Brazil was 36.6 million tons grown on 14 million hectares.The southern, southeastern, and central regions of Brazil accounted for 90% of the total maize porduction in 1995, with the remaining 10% produced by the northern and northeastern regions (3% and 7% respectively). Maize is produced in different agroecological zones in these three regions (Figure 1). These agroecological zones vary in natural aspects such as soil, clime, topography, and vegetation. In addition, social and economic elements constitute a cultural reality and establish limiting factors and potentialities of the area. These agroecological zones are also differentiated by varying degrees of technology.

The area planted with maize is expanding primarily in the States of Mato Grosso, Mato Grosso do Sul, and also in the southeast, where maize has been included in a rotation system with soybean, the major crop in those states.

In certain areas of Brazil it is also common to grow maize as a second season crop, during the "safrinha" season. The total production of the 1995 "safrinha" was about 2.8 million tons, grown on approximately 1.4 million hectares. This accounts for 7.8% of the total maize production in Brazil. The "safrinha" contributes significantly to the total maize production in each state, accounting for 13.1% of the total maize production in the State of Paraná, 20.7% in São Paulo, 6.0% in Goiás, 25.9% in Mato Grosso, and 25.5% in Mato Grosso do Sul. There is no information regarding the State of Minas Gerais, but it is well known that "safrinha" is a normal practice there (Figure 2).

Since the beginning of the 1990s, maize has developed serious disease problems that have caused severe yield losses. These problems are marked by both an increase in the severity and in the spread of disease throughout the country. The increase in maize acreage both in the normal season and in the "safrinha," the intense disease challenge to some commercial hybrids, the intensive maize cultivation in irrigated areas (especially under central pivot), and the non adoption of crop rotation in nontillage systems in certain areas are the most important factors contributing to the problem.

Disease surveys developed by CNPMS/EMBRAPA have shown that phaeosphaeria leaf spot (*Phaeosphaeria maydis*), southern rust (*Puccinia polysora*), tropical rust

[1] Fernandes, F.T. 1998. The Current Status of Maize Diseases in Brazil. *In* Diagnosing Maize Diseases in Latin America (Eds. C. Casela, R. Renfro and A.F. Krattiger). *ISAAA Briefs No. 9*. ISAAA: NY. Pp. 5-7.

Figure 1: Area and Production of Maize in Brazil

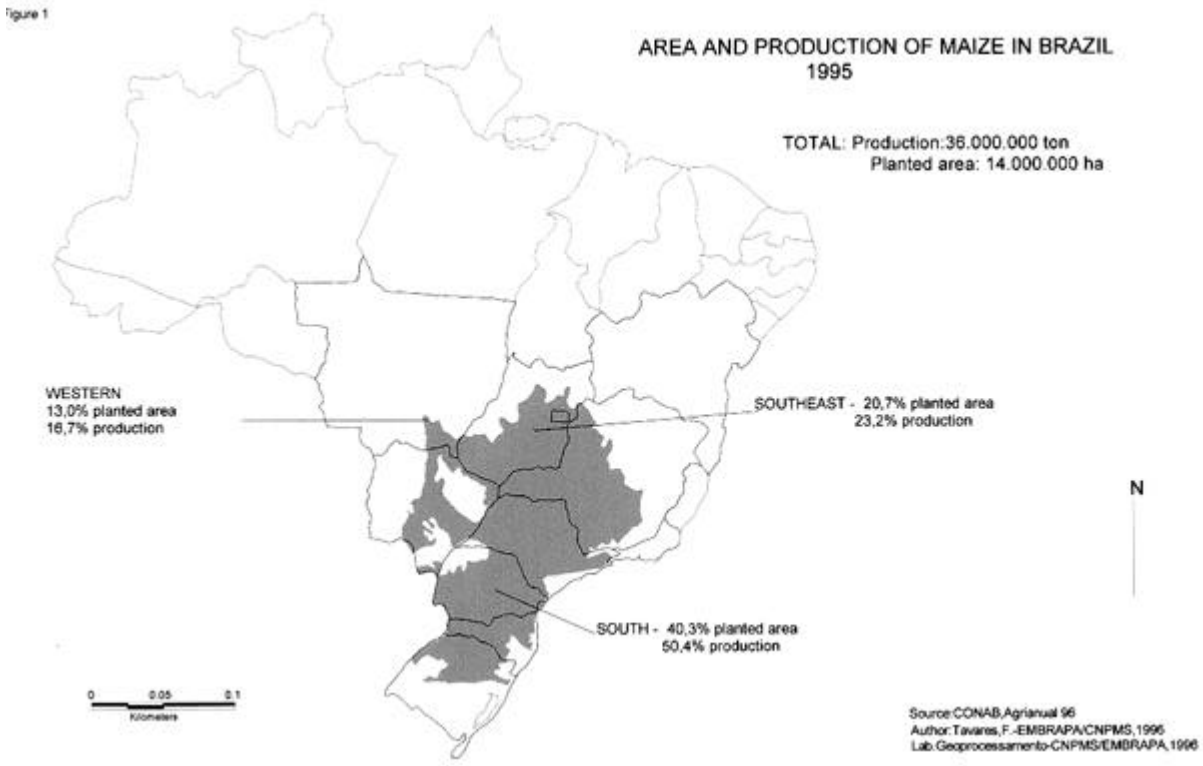
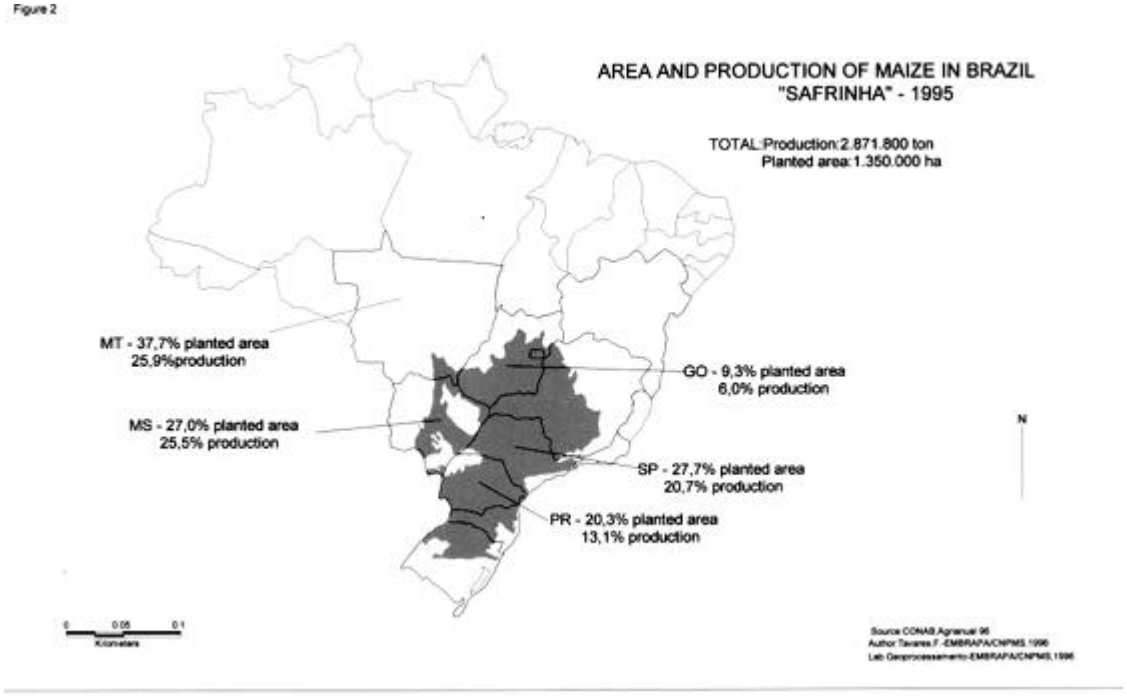


Figure 2: Area and Production of Maize in "Safrinha"



(*Physopella zeae*), common rust (*P. sorghi*), corn stunt (phytoplasm and spiroplasma), maize dwarf mosaic virus, and rayado fino virus are the most important maize diseases in Brazil.

Although present in Brazil since 1902, the phaeosphaeria leaf spot only recently became important. It is now widely spread in Brazil. Because of its secondary importance in other areas of the world, little information is available on phaeosphaeria leaf spot. It is known that the disease is favored by low night temperatures and relative humidity above 60%. Symptoms are first observed on lower leaves, but under favorable conditions the disease can reach young top leaves and even infect the husk. Early plant death or the production of scattered and light grains are observed in late plantings.

The rusts are observed in both the "safrinha" and the normal season in the major maize areas of the States of Minas Gerais, Goiás, São Paulo, and Paraná. Rust, especially the southern and tropical rusts, can cause severe yield reduction in maize in Brazil. Common rust (*P. sorghi*) was the first maize rust observed in Brazil. The disease is favored by temperatures between 16 and 23^{0}C and a high relative humidity. The pathogen has *Oxalis* sp. As an alternate host. Southern rust (*P. polysora*) only recently became important and is favored by high temperatures (27^{0}C) and a high relative humidity. No alternate host is known for this pathogen in Brazil. This disease is more severe in the "safrinha" season. Tropical rust (*P. zeae*) is the latest maize rust found in Brazil and it has increased in severity during the last 4 years. The disease follows the same pattern of distribution as southern rust.

Corn stunt has been observed in Brazil since the 1970s, but like the tropical rust, it has become significant in only the last 4 years. This disease is particularly severe in the "safrinha" season because it provides favorable for vectors and thus for the multiplication and maintenance of pathogens. Under favorable conditions, grain production of infected plants may be completely suppressed. Corn stunt is caused by a spiroplasma organism transmitted in a persistent way by the leafhopper *Dalbulus maidis*.

The corn stunt phytoplasm has the species *Z. mays mexicana* as a collateral host and is transmitted by *D. maidis* and *D. elimatus*. Other vectors are species of the leafhoppers *Graminela nigrifons*, *G. sonora*, and *Baldulus tripsaci*. Infected leaves exhibit a typical reddening at the top, and diseased plants bear numerous small ear shoots.

A high incidence of maize dwarf mosaic virus (MDMV) and rayado fino virus (RFV) has frequently been observed in the same areas where corn stunts occur. There are probably other virus diseases present in Brazil. Similar to corn stunt, virus diseases are of considerable importance in the "safrinha" season due to favorable conditions for insect vectors and their maintenance on alternate hosts. Losses due to incidence of the MDMV can reach 50%. The fact that MDMV is considered a variation of the sugar cane mosaic virus puts maize crops grown close to sugar cane at risk for the disease. Strains of the MDMV can infect more than 250 species of grasses, among them sugar cane, maize, sorghum, wheat, rye, and rice. This virus is mechanically transmitted and is disseminated by more than 20 species of aphids, but primarily by *Rophalosiphum maidis*, *Schizaphis graminum*, and *Myzus persicae* in a non persistent way.

The RFV can cause yield losses between 30 and 50%. It is persistently transmitted by leafhoppers, primarily *D. maidis*. Species of *Zea*, *Tripsacum*, and the species *Roittboelia exaltada* are collateral hosts of this virus.

Information about maize disease distribution in Brazil has been obtained through disease surveys that are made annually by EMBRAPA/CNPMS in all maize regions of Brazil. Through this survey it has been possible to relate disease incidence and severity with environmental conditions, as well as to detect major shifts in pathogen populations. This information is important for the development of integrated disease management strategies for the maize crop in Brazil.

Maize Rusts

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Overview

Three rust diseases of maize reduce yields: common, southern, and tropical rust. Obligate parasites that have difficulty surviving between growing seasons, one or more of these rusts occur wherever maize is grown. The causal fungi have similar host ranges and are not seed-borne, although the urediospores can be carried long distances by winds. And while common and southern rusts cause symptoms and urediospores that are difficult to distinguish from one another, those to tropical rust are quite distinct. Common and southern rusts are more economically important than tropical rust. Only the common rust pathogen is known to be a full-cycle rust: *Oxalis* spp. Are its alternate hosts. Common rust development flourishes in cooler temperatures than the other two rusts, but the three have similar moisture requirements.

Host resistance offers the best control measure, particularly the non-specific or quantitative type. This type of resistance is effective against all known biotypes of the causal fungi and is relatively easy to incorporate into cultivars through recurrent selection. Adequate soil fertility, moisture, and weed control will not control the rusts, but they will reduce plant stress and yield losses. New plantings should not be made adjacent to older infected maize. Foliar sprays with fungicides provide an effective control and can be applied when economically feasible.

Introduction

The three rust diseases that occur on maize (*Zea mays* L.) are caused by fungi in the class Basidiomycetes and order Uredinales. They are obligate parasites, heteroecious, and have narrow host ranges. All produce, sequentially, urediospores, teliospores , and basidiospores. The basidiospores produced by *Puccinia polysora* Underw., the cause of southern maize rust, and *Physopella zeae* (Mains) Cumm. And Ramochar (*Angiopsora zeae* Mains), the cause of tropical maize rust, have no known alternate host to infect and are microcyclic. *Puccinia sorghi* Schwein., the cause of common maize rust, completes its full life cycle by infecting and producing spermatia and aeciospores on an alternate host, *Oxalis* spp. Basidiospores, aeciospores, and urediospores are the

[1] Renfro, R. 1998. Maize Rusts. *In* Diagnosing Maize Diseases in Latin America (Eds. C. Casela, R. Renfro and A.F. Krattiger). *ISAAA Briefs No. 9*. ISAAA: NY. Pp. 8-14.

only spores that can cause infection to host plants. The teliospores serve as only the sexual overwintering or overseasoning stages. On germination and after meiosis, they produce a promycelium (basidium) on which four haploid basidiospores are produced. The rust fungi penetrate their host indirectly through stomata. Bulow (1966b) found the hyperparasite, *Darluca filum* in uredia of *P. polysora* and *P. sorghi* in south-central Brazil and regarded it as a limiting factor for urediospore dissemination.

Wind dissminates the spores of these fungi. Some of their spores are carried several hundred kilometers, and upon being scrubbed from the air by rain, can start new infections. The rusts may debiliate and kill young plants, but usually they reduce foliage, root growth, and yield by reducing the photosynthetic rate. They also increase the rate of respiration, decrease the translocation of photosynthates from diseased tissue, and significantly increase water loss through ruptured pustules. Host resistance of the quantitative type represents the best control measure and reduces the size and number of pustules as well as leaf chlorosis and necrosis. Infection types of the qualitative types of the maize rusts are not as easily discerned as those of the small grain cereals. The excellent reviews by Hooker (1985) and Melching (1975) are used extensively in this paper.

Melching (1975) discussed reasons that the rust diseases have not historically caused severe damage to maize. The most significant reason is the presence of non-specific resistance. Also important is the C4 metabolic pathway of photosynthesis, which, of the major crop plants, is possessed only by maize, sorghum, and sugarcane. This pathway converts carbon dioxide into photosynthate much more efficiently with a lower rate of photorespiration than C3 crops. An indirect result of breeders developing high yield varieties with enhanced photosynthesis is that the plants may better withstand attack from the rusts. Another reason given is the rapid development of the maize plant, which has diluted the severity of the rusts and lessened yield losses. In addition, the spatial relationship has also reduced the effects of the rusts. There is little contact between the leaves of plants grown in different rows for many weeks after emergence; the wider spacing between plants and the corresponding rapid growth makes a much less hostile microclimate than that existing in most other crop plants.

Southern Rust

Puccinia polysora was named by Underwood (1897) from a herbarium specimen of *Tripsacum dactiloides* collected in Alabama, USA in 1891. Cummins (1941) also examined herbarium specimens and found that *P. polysora* was present earlier than 1891 and that it was widespread in Central and South America, as well as in Massachusetts, USA in 1879. Southern rust became prominent in the literature after 1949 when losses of more than 50% occurred over wide areas in West Africa. The first report from Africa was from Sierra Leone. Before this it was known to occur only in the western hemisphere. Southern rust now occurs in most of sub-saharan Africa, southeast Asia, Australia, Mauritius, the Philippines, Indonesia, Taiwan, and it has recently been reported in Southern India (Payak, 1994). Collateral hosts are maize, three *Tripsacum* spp., two *Erianthus* spp. And teosinte. Yield losses reported from the Philippines (Reyes, 1953) on some susceptible varieties were 80-84%. Bulow (1966a) placed losses in Brazil at 40% on susceptible varieties and only 0.5 to 1% from common rust. Bulow (1967b), however, measured a 35% loss in yield when maize was inoculated with *P. sorghi*. The yields and quality may be reduced by southern rust, and infected plants are more likely to develop stalk rot and to lodge.

Symptoms and signs are uredia occurring on both leaf surfaces, the leaf sheathes, husks, and sometimes stems. These pustules are orange-red and later light cinnamon brown (without paraphyses), circular, and 0.2-2.0mm long. They are generally smaller, more circular, and lighter in color than those of the common rust. The leaf epidermis is retained over the pustules longer than with common rust. Telia are brownish-black and most often form along the mid-rib on the underside of the leaf and may develop in a circle around the uredium. Cammack (1958a) reported that urediospores are produced for 18-20 days from a single pustule and that they release 1500-2000 and 600-1150 spores daily from susceptible and resistant plants, respectively. They are yellowish to golden and measure 23-29 X 29-36µ, sparce echinulate, and have 4 to 5 equatorial pores. The teliospores are two-celled, chestnut brown, angular to ellipsoid or oblong, and measure 20-27 X 29-31µ. These more angular teliospores with only slightly thickened apical walls help to distinguish *P. polysora* from *P. sorghi* (Hooker, 1985). *P. polysora* is perpetuated through the repeating uredial

stage as the teliospores are not known to be functional. It overseasons on its hosts and the urediospores are disseminated over long ranges. Cammack (1959), working in Nigeria, did research on urediospore survival and considered that their air transport to Africa from a Caribbean island was the most likely means of introduction. Cammack (1958b) also examined all herbarium specimens at C.M.I. and found two spore sizes. The smaller group was found in material from southeast Asia and neighboring islands, with the exception of Borneo (Kalimantan), and the larger urediospore group was found in the West Indies, Africa, and the South Indian Ocean.

The optimum temperature for germination of urediospores is 23-28°C. Germination is drastically reduced at 13 and 30°C, and few germinate at 34°C (Melching, 1975). Hollier and King (1985) reported the optimum temperature at 26°C with a 16 hour dew period (double the infection of a 12 hour dew period) and no infection at 8,12,36 or 40°C regardless of the length of the dew period. The disease is primarily tropical and sub-tropical, occurring in its most prevalent and severe form below 900m and rarely at all above 1200m. Free water on the plant surface is necessary for germination and host penetration. Pustule development has an optimum of 25-29°C and does not develop at 7C or 31°C. Melching (1975) found that 24-37 urediospores of 87% viability were required for each pustule produced. Cammack (1958a) obtained 2% and 15% infection with single spore inoculations of resistant and susceptible seedlings, respectively, and he found that pustules and urediospores developed best around 27°C. Pustules develop in 6-10 days following infection. The rate is primarily dependent upon the temperature, but light conditions and the plant's moisture stress also contribute. Numerous physiological races of *P. polysora* have been reported; Bulow (1967a) reported 13 races in a study of 55 monospore isolates from South-Central Brazil.

Tropical Rust

Tropical maize is not known to exist outside the western hemisphere. It occurs in warm, humid areas of Mexico, the Caribbean, Central America, and South America to 5 degrees south latitude (Melching, 1975). However, Brazilian scientists at the workshop have observed tropical rust to be widely prevalent and causing damage to maize in south Brazil to about 30 degrees south latitude (unpublished). Cummins (1971) reported it in Florida on teosinte, but this has not been reported thereafter. In addition to maize and teosinte, it has four *Tripsacum* spp. Hosts. The disease is not known to be economically important on these hosts. Much less research has been done on tropical rust than on the other two maize rusts.

Uredia are round to oval, 0.3 – 1.0mm long on the upper leaf surface, often small and beneath the epidermis (except for a small pore or slit), without paraphyses, and tend to occur in groups. The margin of the uredium is often black, while the center is white to pale yellow in color. These pustules develop into dark purple, circular or oblong blotches with cream colored centers, 0.6 cm in diameter. They are larger than those of *P. polysora* and are elongated parallel to the leaf veins. The urediospores are echinulate, hyaline to yellow, elliptical to oviod, 12-20 X 18-30 um, and have five equatorial pores. The telium remains covered and is brown to black. The teliospores are uni-cellular, golden to light chestnut brown, cubiod or oblong, occur in chains of 2 to 4 spores without pedicels, and measure 10-18 X 12-29µ. The side and apical walls measure 1.5-2 and 3-4µ, respectively (Hooker, 1985). There is no known alternate host and therefore no aecial stage.

Spores are released when the host tissue splits. Secondary spread is by urediospores. Disease outbreaks of tropical rust are sporadic, although it occasionally has occurred severely in individual fields in Guatemala and Venezuela. Losses, however, were not given (Melching, 1975). Bonde, and co-workers (1982) obtained germination of urediospores in 1-2 hours at 22°C with free moisture on maize leaves. Large appressoria developed by the fifth hour, and penetration by infection pegs was observed by the twelfth hour. Primary hyphae (avg. 9.8µ width) grew from the infection peg by the 28th hour and colonized epidermal and mesophyll tissue as intracellular hyphae. Pustules appeared 7-9 days following infection. The cardinal temperatures for *P. zeae* are about the same as for *P. polysora*, but urediospores did not germinate below 8°C and only occasionally at 34°C (Melching, 1975). At least two physiological races are known.

Maize is known to possess resistance to tropical rust. Resistance is expressed as chlorotic flecks, small uredia, and mixed or mesothetic (type X) infection types.

Common Rust

Common rust is probably not a threat to maize in Brazil. This is based on Bulow (1966a), who estimated a loss of 0.5-1%, although Bulow (1967b) measured a 35-36% loss after plants were inoculated by syringe injection. In addition, Brazil is situated between about 4 degrees N and 34 degrees S latitude – tropical and sub-tropical – and has little highland maize growing area. Maize grown during the winter season would appear to be the most vulnerable. Yield losses from common rust worldwide are modest on field maize because the cultivars grown have polygenic resistance. Epidemics do occur when susceptible cultivars are grown, such as in Northern India during the winter season, where 6-32% yield losses are reported (Sharma, et al., 1982). The disease occurs wherever maize is grown and is often severe on sweet corn and popcorn.

The causal fungus, *P. sorghi*, was described by Schweinitz from tissue thought to be from sorghum. Species of sorghum, however, are not hosts. The uredia and telial hosts are maize and annual and perennial teosintes (*Zea mexicana* Schrad. And *Z. perennis* Hitch., respectively). The aecial hosts are several species of *Oxalis* which infrequently become naturally infected in the temperate regions of Europe, India, Mexico, Nepal, some nations of the former USSR, South Africa, and the United States.

Uredia (pustules) are circular to elongate, cinnamon brown, without paraphyses, and develop on both leaf sidesf and on leaf sheathes, husks, and sometimes stems. The urediospores are moderately echinulate, cinnamon brown, globoid to ellipsoid, have 3-4 equatorial pores, and measure 21-30 X 24-33µ. They are one celled with two nuclei, as is the mycelium that develops after germination. The daily peak release is about 1300 hr. The pustules become brownish to black as the plants mature due to dark colored teliospores replacing urediospores. Teliospores are two celled, attached to pale yellow to brown pedicles up to 80um long, slightly constricted at the septum, have a thickened apex, and measure 14-25 X 28-54µ. Two haploid nuclei in each cell of the teliospore fuse at the time of germination. Meiosis occurs and a basidium is formed on which small, hyaline haploid basidiospores (sporidia) are borne. The sporidia can infect *Oxalis* directly through the epidermis, but not maize. The urediospores were demonstrated by Wechmar, et al. (1992), and were first reported to successfully transmit maize dwarf mosaic potyvirus-B from virus infected maize to non-infected maize plants at the fifth Congress of Plant Pathologyheld in South Africa, 1988,. The aeciospores are pale yellow, verrucose, spherical to ellipsoid, occur in cluster cups on the lower side of *Oxalis* leaves, and measure 13-19 X 18-26µ.

The development and spread of the disease are favored by cool, moist weather. The cardinal temperatures for urediospore germination are a minimum near 4°C, an optimum of 17°C, and a maximum of about 32°C (Weber, 1922); good germination occurs at 13 to 27°C. However, Smith (1926) reported the optimum temperature for germination is 25°C. A minimum of 4 hours is required for infection, with the rate of infection increasing in increments of time to 12-16 hours. The time for pustule formation is about 16, 10, 7 and 5 days at 10, 15, 20 and 25°C, respectively (Hooker, 1985). Melching (1975) obtained 4-5 times the number of pustules at 23-28°C than at 29.5 or 13°C and practically no infection at 7 and 30°C. He (1975) also reported that temperatures can change the infection type; one inbred displayed a resistant reaction at 16, 20 and 24°C, but a susceptible reaction at 28°C. Yield losses of 20-25% on susceptible varieties are not uncommon. Kim and Brewbaker (1976) measured in Hawaii an average reduction over two seasons of 35% for grain yield, 27% for fresh plant weight, 11% for ear length, 10% for kernel weight and ear diameter, and less than 5% for plant and ear height and days to silk.

Isolates of *P. sorghi* differ in virulence to plants having specific resistance, and these genes for virulence are not randomly distributed in the world. Biotypes produce different antigens which can be distinguished by serology (Hooker, 1985).

Resistance has been successfully incorporated into maize cultivars in many breeding programs. At CIMMYT, Ceballos, et al. (1991) used four cycles of S_1 selection to incorporate non-specific or polygenic resistance into eight subtropical open-pollinated varieties. The incidence of common rust was reduced by 6% per cycle on the eight varieties. For fungicidal control, Dillard and Seem (1990) propose that the initial spray be applied at 80% incidence (percent diseased leaves) in their work with sweet corn.

Inheritance of Resistance to Rusts

The inheritance of resistance of tropical rust has not been determined. Both monogenic (also referred to as vertical, specific, qualitative, and differential resistance) and polygenic or mature plant (also referred to as horizontal, non-specific, quantitative, generalized, uniform, and field resistance) resistance are known to exist for common rust and southern rust. These produce different infection types (qualitative) and a reduction in pustule number (quantitative). The resistant reaction conferred by monogenes is expressed qualitatively as chlorotic or necrotic hypersensitive flecks with little or no sporulation. Seedlings are more susceptible than older plants, and plants with quantitatively inherited resistance are generally fully susceptible as seedlings. The nature of resistance in mature plants is unknown, but now appears to be the same as slow rusting described in some other cereal crops. The uredia are either reduced in number or their appearance is delayed on resistant plants (Hooker, 1985). This type of resistance is present in maize cultivars in areas where common and southern rusts are potentially important, and these materials have kept losses low. Additional details are provided in reviews by Hooker (1985) and Renfro (1985).

Southern Rust

Polygenic resistance to southern rust is expressed quantitatively in uredia number. Although this type or resistance has not been studied thoroughly, it has been selected for and widely deployed. It is credited with stabilizing the incidence of southern rust at rather low economic levels in disease-prone areas (Hooker, 1985; Renfro, 1985). This generalized form of resistance was responsible for controlling southern rust epidemics in Africa during the 1950's, where specific genes failed to provide control (Robinson, 1976). Slow-rusting resistance has been identified in the inbred line B37 almost entirely from factors located on chromosome 4, although factors on chromosome 8 were also implicated (Beckett, 1971). There is a large variation among cultivars for the slow-rusting trait, and it can be identified by measuring the area under the disease progress curve, or more easily by making weekly assessments (Bailey et al., 1989). Scott and Zummo (1989) found that slow rusting traits possessed fewer and smaller pustules that ruptured later, reducing the destruction of leaf tissue and producing fewer urediospores.

Eleven loci in maize have been found or asserted to have (some may be duplicates) specific genes for resistance to *P. polysora*; these loci have been designated *Rpp*1 to *Rpp*11. Few allelic or linkage tests have been made, unlike what has been done for common rust. Genes R*pp*1, *Rpp*2, *Rpp*10 and *Rpp*11 were identified in Kenya. *Rpp*1 is fully dominant; *Rpp*2 conditions an intermediate reaction, which is modified by other host genes; *Rpp*10 is fully dominant; and Rpp11 is partially dominant. *Rpp*1 and *Rpp*2 are linked with a recombination value of 12.23%, while these and the other two genes have no apparent linkage (Hooker, 1985). Genes *Rpp*3 to *Rpp*8 were inferred from varying reactions of maize inbred lines to a number of USA isolates of *P. polysora*. Subsequently, 6 of these isolates were named races PP3 through PP8 (Roberts, 1962). *Rpp*9 is a single dominant gene found in PI186208 in Indiana, USA by Ullstrup (1965) to a physiological race termed PP9. The gene was found to be located about 1.6 crossover units from the *Rp*1d locus for *P. sorghi* on chromosome 10.

Common Rust

Polygenic resistance to common rust is conditioned by a large number of genes. It is expressed quantitatively as a reduction of uredia or pustule number, varying from a little to most of the leaf area covered with uredia. In older plants this resistance is effective for all of the numerous races of *P. sorghi*. The genes are largely additive in effect, and both high heritability estimates and high general combining ability have been detected for resistance. Slow rusting has been suggested, but this has not been distinguished from adult plant resistance (Hooker, 1985).

Specific monogenic resistance to common rust is expressed as chlorotic or necrotic hypersensitive flecks, which support little or no sporulation on either seedlings or adult plants. At least 6 loci located on four chromosomes have been found to carry resistance genes to *P. sorghi*. Genes *Rp*1, *Rp*5 and *Rp*6 as well as *Rpp*9 for resistance to *P. polysora* are located on chromosome 10S in a map distance of about 3.0 units. At the *Rp*1 locus, 14 alleles or in some cases closely linked genes for resistance (designated *Rp*a to *Rp*n and all dominant to *rp*1) have been identified. Hooker (1985) reported that the recombination values between *Rp*q and *Rp*1, *Rp*a and *Rp*k, *Rp*a and *Rp*c, *Rp*c and *rp*k, and *Rp*b and *Rp*c are 0.37, 0.27, 0.22, 0.16, 0.10%, respectively, and that no recombination was detected between *Rp*d and other alleles.

Resistance in the sweet corn inbred IL677a was found to be controlled by a single recessive gene, designated

rp677a, that is closely linked or allelic to *Rp*d on chromosome 10 (Kim and Brewbaker, 1987). Pryor (1987) tested the stability of 14 alleles at the *Rp*1 locus and placed them in four classes in which *Rp*m was the most stable and had no susceptible variants; *Rp*q and *Rp*c-k were the least stable.

The *Rp*3 locus is located on chromosome 3 at position 49, where six alleles have been identified. Alleles *Rp*3b and *Rp*3c exhibit a reversal of dominance under challenge by two biotypes of *P. sorghi*, which may also be attributed to either a dosage effect or an interaction of closely linked dominant and recessive genes (Hooker, 1985). The *Rp*4 locus is located on chromosome 4 at position 27, where two alleles for resistance are known to exist. Specific resistance has also been attributed to control by recessive alleles at three loci, which act in a complementary manner. One of these genes (*rp*7) is located in chromosomes 2 at position 11+ (Hooker, 1985).

A difference has been observed between partial resistance and adult plant resistance (Headrick and Pataky, 1987). Partial resistance, also referred to as slow-rusting, rate-reducing, or generalized resistance, is quantitative in expression. Partial resistance is a genotype-specific trait and functions at all growth stages, although plants are most susceptible at the five to six-leaf stage. Adult plant resistance, on the other hand, is a universal property of maize and is a function of plant age. Both types reduce uredial number and should be more durable than monogenic, race-specific resistance.

References

BAILEY, B.A., SCHUH, W., FREDERIKSEN, R.A., AND BERGSTROM, G.C. 1987. Identification of slow rusting resistance to *Puccinia polysora* in maize inbreds and single crosses. *Plant Dis.* 71:518-521.

BECKETT, J.B. 1971. Classification of male-sterile cytoplasms in maize. *Crop Sci.* 11:724-727.

BONDE, M.R., BROMFIELD, K.R. AND MELCHING, J.S. 1982. Morphological development of *Physopella zeae* on corn. *Phytopathology* 72:1489-1491.

BULOW, J.F.W.V. 1966a. As ferrugens (*Puccinia sorghi, P. polysora,* Physopella zeae) do milho (*Zea mays*). I. *Revisio Bibliografica. Pesq. Agropec. Bras*. 1:249-262.

BULOW, J.F.W.V. 1966b. As ferrugens (*Puccinia sorghi, P. polysora*, *Physopella zeae*) do Milho (*Zea mays*). II. *Estudo Comparativo e inimigos naturais. Pesq. Agropec. Bras*. 1:289-293.

BULOW, J.F.W.V. 1967a. As ferrugens (*Puccinia sorghi, P. polysora*, *Physopella zeae*) do Milho (*Zea mays*).III. Racas da ferrugem comum (*P. sorghi*) e linhagens differenciais de milho. *Pesq. Agropec. Bras*. 2:229-235.

BULOW, J.F.W.V. 1967b. As ferrugens (*Puccinia sorghi, P. polysora*, *Physopella zeae*) do Milho (*Zea mays*). IV. Avaliacao das perdas causada Pela ferrugem comum (*P. sorghi*). *Pesq. Agropec. Bras*. 2:277-280.

CAMMACK, R.H. 1958a. *Puccinia polysora*: A review of some factors affecting the epidemiology in West Africa. *Rpt. Sixth Commonwealth Mycol. Conf.* 1960:134-138.

CAMMACK, R.H. 1958b. Studies on *Puccinia polysora* Underw. I. The world distribution of forms of *P. polysora*. *Trans. Mycol. Soc*. 41:89-94.

CAMMACK, R.H. 1959. Studies on *Puccinia polysor*a Underw. II. A consideration of the method of introduction of *P. polysora* into Africa. *Trans. Br. Mycol.* 42:27-32.

CEBALLOS, H., DEUTSCH, J.A., AND GUTIERREZ, H. 1991. Recurrent selection for resistance to *Helminthosporium turcicum* in eight subtropical maize populations. *Crop Sci.* 31:964-971.

CUMMINS, G.B. 1941. Identity and distribution of three rusts on corn. *Phytopathology* 31:356-357.

CUMMINS, G.B. 1971. *The Rust Fungi of Cereals, Grasses, and Bamboos*. Springer-Verlag, N.Y. 570pp.

DILLARD, H.R. AND SEEM, R.C. 1990. Incidence-severity relationships for common maize rust on sweet corn. *Phytopathology* 80:842-846.

HEADRICK, J.M. AND PATAKY, J.K. 1987. Expression of partial resistance to common rust in sweet corn hybrids at various host growth stages. *Phytopathology* 77:454-458.

HOLLIER, C.A. AND KING, S.B. 1985. Effect of dew period and temperature on infection of seedling maize plants by *Puccinia* polysora. *Plant Dis.* 69:219-220.

HOOKER, A.J. 1985. Corn and sorghum rusts. In: *The Cereal Rusts, Vol. II. Diseases, Distribution, Epidemiology and Control.* Roelfs, A.P. and Bushnell, W.R. (Eds.), pp.207-236. Academic Press, Inc., NY.

KIM, S.K. AND BREWBAKER, J.L. 1976. Effects of *Puccinia sorghi* rust on yield and several agronomic traits of maize in Hawaii. *Crop Sci.* 16:874-877.

KIM, S.K. AND BREWBAKER, J.L. 1987. Inheritance of resistance of sweet corn inbred IL677a to *Puccinia sorghi* Schw. *Hort. Science* 22:1319-1320.

MELCHING, J.S. 1975. Corn Rusts: types, races, and destructive potential. In: *Proc. 30th Annual Corn and Sorghum Res. Conf.*, pp. 90-115.

PAYAK, M.M. 1994. Introduction of *Puccinia polysora*, polysora rust of maize in India. *Current Sci.* 66:317-318.

PRYOR, T. 1987. Stability of alleles of Rp (resistance to *Puccinia sorghi*). *Maize Genet. Coop Newsl.* 61:37-38.

RENFRO, B.L. 1985. Breeding for disease resistance in tropical maize and its gentic control. In*: Breeding Strategies for Maize Production Improvement in the Tropics.* Brandolini, A. and Salamini, F. (Eds.), pp. 344-365. FAO of UN Instituo Agron. Pes L'oteremare Firenze.

ROBERTS, A.L. 1962. Host ranges and races of corn rusts. *Phytopathology* 52:1010-1012.

ROBINSON, R.A. 1976. Horizontal Pathosystem Management. In: *Plant Pathosystems*. Pp.96-111. Springer-Verlag, Berlin.

SCOTT, G.E. AND ZUMMO, N. 1989. Effects of genes with slow-rusting characteristics on southern corn rust in maize. *Plant Dis.* 73:114-116.

SHARMA, R.C., PAYAK, M.M., SHANKARLINGAM, S. AND LAXMINARAYAN, C. 1982. A comparison of two methods of estimating yield losses in maize caused by common rust of maize. *Indian Phytopathol.* 35:18-20.

SMITH, M.A. 1926. Infection and spore germination studies with Puccinia sorghi. *Phytopathology*. 16:69.

ULLSTRUP, A.J. 1965. Inheritance and linkage of a gene determining resistance in maize to an American race of *Puccinia polysora*. *Phytopathology* 55:425-428.

UNDERWOOD, L.M. 1897. Some new fungi chiefly from Alabama. *Bull.* Torrey Bot. Club 24:81-83.

WEBER, G.F. 1922. Studies on corn rust. *Phytopathology* 12:89-97.

WECHMAR, M.B. Von, CHAUHAN, R. AND KNOX, E. 1992. Fungal transmission of a potyvirus: urediospores of *Puccinia sorghi* transmit maize dwarf mosaic virus. *Archives of Virology, Supplementum* 5:239-250.

The Phaeosphaeria Leaf Spot

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Introduction

Phaeosphaeria leaf spot is caused by the fungus *Phaeosphaeria maydis*. This disease was first reported by Rane et al., (1965) in India and has since been reported in the Himalayas, Costa Rica, Colombia, and the United States (Shrtleff 1980; Carson et al., 1991). In Brazil, the incidence and severity of the disease have dramatically increased the last five years throughout the major corn areas of the country, including Central, Southeastern, and Southern Brazil, where it has already caused severe yield losses on susceptible cultivars. Phaeosphaeria leaf spot and the leaf blight caused by *Helminthosporium turcicum* are major problems in Central Brazil, especially when the crop is sown in the "safrinha" season (February-March).

The Disease

Symptoms

Spots first appear as pale green or chlorotic areas. As the disease progresses these turn into larger bleached or dried lesions with dark brown irregular margins. The spots are round, elongated to oblong, measuring 0.3-2.0cm, and are scattered over the leaf. Lesions may coalesce and become irregularly shaped (Shrtleff, 1980; Rane et al., 1965; Parentoni et al., 1994).

Etiology

Phaeosphaeria maydis (P. Henn.) Rane, Payak & Renfro (syn. *Sphaerulina maydis* P. Henn.) is the organism that causes this disease. In culture the fungus produces white mycelium that becomes dark with the presence of numerous pycnidia. The pycnidia are dark brown and measure 74-151µm in height and 67-159µm in width. Conidia are hyaline, uni- or bi-gutullate, ellipsoid, round and measure 2.4-5.0µm x 1.6-3.2µm (Parentoni et al., 1994). According to the observations of the author, if the mycelia of a colony grown on oatmeal agar under continuous light are removed, the fungus can sporulate profusely within 4-5 days of replication. The asci are hyaline, clavate or cylindrical, bitunicate, truncate at the base, thickened at the apex, straight or curved, and eight-spored, measuring 44.5-70.0µm x 7.5-8.5µm. Ascospores are biseriate but may become overlappingly uniseriate,

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hyaline, fusoid, straight or slightly curved, 3-septate, and slightly constricted at the septa, measuring 14.4-17.5 x 3.5-5.0 µm. In culture the fungus produces white mycelium that later becomes dark gray.

Epidemiology

The fungus persists in cultural debris in diseased plants in the field. Under favorable environmental conditions, high rainfall and relatively low night temperatures, the spores can germinate and infect maize leaves. Results indicated that under the conditions of Sete Lagoas the disease was more severe when crops were sown between May and October. A high correlation between plant age, temperature (maximum and minimum), relative humidity, and disease incidence was found. Night temperatures above 14^{0}C and relative humidity above 70% are sufficient for development (Fernandes and Sans, 1994).

Control

Genetic resistance

Of all possible control methods for this disease, the most effective strategy is the use of genetic resistance. Although there are few studies on this disease in Brazil, a number of experimental hybrids have been developed by CNPMS/EMBRAPA, especially some quality protein maize (QPM) materials, and these possess good levels of resistance (Table 1). Given the severity of the disease in maize production areas of Brazil, avoiding susceptible genotypes is advisable. In a diallel cross of eight open pollinated varieties of maize exposed to natural infection, Das et al., (1989) found the presence of dominance variance at higher levels than additive effects on the genetic control of resistance. The crosses Nabin x Comp H3, Diara x Vijay, and Super l x Vijay presented the highest levels of resistance to *P. maydis*. In another series of diallel crosses for the evaluation of resistance to *P. maydis*, one involving six Tuxpeño and another with eight flint lines, Parentoni et al., (1984), measured the general combining ability (GCA) between 1.04 and 1.03 for the Tuxpeño genotypes and from 1.83 to 1.67 for the flint lines. There was a trend for less disease severity when the cross involved lines with negative values of GCA, and the resistance was determined by a recessive gene.

Chemical Control

The urgent need for a short term control strategy suggested to CNPMS/EMBRAPA that there was a need for research on fungicidal control. A trial with six treatments, involving combinations of three fungicides at different doses, indicated that Mancozeb (2.4Kg/ha) gave the best control of the disease. No phytotoxic effect was observed in plots treated with this fungicide (Pinto, in press).

Comments

In the near future Phaeosphaeria leaf spot is expected to become a major problem for the maize crop in Brazil. The results presented in this paper indicate that much work on the establishment of adequate control strategies remains to be done. Clearly, breeding for resistance is the best strategy for the control of this disease, and the identification of maize resistant genotypes, as presented here, indicates that more intense research efforts in this direction will produce good results. It is also important to stress the need for genetic studies on the resistance to this disease and on pathogen variability, so that breeding programs can be better oriented. Studies on the pathogenicity and host range of *Phaeosphaeria maydis* are also needed. Because no genetic resistance is available yet, for the short term the use of commercial varieties of fungicides is an alternative to be considered.

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Table 1: Reaction of EMBRAPA experimental hybrids to Phaeosphaeria leaf spot in 8 locations in Brazil [1]

	Location[2]								
Cultivar	**A[1]**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**Mean**
BR201	4.0	2.0	3.0	3.0	3.0	1.0	3.0	4.0	2.87
HD1X	4.0	3.0	4.0	3.0	3.0	3.0	4.0	4.0	3.50
HD2X	4.0	3.0	3.0	3.0	4.0	2.0	3.0	4.0	3.25
HD9176	4.0	2.0	4.0	3.0	4.0	1.0	4.0	4.0	3.25
HD9267	2.0	2.0	3.0	2.0	2.0	1.0	3.0	3.0	2.25
HDM9274	2.0	3.0	3.0	3.0	3.0	2.0	3.0	4.0	2.87
HD9230	4.0	3.0	4.0	3.0	4.0	2.0	4.0	4.0	3.50
HDMS01	2.0	3.0	3.0	2.0	2.0	1.0	3.0	3.0	2.37
HD9226	2.0	3.0	4.0	3.0	4.0	1.0	4.0	4.0	3.12
93HD3QPM	2.0	2.0	2.0	2.0	2.0	1.0	3.0	4.0	2.25
93HD30QPM	2.0	2.0	2.0	2.0	2.0	1.0	3.0	2.0	2.00
94HD32QPM	2.0	2.0	2.0	3.0	2.0	1.0	3.0	3.0	2.25
94HT31QPM	1.0	2.0	2.0	2.0	2.0	1.0	3.0	3.0	2.00
92HD1QPM	2.0	2.0	3.0	2.0	2.0	1.0	3.0	4.0	2.37
BR206	4.0	3.0	4.0	3.0	4.0	1.0	4.0	4.0	3.37
BR205	4.0	3.0	4.0	3.0	4.0	2.0	4.0	4.0	3.50
HT2X	2.0	4.0	4.0	3.0	4.0	2.0	4.0	4.0	3.37
AG122	3.0	2.0	3.0	3.0	4.0	1.0	3.0	4.0	2.87
P3041	2.0	2.0	3.0	2.0	3.0	1.0	3.0	4.0	2.50

1. 0 = No lesions formed; 1 = lesions sparsely distributed; 2 = lesions on 50% of the leaves and 25% disease severity; 3 = lesions on 75% of the leaves and 50% disease severity; 4 = lesions on 100% of the leaves and 75% disease severity; 5 = lesions on 100% of the leaves and 100% disease severity.
2. A – Goianésia; B – Goiânia (EMGOPA); C – Goiânia (CNPAF); D – Rio Verde; E – Santa Helena; F – Campo Grande de Hatã; G – Uberlândia; H – Goiatuba.

References

CARSON, M. L., GOODMAN, M. M., GLAWE, D. A. 1991. Phaeosphaeria leaf spot of maize in Florida. *Plant Disease* 75: 968.

DAS, S. N., SINHAMAHAPATRA, S. P., and BASK, S. L. 1989. Inheritance of resistance to Phaeosphaeria leaf spot of maize. *Annals of Agric. Res*. 10:182-184.

FERNANDES, F. T., SANS, L. M. A. 1994. Influência das condições climáticas na ocorrência das lesões foliares por Phaeosphaeria maydis. *XX Congresso Brasileiro de Milho e Sorgo. Resumos*. Goiânia. 1994. P. 136

PARENTONI, S. N., TAVARES, F., MAGNAVACA, R., GAMA, E. E. G., CASELA, C. R., LOPES, M. A., EVARISTO, P. E. O., SANTOS, M. X., ELEUTÉRIO, A. S., OLIVEIRA, A. C. 1994. Avaliação de cruzamentos dialélicos para tolerância a Phyllosticta sp. Em milho. XX Congresso Brasileiro de Milho e Sorgo. Resumos. Goiânia. 1994. P. 18.

PINTO, N. F. J. A. Avaliação da eficiência de fungicidas no controle das doenças foliares de milho. *Summa Phytopathologica* (in press).

RANE, M. S., PAYAK, M. M., and RENFRO, B. L. 1965. A Phaeosphaeria leaf spot on maize. *Indian Phytopathological Soc. Bull.* 3:7-10.

SHRTLEFF, M.C. 1980. (ed). *Compendium of corn diseases*. Phaeosphaeria leaf spot. *The American Phytopathological Society*, St. Paul. Pp. 23-24.

Dalbulus maidis Identification, Biology, Ecology and Pest Status

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Identity

Taxonomy And Nomenclature

The corn leafhopper was described by DeLong and Wolcott in 1923 from specimens collected from Puerto Rico. The leafhopper was described as *Cicadula maidis* but later transferred to the genus *Baldulus*. In 1960 DeLong described several new leafhopper species and erected the genus *Dalbulus*. He transferred two species, the Mexican corn leafhopper, *D. elimatus*, and the corn leafhopper (from *Baldulus* to *Dalbulus*).. In addition to these maize specialists, 15 related leafhopper species have been described.

Host Plants

Field hosts are members of the genus *Zea,* and include annual and perennial wild teosinte species in addition to the domesticated maize or corn, *Zea mays*. Occasionally breeding populations may be found on the gamagrasses (*Tripsacum*) that are close relatives of *Zea*. Most *Dalbulus* and *Baldulus* species specialize on the gamagrasses where they produce populations. A few of these *Dalbulus* species use maize as a secondary breeding host in Mexico and Guatemala.

Geographical Distribution

The corn leafhopper is found (or likely to be found) everywhere in the neotropics where maize is grown. The leafhopper can be found at all elevations where maize is found. In Peru, for example, the corn leafhopper has been found in habitats ranging from sea level near Lima to 3,200 m above sea in the Andean valleys.

Biology And Ecology

Throughout its range of distribution, a minimum of two generations of corn leafhoppers develop on a single maize crop. This would be the situation at high elevations or where there is a short rainy season. Where there is a long rainy season and several overlapping crops are grown, many more leafhopper generations are produced. In regions where maize is grown year round with irrigation during the dry season, theoretically more than a dozen generations could develop.

At 25C the generation time for the corn leafhopper is approximately 25-30 days. Eggs hatch about 9 days after being laid and development through the five nymphal stages takes approximately 17 days. Adult females will mate and begin laying eggs within 1-2 days after eclosure. The mean survival of adults is 7-8 weeks at 26C with some individuals living 15 weeks. Females lay 400-500 eggs over their life-time.

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In regions where maize is grown continuously with assistance from irrigation, the corn leafhopper maintains breeding populations year-round. In such cultivation practices the leafhopper populations become very high and the insect has a significant economic impact. In regions where there is a dry season and irrigated corn is not grown, leafhoppers leave corn fields after the crop matures to over-season in as yet undiscovered habitats.

Experiments have been shown that adult leafhoppers developing on maturing corn under late-season environmental conditions can survive for many weeks without hosts so long as they have access to some moisture in the form of free water or a dry-season food host.

In maize planted at the beginning of the rainy season, immigrating adults infest the whorls of seedlings where they feed and lay eggs. Observations suggest that these are local immigrants that may have over-seasoned in nearby protected habitats. First activity and host searching is stimulated by spring rains. Other observers have suggested that leafhoppers also may migrate or be carried great distances by tropical storms. The latter possibility was suggested for epidemics of corn stunt in southern Florida; leafhoppers appearing in Florida were thought to originate from the Caribbean Islands.

Natural Enemies

Little is known about the predators of the corn leafhopper except that generalists would presumably have some impact on populations. Hymenopteran parasites have been reported to attack nymphs, adults, and eggs, but it is not known if these enemies will prove to be effective in control programs.

Pest Significance and Economic Impact

The corn leafhopper has only rarely been reported a direct pest of maize (i.e. by damage caused by feeding and decline of its host by removal of plant sap). The leafhopper is a significant pest because it is a vector for three stunting pathogens: the corn stunt spiroplasma (CSS) (*Spiroplasma kunkelii*), the maize bushy stunt phytoplasma (MSBSP), and the maize rayado fino marafivirus (MRFV). At least one if not all three pathogens have been reported present wherever the corn leafhopper is found. The diseases frequently occur sporadically in many regions, but they can cause widespread and serious disease in newly introduced non-adapted varieties—especially where maize is grown year round with irrigation during the dry season. The most severe damage occurs when plants are inoculated in the seedling stage; however, grain yield may be significantly decreased even when plants are infected at the 16-leaf-stage.

All three pathogens are persistently transmitted by the corn leafhopper (i.e. once an insect becomes a vector it transmits for life). All three pathogens multiply in the vector and undergo a latent period. After the corn leafhopper acquires the pathogen by feeding on an infected plant, a period of 2-weeks for MRFV and 3-4 weeks for CSS and MBSP must pass before vectors become inoculative.

Identification

Symptoms of Stunting Diseases on Maize

CSS is characterized by broad yellow streaks that first appear on the bases of newly infected leaves and on all leaves that subsequently develop. At about the same time, the older leaves may turn red or a golden yellow color. First symptoms appear 3-4 weeks after plants are inoculated by infective corn leafhoppers. Mature infected plants are stunted, tassels are deformed, and ears are small, deformed, or absent. When older plants are infected, the characteristic yellow streaks may be confined to the bases of the oldest leaves, to the busk leaves on the ear, or they may be absent. Other symptoms would include some slight stunting and reddening of older leaves similar to symptoms caused by MBSP.

MBSP is characterized by an extensive reddening and yellowing of leaves (more so than with CSS infections), stunting of plants, proliferation of basil tillers and axillary shoots, and the formation of many small barren ears giving plants a bushy appearance. The tassel is often absent. First symptoms, a reddening of older leaves, appear 2-3 weeks after plants are inoculated. The yellow streaks produced by CSS infections are not manifested in plants infected with MBSP. The symptoms in late infections are difficult to distinguish from CSS infection.

MRFV symptoms appear in maize seedlings 7-10 days after inoculation as a series of small chlorotic spots that soon coalesce to form fine chlorotic streaks, thus the spanish name rayado (rays or streaks) fino (fine). Susceptible genotypes are stunted and may not produce ears or tassels. The extensive reddening and yellowing associated with CSS and MBSP infections does not occur in maize infected with MRFV.

Morphology Of Corn Leafhopper

Females insert eggs with their ovipositor, usually into the leaf and onto the midvein. Several eggs may be laid in a row. Ovipositing females frequently can be observed in the whorls of corn seedlings where they prefer to lay eggs. The oval eggs, less than 1 mm long and 1/5 mm wide, are practically colorless and difficult to see when first laid. After 7-10 days they become white and red eye spots develop, making them easier to see. A few days after eggs are laid, a tuft of diagnostic microfilaments develop and protrude from the proximal end of the egg. Filaments can be readily seen with a 10x hand lens. Under warm summer temperatures eggs hatch in 9-12 days, but they may take several weeks at cooler temperatures.

There are five nymphal instars. First instars are less than 1 mm long whereas the last instar is just under 4 mm long. The nymphs are pale yellow and the eyes are black. A pair of irregular black spots appear on the anterior margin of the last two abdominal tergites in the second through fifth instars. In the fifth instar the mesothoracic and metathoracic wing buds extend posteriorly over the abdomen.

The length of adults varies from 3.7-4.3 mm. Background color is light yellow with variable black spots on the abdomen. Specimens from higher elevations and those that develop at cool temperatures have more and larger spots than those that are taken from lower elevations or that develop at warm temperatures. Two large spots more than twice the diameter of the ocelli are always present on the head over the ocelli. Serveral other spots may occur on the head of specimens whose overall color is dark. The corn leafhopper can be distinguished from its congeners that regularly or occasionally occur on maize by the number and size of spots on the head and by the shape of the aedeagus in the male.

Detection And Inspection Methods For Leafhopper

Corn leafhopper adults can be readily seen infesting the whorls of seedling maize plants as well as in older plants when the whorl can be readily observed from above. Inspection of plants in the early morning hours when leafhoppers are less active facilitates detection of the insects.

Diagnostic Methods For Pathogens

As noted earlier, the identification of stunting pathogens by symptomatology in plants is difficult. Not only can there be an overlap in symptom expression by these three pathogens but in plants infected by two or more pathogens, symptoms of one disease can mask the others. Dark field light microscopy can be used to detect the spiroplasmas associated with CSS, but enzyme-linked immunosorbant assay (ELISA) is a more reliable method. Recombinant DNA techology has provided probes useful for detecting MBSP by dot blot and Southern blot assays. ELISA has been used to detect MRFV in field-collected plants and leafhoppers.

Control

Suprisingly little information has been published on control of the corn leafhopper and corn stunting diseases. Control tactics can be directed at the vector, the pathogens, or both. The systemic oxydemeton-methyl and the contact insecticide, carbaryl, provided the best control of the corn leafhopper. Using pesticides to control the leafhopper when maize plants are young can greatly reduce disease incidence, but in most maize growing regions in Latin America such practice is probably not economically feasible.

Perhaps the most effective measure to avoid serious disease outbreaks is to avoid planting maize year round. Planting maize under irrigation during the dry season encourages the build-up of vectors and the spread of disease. If irrigation is used, a one or two month maize-free period prior to the rainy season will help to decrease leafhopper populations.

A series of local varieties tolerant to stunting disease have been identified in Nicaragua and elsewhere. Whether or not the tolerance is to the vector, to the pathogens, or to both is not known. Introduced varieties often prove highly susceptible to corn stunt. It has been suggested that rouging diseased plants when leafhopper densities and ambient temperatures are high will also reduce disease losses. In Central America, nitrogen fertilization, varying plant densities, and intercropping maize with beans does not reduce disease incidence.

References

ALIVIZATOS, A.S. 1983. Acquisition of corn stunt spiroplasma by the leafhopper *Dalbulu maidis*. *Annales de I'Institur Phytopathologique* Benaki 14:101-109.

BAJET, N.B., RENFRO, B.L. 1989. Occurence of corn stunt spiroplasma at different elevations in Mexico. *Plant Disease* 73:926-930.

BARNES, D. 1954. Biologia, ecologia, Y distribucion de las chicharritas, *lus elimarus* (Ball) y *Dalbulus maidis* (Del. And W.). *Follero Technico Nº 11, Secretaria de Agricultura y Ganaderia*. Oficina de Estudios Especiales, Mexico, D.F., 112pp.

BUSHING, R.W., BURTON, V.E. 1974. Leafhopper damage to silage corn in California. *J. Economic Entomology* 67:656-658.

CASTRO, V., RIVERA, C., ISARD, S.A., GAMEZ, R., FLETCHER, J., IRWIN, M.E. 1992. The influence of weather and microclimate on *Dalbulus maidis* (*Homoptera: Cicadellidae*) flight activiry and the incidence of diseases within maize and bean monocultures and bicultures in tropical America. *Annals Applied Biology* 121:469-482.

DAVIS, M.J., TSAI, J.H., COX, R.L., McDANIEL, L., HARRISON, N.A. 1988. Cloning of chromosomal and extra-chromosomal DNA of the mycoplasma-like organism that causes maize bushy stunt disease. *Molecular Plant-Microbe Interactions* 1:295-302.

GAMEZ, R., LEON, P. 1983. Maize rayado fino virus: evolution with plant host and insect vector. *Current Topics in Virus Research* 1:149-168.

GLADSTONE, S.M., de la LLANA, A., RIOS, R., LOPEZ, L. 1994. Egg parasitoids of the corn leafhopper *Dalbulus maidis* (DeLong and Wolcott) (*Homoptera: Cicadellidae*) in Nicaragua maize. *Proceedings Entomological Society of Washington* 96:143-146.

GORDON, D.T., NAULT, L.R., GORDON, N.H., HEADY, S.E. 1985. Serological detection of corn stunt spiroplasma and maize rayado fino virus in field-collected *Dalbulus* spp. From Mexico. *Plant Disease* 69:108-111.

HEADY, S.E., NAULT, L.R. 1984. Leafhopper egg microfilaments (*Homoptera: Cicadellidae*). *Annals Entomological Society of America*. 77:610-615.

LARSEN, K.J., NAULT, L.R. 1994. Seasonal polyphenism of adult *Dalbulus* leafhoppers (*Homoptera: Cicadellidae*). *Annals Entomological Society of America* 87:355-362.

LARSEN, K.J., NAULT, L.R., MOYA-RAYGOZA, G. 1992. Overwintering biology of *Dalbulus* leafhoppers (*Homoptera: Cicadellidae*). Adult populations and drought hardiness. *Environmental Entomology* 21:566-577.

NAULT, L.R. 1980. Maize bushy stunt and corn stunt: A comparison of disease symptoms, pathogen host ranges and vectors. *Physopathology* 70:659-662.

NAULT, L.R. 1990. Evolution of an insect pest: maize and the corn leafhopper, a case study. *Maydica* 35:165-175.

NAULT, L.R., BRADFUTE, O.E. 1979. Corn stunt: Involvement of a complex of leafhopper-borne pathogens. In: *Lefhopper vectors and plant disease agents* (Ed. By Maramorosch, K., Harris, K.F.) pp. 561-586. Academic Press, N.Y.

NAULT, L.R., GORDON, D.T., GINGERY, R.E., BRADFUTE, O.E., CASTILLO LOAYZA, J. 1979. Identification of maize viruses and mollicutes and their potential insect vectors from Peru. *Phytopathology* 69:824-828.

NAULT, L.R., GORDON, D.T., CASTILLO LOAYZA, J. 1981. Maize virus and mycoplasma diseases in Peru. *Tropical Pest Management* 27:363-369.

NAULT, L.R., KNOKE, J.K. 1981. Maize vectors. In: Virus and viruslike diseases of maize in the United States (Ed. By Gordon, D.T., Knoke, J.K., Scott, G.E.) pp. 77-84. Southern Cooperative Series Bulletin 247. June 1981. *Ohio Agricultural Research and Development Center*, Wooster, Ohio, USA.

NAULT, L.R., MADDEN, L.V. 1985. Ecological strategies of *Dalbulus* leathoppers. *Ecological Entomology* 10:57-63.

POWER, A.G. 1989. Influence of plant spacing and nitrogen fertilization in maize on *Dalbulus maidis* (*Homoptera: Cicadellidae*) vector of corn stunt. *Environmental Entomology* 18:494-498.

POWER, A.G. 1992. Host plant dispersion, leafhopper movement and disease transmission. *Ecological Entomology* 17:63-68.

TAYLOR, R.A.J., NAULT, L.R., STYER, W.E. 1993. Experimental analysis of flight activity of three *Dalbulus* leafhoppers (*Homoptera: Auchenorrhyncha*) in relation to migration. *Annals Entomological Society of America* 86:655-667.

TODD, J.L., MADDEN, L.V., NAULT, L.R. 1991. Comparative growth and spacial distribution of *Dalbulus* leafhopper populations (*Homoptera: Cicadellidae*) in relation to maize phenology. *Environmental Entomology* 20:556-564.

TRIPICHORN, B.W., NAULT, L.R. 1985. A phylogenetic classification of the genus *Dalbulus* (Homoptera: Cicadellidae) and notes on the phylogeny of the Macrostelini. *Annals Entomological Society of America* 78:291-315.

TSAI, J.H., DeLONG, D.M. 1990. Descriptions of new species of *Dalbulus* and *Balclurha* from Equador (*Homoptera: Cicadellidae: Deltocephalinae*). *Entomotaxonomia* 11:283-288.

TSAI, J.H., STEINBERG, B., FALK, B.W. 1990. Effectiveness and residual effects of seven insecticides on *Dalbulus maidis* (*Homoptera: Cicadellidae*) and *Peregrinus maidis* (*Homoptera: Delphacidae*). *Journal of Entomological Scien*ce 25:106-111.

VANDERMEER, J., POWER, A. 1990. An epidemiological model of the corn stunt system in Central America. *Ecological Modelling* 52:235-248.

VEGA, F.E., BARBOSA, P., PÉREZ, P.A. 1991. Eudorylas (Matadorylas) sp. (*Diptera: Pipunculidae*): a previously unreported parasitoid of *Dalbulus maidis* (DeLong and Wolcott) and *Dalbulus elimatus* (Ball) (*Homoptera: Cicadellidae*). *Canadian Entomologist* 123:241-242.

Maize Viruses and Mollicutes: Interactions Between the Host and Pathogens

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Introduction

Most of the viruses and mollicutes referred to as etiological agents of diseases on maize (*Zea mays* L.) have been found in several parts of Brazil. These include maize rayado fino virus (MRFV), a *marafivirus* (Gamez, 1980) in the states of the São Paulo (Costa et al., 1971), Ceará (Lima and Gamez, 1982), Paraná (Kitajima and Nazareno, 1985), and Rio de Janeiro (Kitajima et al., 1986); maize mosaic virus (MMV), a *rhabdoviridae* (Herold, 1972) in the states of São Paulo (Costa et al., 1971), Rio de Janeiro (Kitajima et al., 1986), Amazonas, the Federal District (Kitajima and van der Pahlen, 1977), and Rio Grande do Norte (Oliveira et al., 1992); maize dwarf mosaic virus (MDMV), a *potyvirus* (Shukla et al., 1989) in the states of São Paulo (Costa et al., 1971), Rio Grande do Sul (Hagedorn et al., 1969), Rio de Janeiro (Kitajima et al., 1985), and Paraná (Kitajima and Nazareno, 1986); cucumber mosaic virus (CMV), a *cucumovirus* in the state of São Paulo (Costa and Kitajima, 1972); corn stunt spiroplasma (*Spiroplasma kunkeli*) in the states of São Paulo (Costa et al., 1971), Pernambuco (Costa and Kitajima, 1973), Paraná (Kitajima and Nazareno, 1985), Rio de Janeiro (Kitajima et al., 1986), and Santa Catarina (Milanez, 1985); and maize bushy stunt phytoplasma in the states of São Paulo (Costa et al., 1971) and Pernambuco (Costa and Kitajima, 1973). There is an unconfirmed report of the presence of a virus similar to maize rough dwarf virus (MRDV), a *fijivirus* (Lovisolo, 1971) in the state of São Paulo (Trevisan et al., 1986). This virus is already known in Argentina, where it causes a disease known locally as "mal de Rio IV" (Nome et al., 1981; Fernandez-Valiela, 1995). A list of publications on maize viruses and mollicutes found in Brazil is available (Kitajima, 1986, 1995).

Other maize viruses described on the American continents are maize stripe virus (MStV, *tenuivirus* [Gingery, 1985]), maize chlorotic mottle virus (MCMV, m*achlovirus* [Gordon et al., 1984]), maize chlorotic dwarf virus (MCDV, s*equiviridae, waikavirus* [Gingery, 1988]), and maize white line mosaic virus (MWLMV, [de Zoeten and Reddick, 1984]) have not yet been found in Brazil. Viruses found infecting maize on the African continent include: maize streak virus (*geminvirus*), maize mottle/chlorotic stunt virus, maize eyespot virus, guinea grass mosaic virus (*potyvirus*), cynodon chlorotic streak virus (*rhabdoviridae*), maize yellow stripe virus (*tenuivi-*

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rus), brome mosaic virus (*bromoviridae* and *bromovirus*), barley stripe mosaic virus (*hordeivirus*), and barley yellow dwarf virus (*luteovirus* (Thottappilly et al., 1993). None of these have been described in Brazil.

In past years, there were no significant losses in yield caused by viral diseases in maize. Their incidence was usually below 1%. The corn stunt spiroplasma has caused some recent losses when maize crops were planted out of season to gain cycles during breeding programs or to produce corn for *in natura* consumption (A.S. Costa, personal communication). In recent years, however, the so-called "safrinha" system was established, in which maize is planted practically all year and thus continuously maintains both inocula and vectors. As a result, significant losses have occurred from an unidentified disease that produces symptoms (stunting and reddening of leaves) resembling those of maize bushy dwarf (Oliveira et al., 1995).

All these pathogens represent potential threats to maize productivity. In the event of epidemiological surges, the ability to rapidly and precisely diagnose and identify the causal agents will be necessary to design control measurements.

The following is a review of the research carried out in Brazil on the interaction of these pathogens (virus, spiroplasma, and phytoplasma) with maize plants and their vectors, and the progress of this work towards their characterization and pathogenesis.

Maize rayado fino virus (MRFV)

This virus has not been important in Brazil (Costa et al., 1971), but there are reports of important losses in Central America, especially in varieties which are not locally adapted (Gamez, 1980). It is an isometric virus *ca*. 30nm in diameter, not transmissible mechanically, and has a single ssRNA genome. It is primarily spread by the leafhopper *Dalbulus maidis* (De Long and Walcott). The molecular aspects of MRFV are well characterized (Leon and Gamez, 1986). In Brazil, the virus is known as "risca do milho." It is indistinguishable serologically from the MRFV originally described in Costa Rica and has similar cytopathic effects.

Presumed viral particles have been observed in the cytoplasm and vacuoles of infected cells, which occasionally produce crystalline arrays in cells next to necrotic ones. These cells also commonly contain groups of vesicles, possibly associated with viral replication (Kitajima et al., 1976; Kitajima and Gamez, 1977). Similar particles were observed in several organs (midgut, fat bodies, and salivary gland) of viruliferous *D. Maidis*, usually in cavities resembling lysosomes, but no cell change suggesting viral replication was noticed (Kitajima and Gamez, 1983). Rivera and Gamez (1986), however, have presented evidence that this may occur.

Maize dwarf mosaic virus (MDMV)

MDMV is a potyvirus, non-persistently transmitted by aphids, and mechanically transmissible. For a long time it was considered a strain of the sugarcane mosaic virus (SCMV); in fact, SCMV is known to infect maize under experimental conditions when it has been used as an indicator plant (Costa and Penteado, 1951). However, recent molecular work indicates that it is sufficiently different and should be considered a distinct virus (Shukla et al., 1989). Their particles are long and flexuous (10 x 750 nm). Like other potyviruses, it induces in infected cells the appearance of typical lamellar inclusion that depending on the section angle produces the so called "pinwheel" configuration. These inclusions were classified by Edwardson (1974) and those induced by MDMV fit in type 3. No critical work characterizing the Brazilian isolates of MDMV has been done.

Maize mosaic virus (MMV)

2-5 mm wide chlorotic stripes along the leaf veins are the characteristic symptoms caused by MMV in infected maize plants. For this reason the disease has been referred to as "chlorotic vein banding" in Brazil (Costa et al., 1971). Infected plants are also stunted. If the infection occurs in young plants, no ears develop.

MMV is a rhabdovirus transmitted in a circulative-propagative manner by the leafhopper *Peregrinus maidis* Ashmead, but it is not mechanically transmissible. Particles of Brazilian isolates of MMV are membrane-bound, *ca.* 50-70 x 300 nm, bacilliform in shape and contained within the cytoplasm in endoplasmic reticulum cavities which may form a complex three dimensional array of tubules. Amorphous masses (viroplasm), possibly of nucleocapsids, are commonly present in nearby viral aggregates. Chloroplasts often present a disorganized lamellar system. Presumed MMV particles were also found in many organs (midgut, fat bodies, muscle, salivary gland, and nervous ganglia) of viruliferous *P. maidis* (Kitajima and Costa, 1982). In the last report of the International Committee for Virus Taxonomy, (Murphy et al., 1995) MMV is included in the genus *Nucleorhabdovirus,* but the Brazilian isolate must be a *Cytorhabdovirus*.

Mollicutes (corn stunt spiroplasma and maize bushy dwarf phytoplasma)

The early descriptions of corn stunt disease made a distinction between the so-called Rio Grande type (causing chlorotic stripes in the leaves) and the Mesa Central type (causing reddening of the leaves). Both are transmitted by leafhopper; *Dalbulus maidis* is the best known vector. After the discovery of mycoplasma-like organisms (MLO) associated with several yellows type diseases in plants (Doi et al., 1967), Granados et al.(1968) demonstrated the presence of MLO in the sieve tubes of maize plants affected by Rio Grande type corn stunt. Soon after, Davis and Worley (1973) showed that the mollicute associated with this disease has a helical shape and they coined the term spiroplasma, which became the genus name (*Spiroplasma*) for this type of cultivable organism. Subsequent works revealed that the Mesa Central type was a pleomorphic MLO (now designated *Phytoplasma*) and designated maize bushy dwarf (Bradfute et al., 1977) , which is related to the aster yellows agent (Lee et al., 1993). Both corn stunt spiroplasma (CSS) and maize bushy dwarf phytoplasma (MBDP) are easily observed in sieve tubes of infected plants in thin sections. They appear as single membrane bounded bodies containing DNA filaments and ribosome granules. Although in thin sections both spiro- and phytoplasmas appear similar, in thicker sections the helical form of CSS may be seen (Davis and Worley, 1973). This is also clearly seen by scanning electron microscopy (Massola and Kitajima, 1997). Antibodies raised against CSS labelled specifically its membrane in *in situ* immunocytochemical experiments (Massola and Kitajima, 1997). The thin section of *D. Maidis*, viruliferous for corn stunt, revealed the presence of wall-less prokaryotes in several tissues, including the salivary gland (Kitajima and Costa, 1982). CSS is known to be deleterious to the leafhopper vector (Banttari and Zeyen, 1979).

Final comments

Studies on the interaction of viruses and mollicutes with maize plants or their vectors are producing important data that complements the characterization of these pathogens and assists in their detection through diagnostic procedures. For example, symptoms induced by MRFV and MMV sometimes may be confusing, but their cytopathic effects are clearly distinct enough to permit their correct identification. Confirmation of the presence of mollicutes can be made easily by the observation of the pleomorphic or helical bodies in the sieve tubes. Detection of viruses or mollicutes in the vectors not only helps us understand the pathogen cycle in these organisms, but also provides important information for epidemiological studies. On the other hand, immunocyto-chemical and *in situ* hybridization experiments provide clues to follow the replicative processes of the pathogens and the phenomena related to resistance against them.

References

BANTTARI, E.E. AND ZEYEN, R.J. 1979. Interactions of mycoplasmalike organisms and viruses in dually infected leafhoppers, planthoppers and plants. *In* Maramorosch, K. and Harris, K.F. eds. Leafhopper vectors and plant disease agents. New York, Academic Press. Pp. 327-347.

BRADFUTE, O.E., NAULT, L.R., ROBERTSON, D.C. and TOLER, R.W. 1977. Maize bushy stunt: a disease associated with a non-helical mycoplasma-like organism. Amer. Phytopath. Soc. Proc. 4: 171.

COSTA, A.S. and KITAJIMA, E.W. 1972. Ocorrência do mosaico em milho no Estado de São Paulo, causado pelo vírus do mosaico do pepino. (abstr.) Rev.Soc. Bras. Fitopatol. 5: 159-160.

COSTA, A.S. and KITAJIMA, E.W. 1973. Ocorrência do enfezamento do milho em plantações de Petrolina, PE. (abstr.) Fitopatologia (Lima) 8: 7.

COSTA, A.S. and PENTEADO, M.P. 1951. Corn seedlings as test plants for the sugarcane mosaic virus. Phytopathology 41: 758-763.

COSTA, A.S., KITAJIMA, E.W. and ARRUDA, S.C. 1971. Moléstias de vírus e de micoplasma do milho em São Paulo. Rev. Soc. Bras. Fitopatol. 4: 39-41.

DAVIS, R.E. and WORLEY, J.F. 1973. Spiroplasma: motile, helical microrganism associated with corn stunt disease. Phytopathology 63: 403-408.

DE ZOETEN, G.A. and REDDICK, B.B. 1984. Maize white line mosaic virus. CMI/AAB Description of plant viruses no. 283. 4pp.

DOI, Y, TERANAKA, M., YORA, K. and ASUYAMA, H. 1967. Mycoplasma- or PLT-like organism found in the phloem elements of plants infected with mulberry dwarf, potato "witches' broom", aster yellows, or Paulownia "witches' broom. Ann. Phytopathol. Soc. Japan 33: 259-266.

EDWARDSON, J.R. 1974. Some properties of the potato Y virus-group. Fla. Agric. Expt. Sta. Monog. Series. Gainesville, IFAS/Ufla. 398pp.

FERNANDEZ-VALIELA, M.V. 1995. Virus patógenos de las plantas y su control. 4a ed. Serie no.14, Buenos Aires, Academia Nacional de Agronomia y Veterinaria. 2 vols.

GAMEZ, R. 1980. Maize rayado fino virus. CMI/AAB Description of plant viruses no. 220. 4pp.

GINGERY, R.E. 1985. Maize stripe virus. CMI/AAB Description of plant viruses no. 300. 4pp.

GINGERY, R.E. 1988. Maize chlorotic dwarf and related viruses. *In* Koenig, R. ed. Plant viruses with monopartite RNA. Vol.3. New York, Plenum Press. Pp. 259-272.

GORDON, D.T., BRADFUTE, O.E., GINGERY, R.E., NAULT, L.R. and UYEMOTO, J.K. 1984. Maize chlorotic mottle virus. CMI/AAB Description of plant viruses no. 284. 5pp.

GRANADOS, R.R., MARAMOROSCH, K. and SHIKATA, E. 1968. Mycoplasma, suspected etiologic agent of corn stunt. Proc. Natl. Acad. Sci. U.S. 60: 841-844.

HAGEDORN, D.J.,GRILL, J.P. and DAVIS, J.R. 1969. Diseases of corn and soybeans in Rio Grande do Sul. Plant Dis. Reptr. 53: 165-168.

HEROLD, F. 1972. Maize mosaic virus. CMI/AAB Description of plant viruses no. 94. 4pp.

KITAJIMA, E.W. 1986. Lista de publicações sobre viroses e enfermidades correlatas de plantas no Brasil (1911-1985). Fitopatol. Bras. Supl. 95. 23pp.

KITAJIMA, E.W. 1995. Lista de publicações sobre viroses e enfermidades correlatas de plantas no Brasil (1986-1993). Fitopatol. Bras. Supl.

KITAJIMA, E.W. and COSTA, A.S. 1972. Microscopia eletrônica de organismos do tipo micoplasma nos tecidos do milho afetado pelo enfezamento e nos orgãos da cigarrinha portadora. Bragantia 31: 76-82.

KITAJIMA, E.W. and COSTA, A.S. 1982. The ultrastructure of the corn chlorotic vein banding (Brazilian maize mosaic) virus-infected corn leaf tissues and viruliferous vector. Fitopatol. Bras. 7: 247-259.

KITAJIMA, E.W. and COSTA, A.S. 1983. Maize viruses and mycoplasma in Brazil. *In* Gordon, D. et al. Eds. Proc. 2nd Int.Colloq.Workshop on Maize Viruses and Mycoplasma. Wooster, OARDC. 1983. Pp.100-103.

KITAJIMA, E.W. and GAMEZ, R. 1977. Histological observatons on maize leaf tissues infected with rayado fino virus. Turrialba 27: 71-74.

KITAJIMA, E.W. and GAMEZ, R. 1983. Electron microscopy of rayado virus in the internal organs of its leafhopper vector. Intervirology 19: 129-134.

KITAJIMA, E.W. and NAZARENO, N.R.X. 1985. Levantamento de virus e molicutes do milho no Estado do Paraná. Fitopatol. Bras. 613-625.

KITAJIMA, E.W. and VAN DER PAHLEN, A. 1977. Ocorrência do virus da faixa clorótica das nervuras do milho no Distrito Federal e em Manaus. (abstr). Fitopatol. Bras. 2: 83-84.

KITAJIMA, E.W., LIN, M.T., and GAMEZ, R. 1972. Serological and histological comparison between maize "rayado fino" and Brazilian corn streak viruses. (abstr.). Proc. Amer. Phytopathol. Soc. V.2. C-15.

KITAJIMA, E.W., YANO, T. and COSTA, A.S. 1976. Purification and intracellular localization of isometric viruslike particles associated with Brazilian corn streak virus infection. Cien. Cult. 28: 427-430.

KITAJIMA, E.W., RIBEIRO, R.L.D., LIN, M.T., RIBEIRO, M.I.S.D., KIMURA, O., COSTA, C.L. and PIMENTEL, J.P. 1986. Lista comentada de virus e organismos do tipo micoplasma em planas cultivadas e silvestres do Estado do Rio de Janeiro. Fitopatol. Bras. 9: 607-625.

LEE, I.M., HAMMOND, R.W., DAVIS, R.E. and GUNADERSEN, D.E. 1993. Universal amplification and anaysis of pathogen 16S rDNA for classification of mycoplasmalike organisms. Phytopathology 83: 834-842.

LEON, P. and GAMEZ, R. 1986. Biologia molecular del virus del rayado fino del maiz. Rev. Biol. Trop. 34: 111-114.

LIMA, J.A.A. and GAMEZ, R. 1982. Identificação sorológica do vírus do rayado fino do milho. No Estado do Ceará. (abstr.). Fitopatol. Bras. 7: 535.

LOVISOLO, O. 1971. Maize rough dwarf mosaic virus. CMI/AAB Description of plant viruses no. 72. 4pp.

MASSOLA FO., N. and KITAJIMA, E.W. 1977. Scanning electron microscopy and *in situ* immunolabelling of the corn stunt spiroplasma. Acta Microscopica 6 (Supl.B): 176-177.

MILANEZ, J.M. 1985. Ocorrência do enfezamento do milho em Santa Catarina. Com.Tecn. 90.EMPASC. 11pp.

MURPHY, F.A., FAUQUET, C.M., BISHOP, D.H.J.L., GHABRIAL, S.A., JARVIS, A.W., MARTELLI, G.P., MAYO, M.A. and SUMMERS, M.D. 1995. Virus taxonomy. Sixth Report of the International Committee on Taxonomy of Viruses. Arch.Virology Supl.10. 586pp.

NOME, S.F., LENARDON, S.L., RAJU, B.C., LAGUNA, I.G., LOWE,S.K. and DO CAMPO, D. 1981. Association of reovirus-like partaicles with "Enfermedad del Rio IV" of maize in Argentina. Phytopathol. Z. 101: 7-15.

OLIVEIRA, E., WAQUIL, J.M., FERNANDES, F.T., PAIVA, E., RESENDE, R.O. and KITAJIMA, E.W. 1995. Doenças de enfezamento na cultura do milho no Brasil Central. (abstr.). Fitopatol. Bras. 20: 283.

OLIVEIRA, F.C., PINHEIRO, C.S.R. and KITAJIMA, E.W. 1992. Ocorrência do vírus da faixa clorótica das nervuras do milho no Estado do Rio Grande do Norte. Fitopatol. Bras. 17: 339-342.

RIVERA, C. and GAMEZ, R. 1992. Multiplication of maize rayado fino virus in the leafhopper vector *Dalbulus maidis*. Intervirology 25: 339-342.

SHUKLA, D.D., TOSIC, M., JILKA, J., FORD, R.E., TOLE, R.W. and LANGHAM, M.A.C. 1989. Taxonomy of potyviruses infecting maize, sorghum and sugarcane in Australia and the United States as determined by reactivities of polyclonal antibodies directed towards virus-specific N-termini coat proteins. Phytopathology 79: 223-229.

THOTTAPPILLY, G., BOSQUE-PEREZ, N.A. and ROSSEL, H.W. 1993. Viruses and virus diseases of maize in tropical Africa. Plant Pathol. 42: 494-509.

TREVISAN, W. L., GASPAR, J. O., and COSTA, A. S. 1986. Ocorrência de moléstias semelhantes ao nanismo rugoso do milho no Braxil. (abstr.). Fitopatol. Bras. 11: 273-274.

Characteristics of the High Plains Virus (HPV) and Breeding for Resistance in Maize

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Overview

The High Plains virus (HPV), causes a severe disease on susceptible maize and wheat genotypes and has been confirmed in 10 states in the USA and other countries since its discovery in 1993. HPV is vectored by the eriophyid mite, *Aceria tossichella* Keifer, the wheat curl mite. This mite also transmits wheat streak mosaic virus (WSMV), another important pathogen of wheat and maize. Plants are often infected with both viruses. HPV symptoms on susceptible maize include chlorotic spots, flecks and/or streaks, reddening of leaf margins, leaf necrosis, stunting, and plant death. Symptoms are more severe on lower leaves and leaf tips. The virus has an associated 32 kDa nucleoprotein. Five species of dsRNA have been recovered, cloned, and sequenced from this viral nucleoprotein. Viroplasms and spherical double membrane bound bodies 150 nm in diameter characterize the ultrastructure of HPV infected cells. Virus resistance in maize was identified by challenging maize inbreds with viruliferous mites carrying both HPV and WSMV. A symptom rating scale and enzyme-linked immunosorbent assay (ELISA) were used for determining the host reaction and measuring virus titer. Inbreds susceptible to HPV include W64A, Wf9, N194, and H100. Resistant inbreds include B73 and B14. F_1 crosses showed that resistance is partially to completely dominant. Resistance vs susceptibility segregated in a 3:1 ratio in a B73 x Wf9 F_2 population. A B73 x Mo17 recombinant inbred population showed transgressive segregation fitting a two-gene model. Mapping of HPV resistance using restriction fragment length polymorphism markers showed highest probability of linkage with probes BNL 6.29 on chromosome six and UMC 57 on chromosome 10.

Introduction

High Plains virus (HPV) has been recognized as an epidemic pathogen in maize and wheat grown in the central and western part of the USA since its discovery in 1993. It is a serious economic threat to both crops

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(Brown et al., 1994, Jardine et al.,1994 Jensen and Lane, 1994, Jensen, 1994). This article summarizes our current research activities aimed at identifying the pathogen as a virus, the molecular characterization of the virus, distribution of the virus, description of the vector, and genetic analysis of resistance to HPV in maize.

Identification

The Pathogen

The first samples of diseased maize came from the Texas panhandle in June of 1993. Sap from these diseased tissues sometimes reacted with wheat streak mosaic virus (WSMV) antiserum but not with antiserum of other known maize viruses. No characteristic viral particles were seen in the electron microscope. No pathogen was mechanically transmissible that would cause the severe disease symptoms, which indicated that WSMV was not solely responsible for the symptoms. In an initial test using a mini-purification technique (Lane, 1986), a viral-like nucleoprotein of approximately 32 kDa was found. Sucrose density gradient centrifugation of concentrated plant sap showed a diffuse slow sedimenting zone and occasionally a sharp fast sedimenting WSMV zone. It was found that the diffuse slow sedimenting zone contained only the 32 kDa nucleoprotein. This nucleoprotein was collected and concentrated by ultracentrifugation from infected maize and later from HPV infected wheat. This nucleoprotein was then used to produce the antiserum against HPV for ELISA and Western Blot assays.

The concentrated nucleoprotein preparation was extracted by phenol or the methods of Morris and Dodds (1979) to obtain a nucleic acid fraction identified by treatment with DNAse and RNAse as dsRNA. Gel electrophoresis revealed four dsRNA bands. This dsRNA was reverse transcribed into cDNA and cloned. Five dsRNA species have been successfully cloned and sequenced. This indicates that one of the four bands in the gel was a doublet. No sequence homologies with other viruses were obtained when compared to sequences available in the viral gene bank (J. S. Hall, *unpublished data*). Preliminary reports of the molecular biology of the virus have been published (Jensen, 1994; Jensen and Hall, 1995; Jensen et al., 1996). This evidence for the protein and nucleic acid composition of the pathogen has led us to conclude that the pathogen was a virus and to name it the High Plains virus (HPV).

Ultrastructure of HPV infected cells

Electron microscopic studies have also suggested a viral nature for the pathogen. The ultrastructure of HPV infected cells has been described (Ahn et al., 1995; Ahn et al., 1996) and structures resembling those associated with wheat spot mosaic virus (WSpMV), rose rosette, redbud yellow ringspot, fig mosaic, and thistle mosaic pathogens have been observed. Viroplasms and unique ovoid double membranes bound bodies 150 nm in diameter characterize the infected cells of this group of eriophyid mite transmitted pathogens. The ultrastructural characteristics of the large particle of HPV and other members of this group of pathogens are unlike those of any other well characterized virus group and these pathogens may represent a new group of viruses.

Symptom expression of HPV in maize

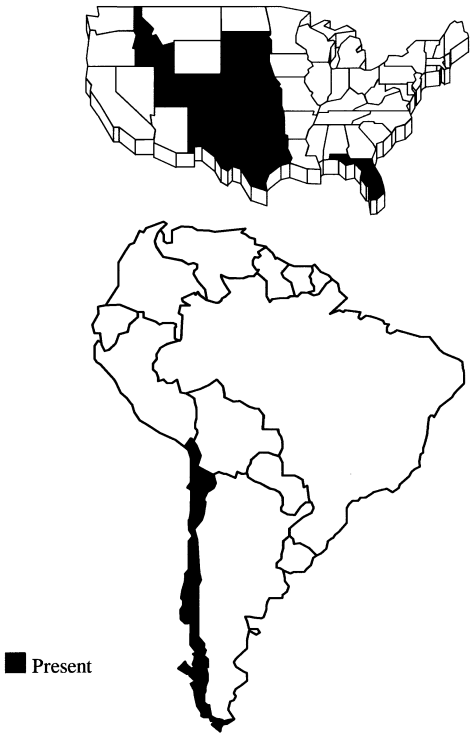
HPV symptoms are variable and dependent upon genotype, time of infection, and time of the year. Symptoms on maize include chlorotic white-spots, flecks or streaks, reddening of leaf margins, leaf necrosis, stunting and plant death. Reddening of the leaf is genotype-dependent, beginning at leaf margins and leaf tips and progressing toward the leaf center and base (Marçon et al., 1995). The size, shape and distribution of the small white spots is the most diagnostic symptom. The spots may range from circular to oval in shape and one to three mm in diameter. Where only a few spots occur they are often arranged in linear arrays along a vascular bundle. Few other viruses are so devastating that they can kill maize seedlings up to one meter in height.

Distribution of HPV

Since 1993 the disease has been found with increasing frequency over a much wider area probably due to a greater awareness and surveillance of the disease symptomatology and mite distribution. Presently HPV has been identified in primarily Maize but it has also been found in wheat in ten states of the USA (Colorado, Florida, Idaho, Kansas, Nebraska, New Mexico, Oklahoma, South Dakota, Texas, Utah) (Figure 1) (Jensen, 1994) as well as in other countries (*unpublished data*). There are also unconfirmed reports of a similar disease in other countries as well.

Yield losses up to 100% have been reported from HPV/WSMV complex in sweet corn. In general, sweet corn appears to be much more susceptible than dent corn. Stunted or deformed ears are not salable so the

Figure 1: High Plains virus 1996 distribution



losses are greater in that crop. With dent corn losses of up to 75% have been reported. Some severely affected fields were chopped for fodder because the grain set was so poor. Fortunately, most highly susceptible hybrids have been recognized and removed from distribution in areas where the disease incidence is high.

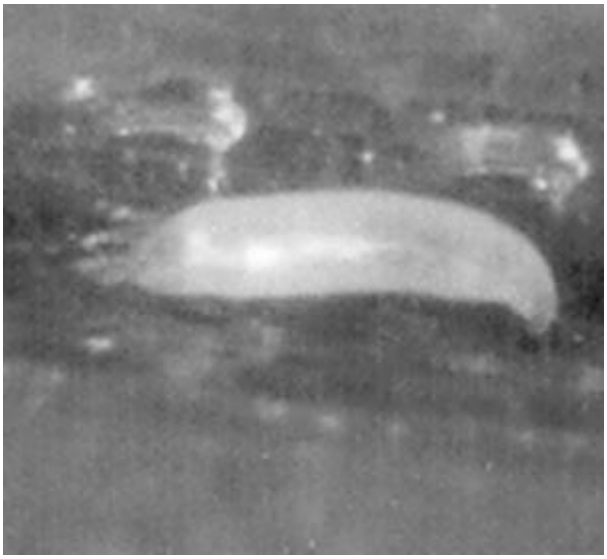
HPV Vector Relations

In early 1994 it was discovered by D. Seifers, T. Harvey and S. Jensen that HPV was transmitted by the wheat leaf curl mite, *Aceria tosichella* Keifer, *(unpublished data)*. This is the same mite that transmits WSMV, which accounts for the frequent mixed infection by the two viruses. Since HPV is not mechanically transmitted the discovery of the mite vector was critical to the continued research on this virus under controlled conditions. The discovery also suggested a natural relationship between HPV and other known mite transmitted pathogens (Slykhuis 1980). The details of the mite transmission of HPV and the interaction of the virus with the mite are not yet available.

The Eriophyid Mite Vector

Hundreds of species of mites are in the superfamily Eriophyoidea. The family Eriophyidae represents all the known acarina vectors of plant disease with economic importance. Eriophyids have only two pairs of legs, and are a unique part of the mite family. The eriophyid mite, *Aceria tossichella* Keifer (formerly *Aceria tulipae* Keifer, *Eriophyes tulipae* Keifer) is the only known vector of HPV (Seifers, Harvey and Jensen *unplublished data*) (Figure 2).

Figure 2: *Aceria tossichella*



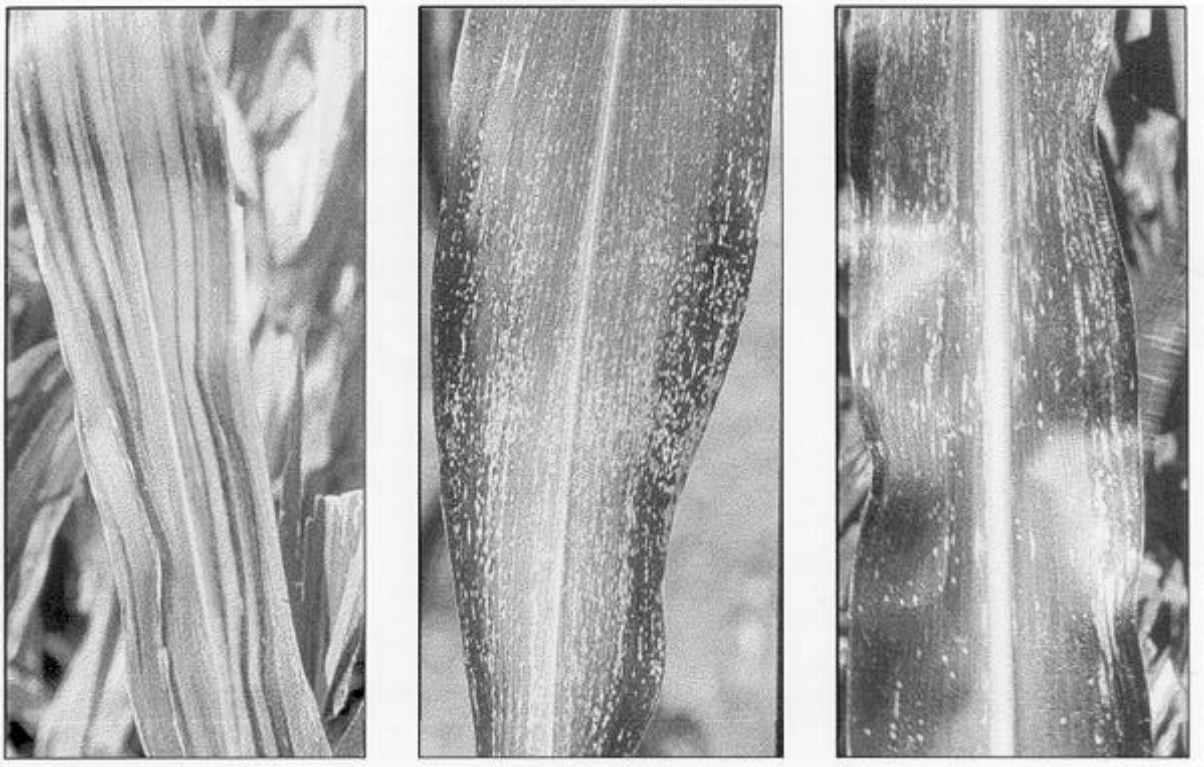
It is also the vector of another important pathogen, wheat streak mosaic virus (WSMV) (Slykhuis, 1953). *A. tossichella* Keifer is also known as wheat curl mite (WCM) due to its feeding process. The leaves upon which the mites feed soon become rolled, especially in wheat. WCM is a very small mite, about 250µ in length, yellow-white in color, cigar-shaped or worm-like, and not visible to the naked eye. In general, it does not cause noticeable injury to its hosts and its presence is usually overlooked. Mites apparently prefer plants with leaves that are easily rolled by its feeding action, which might be the reason for the preference for wheat, barley, and some wild grasses, although corn and sorghum can serve as hosts. Sorghum is suitable for WCM reproduction, but seems to be immune to WSMV (Connin, 1956). One known predator of WCM is *Typhalodromus cucumeris*, Oudms. Mites can be controlled over small areas using tetraethyldithiopyrophosphate (fumigant) at constant interval applications. Controlling mites in commercial maize fields is expensive and ineffective, in part because the mites are protected in the whorl of the plant. Brome mosaic virus (BMV) appears to multiply in WCM (Paliwal, 1972). It is not vectored but is potentially useful if acaricides are applied to the source of the mites (used as a natural acaricide). Adaptation to new hosts and WSMV acquisition-transmission were demonstrated by del Rosario and Sill, 1965. WCM transferred from naturally adapted hosts to other hosts showed high

mortality, but those individuals that survive produce progeny well adapted. After adaptation, vector efficiency increased rapidly. Eriophyid mites have four developmental stages: egg, first nymph, second nymph, and adult. First and second nymphal stages and adult transmit the virus when the pathogen was acquired during nymphal stages. The virus is not transmitted through the eggs, and there is a decline of transmission with increase in time after removal from infected source plants. The possible relationship of these mites with hosts suggests another important factor in disease control and epidemiology.

Genetic Variability Among Inbred Lines

For screening trials we have developed a system to transmit HPV and WSMV by placing wheat plants containing viruliferous mites carrying HPV and WSMV in alternate rows with maize seedlings in a growth chamber. The viruliferous mites naturally move from wheat to maize infecting the test plants during the 4-5 days they were kept in the growth chamber. Following inoculation the plants are fumigated to remove the mites and transferred to the greenhouse for symptom development and ratings. The rate of symptom development and degree of expression has been very consistent indicating that the challenge offered by the mites is very uniform and reproducible. A rating system was developed to evaluate symptom severity and systemic spread of the viruses (Figure 3). More than 30 maize inbred lines, selected for historic as well as common usage, have been tested and evaluated by these criteria. Results from a total of four replications showed that susceptible inbreds include H100, W64A, Wf9, Oh 43, N194, and A188. Resistant inbreds include B73, B14, and N190. Since this was a response to a mixed infection of HPV and WSMV, another experiment was conducted to assess the response of the tested genotypes to WSMV alone. Inbreds were tested for WSMV susceptibility by the mechanical inoculation procedure (McKinney, 1949). Using the scoring system for symptom expression and ELISA to measure virus titer in infected plants, it has become possible to identify the presence of HPV and/or WSMV alone or in mixed infections for inheritance and genetic mapping studies. Results showed that most HPV susceptible inbreds were also susceptible to WSMV, except N194, which showed no WSMV by ELISA. However, WSM symptoms alone were never severe, suggesting that HPV was the critical factor leading to plant death in susceptible genotypes.

Figure 3: Symptom severity and systemic spread of HPV and WSMV



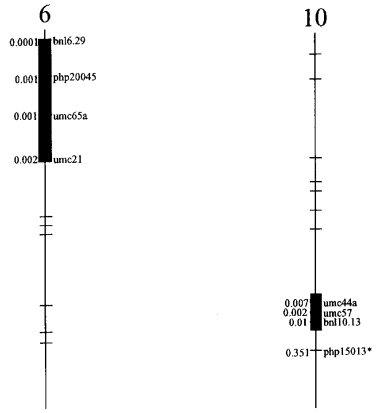
Molecular Markers Linked to Genes Conferring Resistance to HPV in Maize

Crosses between genotypes differing in susceptibility showed symptoms on the hybrids comparable with the more resistant genotype. For example the hybrids B73 x W64A F_1's and B73 x Wf9 F_1's , show that resistance carried by B73 was partially to completely dominant. Crosses involving B14 and B73 showed good resistance to the disease. F_2 populations were produced from the crosses B73 x W64A, and B73 x Wf9. In both F_2 populations, only two classes of HPV reaction were observed: resistant and highly susceptible. The populations show segregation ratios fitting a 3:1 ratio based on X^2 test (P=0.05). A B73 x Mo17 recombinant inbred population was also tested, which suggested a transgressive segregation that indicates a potential two-gene model based on segregation ratios.

Identification of chromosome regions involved in HPV resistance were obtained by correlating HPV symptoms with Restriction Fragment Length Polymorphism (RFLP) marker genotype. Linkage between molecular markers and symptomology were observed in 48 B73 x Mo17 RI's tested. Variation among plants for rating score was partitioned based on marker-genotype using single factor ANOVA. A total of 154 markers, encompassing all maize chromosomes, were tested. Significant correlations between HPV symptoms and genetic markers were found on chromosome 6 (BNL6.29, *Pr.*> *F* = 0.0001) and chromosome 10 (UMC57, *Pr.*> *F* = 0.002) (Figure 4).

The resistance gene correlated with chromosome 6 region imparted the greatest level of resistance. It is particularly interesting that these same chromosome regions have also been correlated with resistance to WSMV (McMullen and Louie, 1991; McMullen et al., 1994). However, N194 was both very susceptible to HPV and also very resistant to WSMV indicating that the loci of the resistance are near each other but not identical. Introgression of HPV resistant alleles from the chromosome 6 and 10 regions should improve HPV resistance in many susceptible inbreds.

Figure 4: Chromosome regions involved in HPV resistance



A resistant hybrid was produced upon crossing the two susceptible lines B79 and Mo17. This indicates an epistatic interaction of two genes and was confirmed by production of an F_2 population (Rodriguez et al., 1996). Inbreds B79 and Mo17 showed high HPV susceptibility under field environment, but under greenhouse studies both inbreds showed an intermediate reaction to HPV (Marçon et al., 1995). It appears that Mo17 susceptibility is confounded with temperature. Susceptibility is higher in locations with higher average temperatures (Rodriguez et al., 1996). Mapping results and field experiments indicate that chromosome 10 allele may be temperature sensitive, providing less protection at higher temperatures and/or providing protection at higher temperatures when in epistatic combination with another resistant allele. Due to those observations, a third gene (possibly in B79) for HPV resistance remains to be mapped.

Conclusions and Goals

It is very unusual for plant viruses to have dsRNA as their genetic material (Jensen, 1994). Only phytoreoviruses have dsRNA and HPV does not share other similarities with phytoreoviruses. The lipid bilayer membrane bound spheres 100-200 nm in diameter are unique to HPV and a group of similar mite transmitted plant viruses. These observations along with other HPV characteristics mentioned above, suggest that HPV belongs to a new group of viruses not yet classified. Other members of this groups would include rose rosette, thistle mosaic, fig mosaic, redbud yellow ringspot, and wheat spot mosaic virus (WSpMV) (Ahn et al., 1996; Nault et al., 1969). Slykhuis (1956) first described the mite transmitted virus, WSpMV which has numerous similarities in host range and symptom expression to HPV. Indeed, HPV may be a variation of WSpMV but there are no cultures of that vi-

rus available for comparisons so any relationship cannot be established and the name cannot be used. Genetic resistance remains the best way to combat the disease. We have identified two major loci that confer resistance to the HPV in maize, and we suspect a third locus exists. Our results indicate that resistance loci are closely linked to/or pleiotropic with genes for resistance to WSMV. According to our results and those of other authors (Findley et al., 1974, Findley, 1984, McMullen and Louie, 1991, and McMullen and Louie, 1994) chromosome 6 plays a major role in HPV, maize dwarf mosaic virus, and WSMV reaction. Results indicating a possible cluster of genes controlling multi disease severity have been reported (Kyle et al., 1986) in other genera of plants. Manipulation of resistant loci by molecular techniques coupled with traditional breeding should improve the resistance of commercial inbreds and subsequently yield.

HPV is a devastating pathogen in susceptible corn genotypes. It has been demonstrated repeatedly that HPV can alternate seasonally between wheat and maize. In areas where it is recognized it has been seen for four consecutive years. It is very unwise to plant susceptible maize genotypes near winter wheat in areas where HPV occurs. In Australia, Brazil, Chile, Israel, and USA, much progress has been made towards an increased awareness and surveillance of the disease. Screening of local inbred lines and hybrids is recommended in areas where the virus is already present to avoid future yield losses and determine suitable genetic material for a particular region. HPV hosts suitable for the mite and/or for the virus replication associated with particular areas (e.g., depending on each country) should be identified for a better understanding of the relationship between host-plant interactions and epidemiology.

Acknowledgements

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References

AHN, K.K., JENSEN, S.G., ANDERSON, E.J., GERGERICH, R.C., and KIM, K.S. 1995. A virus-like disease of corn and wheat in the High Plains: ultrastructural aspects. *Phytopathology* 85:1183.

AHN, K. K., KIM, K.S., GERGERICH, R.C., JENSEN, S.G., and ANDERSON, E.J. 1996. Comparative ultrastructure of double membrane-bound particles and inclusions associated with eriophiyd mite-born plant diseases of unknown etiology: a potentially new group of plant viruses. *J. Subm. Cyt. And Path*. (*in press)*

BRAKKE, M.K. 1971. Wheat streak mosaic virus. *CMI/AAB* Descriptions of Plant Viruses, No.48.

BROWN, W.M.Jr., FRENCH, R.C., HAMMOND, R.W., and JENSEN, S.G. 1994. Occurrence and distribution of wheat viruses in Colorado. *Phytopathology* 84:1167.

CONNIN, R. 1956. The host range of the wheat curl mite, vector of wheat streak mosaic. *J. Econ. Entom*. 49:1-4.

DEL ROSARIO, M.S.E., and SILL, W.H.Jr. 1965. Physiological strains of *Aceria tulipae* and their relationship to the transmission of wheat streak mosaic virus. *Phytopathology* 55:1168-1175.

FINDLEY, W.R., LOUIE, R., KNOKE, J.K., and DOLLINGER, E.J. 1974. Breeding corn for resistance to virus in Ohio. P.123-127 *In*: WILLIANS, L.E., GORDON, D.T., and NAULT, L.R. (eds.) *Proc. Intern. Maize Virus Dis. Colloq. And Workshop, Ohio Agric. Res. Dev. Cent*., Wooster.

FINDLEY, W.R., LOUIE, R., and KNOKE, J.K. 1984. Breeding corn for resistance to corn viruses in *Ohio. Proc. Annual Corn and Sorghum Ind. Res. Conf*. 39:52-67.

JARDINE, D.J., BOWDEN, R.L., and JENSEN, S.G. 1994. A new virus of corn and wheat in western Kansas. *Phytopathology* 84:1117-1118.

JENSEN, S.G. 1994. The High Plains virus – A new threat to corn and wheat production in the *West. Proc. Annual Corn and Sorghum Ind. Res. Conf*. 49:156-164.

JENSEN, S.G., and LANE, L.C. 1994. A new virus disease of corn and wheat in the High Plains. *Phytopathology* 84:1158.

JENSEN, S.G., and HALL, J.S. 1995. Molecular characterization of a viral pathogen infecting maize and wheat in the High Plains. *Phytopathology* 85:1211.

JENSEN, S.G., LANE, L.C., and SEIFERS, D.L. 1996. A new disease of maize and wheat in the High Plains. *Plant Dis*. (*in press*).

KYLE, M., DICKINSON, M.H., PROVIDENTI, R., and MUNGER, H.M. 1986. A major gene for broad spectrum virus resistance in *Phaseolus vulgaris* L. Hort. Sci. 21:1557.

LANE, L.C. 1986. Propagation and purification of RNA plant viruses. In: *Methods in enzymology*. WEISSBACH, A., and WEISSBACH, H. (eds.). 118:687-696.

MARÇON, A., KAEPPLER, S.M., and JENSEN, S.J. 1995. Inheritance of tolerance to the High Plains virus (HPV) complex in corn. *Agronomy Abstr*. 175-176.

MCKINNEY, H.H. 1949. Virus isolates from mosaic wheat in the hard red winter wheat area. *Plant Dis. Reptr*.33;346-349.

MCMULEN, M.D., and LOUIE, R. 1991. Identification of a gene for resistance to wheat streak mosaic virus in maize. *Phytopathology* 81:624-627.

MCMULLEN, M.D., JONES, M.W., SIMCOX, K.D., and LOUIE, R. 1994. Three genetic loci control resistance to wheat streak mosaic virus in the inbred Pa405. *Mol. Pl. Micr. Int*. 7:708-712.

MORRIS, T.J., and DODDS, J.A. 1979. Isolation and analysis of double-stranded RNA from virus-infected plant and fungal tissue. *Phytopathology* 69:854-858.

NAULT, L.R., STYER, W.E., GORDON, D.T., BRADFUTE, O.E., LAFEYER, H.N., and WILLIANS, L.E. 1969. An eriophid-born pathogen from Ohio, and its relationship to wheat spot mosaic virus. *Pl. Dis. Reptr*. 54:156-160.

PALIWAL, Y.C. 1972. Bromo mosaic virus infection in the wheat curl mite, *Aceria tulipae*, A non-vector of the virus. *J.Invertebr.Pathol*. 20:288-302.

RODRIGUES-BALLESTEROS, O.R., MARÇON, A., FREDERIKSEN, R.A., RUSH, C.M., HEIDEL., G., JEFFERS, D., KAEPPLER, S.M., JENSEN, S.G. 1995. Genetics of resistance to High Plains disease in maize. *Proc. High Plains Disease Symp*. Amarillo, Texas. (*in press*)

SLYKHUIS, J.T. 1953. Wheat streak-mosaic in Alberta and factors related to its spread. *Canad. Jour. Agr. Sci*. 33:195-197.

SLYKHUIS, J.T. 1956. Wheat spot mosaic, caused by a mite-transmitted virus associated with wheat streak mosaic . *Phytopathology* 46:682-687.

SLYKHUIS, J.T. 1980. Mites. In: *Vectors of Plant Pathogens*. HARRIS K.F. and MARAMOROSCH, K. (eds.). Academic Press, N.Y. pp.325-356.

Corn Leafhoppers as Vectors of Maize Pathogens in Brazil

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Introduction

There are more than 32 diseases caused by viruses and mollicutes that have been described in maize (*Zea mays L.*) around the world (Damsteegt, 1981; and Brunt et al., 1990). These pathogens are transmitted from diseased plants to healthy plants by leafhoppers, planthoppers, aphids, and beetles. In the USA, Douglas (1966) reported that there are more than 30 species of leafhoppers that feed on maize. The prevalence of these species depends on the locale and the season of the year. Nault and Knoke (1981) cited leafhoppers from the genus *Dalbulus, Graminella, Euscelidus, Stirellus, Exitianus, Baldulus* and *Peregrinus* as major pathogen vectors for maize and sorghum. Other vectors include: 22 aphid species, mainly the corn leaf aphid, *Rhopalosiphum maidis* (Fitch), and the greenbug, *Schizaphis graminum* (Rondani), which is the most efficient vector of the maize dwarf mosaic virus (MDMV). Among beetles, the most important vectors belong to the genus *Diabrotica* and *Choetocnema*, which transmit the maize chlorotic mottle virus (MCMV). Some mite species are also maize disease vectors, such as *Aceria tosichella* Keifer, which transmits the high plains virus (HPV) and wheat streak mosaic virus (WSMV) in the USA (Marçon et al., 1997).

In Brazil, Flechtmann and Santana (1997) reported observing the mites *Catarhinus tricholaenae* and *Oligonychus zeae* (Diptilomiopidae: Tetranychidae) on maize, but there is no information yet as to whether they transmit any pathogens.

The corn leafhopper, *Dalbulus maidis* (DeLong and Wolcott) (Homoptera: Cicadellidae) is reported to be distributed from the southern USA to Argentina (Oman 1948). In the State of São Paulo, Brazil, *D. maidis* was reported by Mendes (1938) and Costa et al. (1971), who described it as a vector of corn stunt spiroplasma (CSS), of maize bushy stunt phytoplasma (MBS), and of the maize "rayado fino" virus (MRFV). They also reported a high incidence (60% of plants) of MRFV on late season planted maize, which they attributed to *Peregrinus maidis* (Ashmead), a vector of maize stripe virus (M Stp V). This pathogen, however, has not been confirmed to be in Brazil. Also, in São Francisco valley, PE, Leão Veiga (1977) reported *P. maidis* infesting irrigated maize through the eggs of the parasite *Anagrus flaveolus* Waterhouse (Hym.: Myrmaridae).

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[1] Waquil, J.M. 1998. Corn Leafhoppers as Vectors of Maize Pathogens in Brazil. *In* Diagnosing Maize Diseases in Latin America (Eds. C. Casela, R. Renfro and A.F. Krattiger). *ISAAA Briefs No. 9*. ISAAA: NY. Pp. 34-42.

In March of 1985 at Sete Lagoas, MG, Brazil, leafhoppers and planthoppers feeding on maize whorl were first recorded on late planted maize at the EMBRAPA Maize and Sorghum experimental fields, but it is possible that these species had existed there for many years before.

Insect samples were collected from maize seedlings and sent to Dr. Max Menezes who identified them as the corn leafhopper *D. maidis* and the planthopper *P. maidis* (Ashmead) (Waquil, 1988).

Sampling Corn Leafhoppers

Leafhoppers were sampled on maize, sorghum (*Sorghum bicolora*) and weeds around the crops. The number of net sweeps, covering a 10 meter line, had no effect on the number of collected insects. *D. maidis* was the most common leafhopper collected on maize (93%), but from sorghum (40%) and weeds (34%) a greater diversity of hoppers was observed. The planthopper species *P. maidis* was the least abundant on all three plant types. The corn leafhopper *D. maidis* was more efficiently collected using the plastic bag method (Waquil and Teetes, 1985) than by using the sweep net.

Weekly surveys carried out for one year using both plastic bag and sweep net methods indicated that under low leafhopper density there was little difference between the two methods; however, under high density the plastic bag method was much more efficient. The highest mean of corn leafhopper density observed over the year was 10 adults/plant. Using the plastic bag method, the leafhopper density was 5 times higher than the number computed with the sweep net (2 adults/plant). In Sete Lagoas, weekly sampling by the plastic bag method for 8 years showed a mean density of one corn leafhopper/plant except for March and April when the density rose up to 12 adults/plant (Fig. 1) (Waquil and Fernandes, 1994). A peak in the leafhopper density mean values occurred for each month in all 8 years, except in 1993. This peak was observed in March or April with variation from 5 to 11 adults/plant. The causes and consequences of these variations are not known. The minimal monthly densities were always under 1 adult/plant with little variation during the year. In Piracicaba, Folegatti and Lopes (1997) studied *D. maidis* densities on maize planted from November to May and reported a peak (average of 3.8 insects/plant) on maize planted in January.

Biology of *Dalbulus maidis*

The corn leafhopper adult can reach more than 4 mm long by 0.85 mm width and its color generally ranges from light to dark pale straw with two dark spots on the head. On the hind (jumping) leg, the tibia has two easily visible lines of spines. The female inserts its eggs into the midveins of the leaves of corn seedlings. After hatching, the nymphs go through 5 instars to become adults (Ortega, 1987). The optimum incubation temperature was 26.5C, when 85% of eggs hatched in 9 days (Figure 2). No eggs hatch below 20C, but the same eggs would hatch when incubated above this temperature (Tsai, 1988). According to the adjusted data from Tsai (1988), the nymph development was shortest at 26.6C and above (Figure 3). The adults longevity was highest at 19.0C. Under controlled conditions of 26.5±2C and 47.5±7.5% relative humidity, at Sete Lagoas eggs hatch in an average of 12.2 days, with the shortest period being 9 days and the longest 20 days. *D. maidis* nymphs under these conditions went through 5 instars during 14 days. The adult longevity mean was 51.9 days. The rate of nymph survival ranged from 90% at 1st instar to 100% at 4th and 5th instar (Waquil et al., 1997a; see Table 1).

To study corn leafhopper morphological variation, Oliveira and Lopes (1997a) collected samples on maize from 27 localities with 5 to 28^{o} latitude south and from 16 to 1578 m altitude in 10 different Brazilian States. *D. maidis* was the only species found. Females were always larger and heavier than males. There was a positive and significant correlation between the morphological variables measured and the latitude and altitude of the location from which the insects were collected.

Table 1: Biology of corn leafhopper on maize seedlings, BR 201, under laboratory conditions with temperature of 26.5±2°C and relative humidity of 47.5±7.5%, CNPMS/EMBRAPA, 1995.

Stage	n. insect	survivor (%)	Developing period	Age (days)
Egg	200	-	12.2	12.2
Nymph I	18	90	3.8±0.01	16.0
Nymph II	17	94	3.1±0.12	19.1
Nymph III	16	94	2.8±0.16	21.5
Nymph IV	13	100	3.3±0.19	24.3
Nymph V	3	100	2.7±0.00	26.3
Adults	17	-	51.9	78.2

Source: Waquil et al., 1997a

Corn Leafhopper Damage on Maize

As mentioned, the species *D. maidis* is considered the major vector of CSS, MBS, and MRFV in Brazil (Costa et al., 1971). The insects cause two types of damage to the plants. In addition to their role as vectors, they also pierce the plant and suck its sap. Losses caused by virus and mollicute diseases in maize can be experimentally measured and range from 9 to 90%, depending on the susceptibility of the cultivar, the involved pathogen, and the environmental conditions (Gordon et al., 1981). In Brazil, Waquil et al. (1996) reported 28.64% yield loss from MRFV infected maize in field experiments. Maize plants are severely damaged when infected by the mollicutes CSS and/or MBS. Direct damage caused by the corn leafhopper on maize was reported in California by Bushing and Burton (1974). In Brazil, Waquil (1997) evaluated the direct damage of corn leafhopper on maize seedlings. Little effect of leafhopper feeding was observed on 15 day-old seedlings. When one, five, and 10 adults/plant were confined on 10-day old plants for a week, a quadratic relationship was observed between corn leafhopper infestation density and canopy and root system dry weight. The infestation of 10 adults/plant reduced the canopy (40%) and root systems dry weight (62%). The recovery of the plants was not evaluated.

Evaluation of Brazilian Maize Hybrids to Virus and Mollicutes

The Embrapa Maize and Sorghum Research Center organized a national trial to evaluate the performance of Brazilian maize hybrids from private companies and official institutions under different conditions. In two trials, the short-season cycle (SSC) and the super-short-season cycle(SSSC) maize varieties were planted in October and in December of 1995 to be evaluated for the incidence of virus in the field under natural infestation conditions (Waquil et al., 1996). In addition, a late season planting (LSP) maize was planted in March of 1995 through the National Net Trials, and was evaluated for the incidence of mollicutes (Waquil et al., 1996b).

In the trials planted in October, the incidence of MRFV was high and the symptoms were strong enough to evaluate the severity of the disease. In the SSC trial the mean incidence for all 30 hybrids was 68.50% of the plants—higher than on SSSC whose mean, of all 49 hybrids, was 57.67 % (Table 2). In both trials, it was possible to estimate the severity of MRFV by using Mckney`s

Table 2: Incidence and severity of MRFV (maize "rayado fino" virus) on the least infected maize hybrids from two Net National Maize Trials (Short cycle and super-short cycle), planted in October(I) and December (II), CNPMS/EMBRAPA, 1995.

Trial/Hybrid	Incidence (%)	Severity (%)
Short cycle (I)		
AGX 5012	6.67	2.22
FT 9043	25.00	8.33
G 153C	26.67	8.89
AGX 5273	30.00	11.11
G 1335	36.67	12.22
Trial Mean	68.50	29.75
Mean of 5 more infected hybrids	96.00	53.33
Super-short cycle (I)		
AGX 9332	5.56	1.85
AGROMEN 3150	15.56	5.19
EXP. 31029 (CAC)	27.78	9.26
HATÃ 3001	28.89	9.63
HATÃ 2020	32.22	11.11
Trial mean	57.67	22.38
Mean of 5 more infected hybrids	89.11	39.70
Super-short cycle (II)		
AGX 9332	0.95	-
EXP. 31029(CAC)	2.49	-
C 806 (Veloz)	2.49	-
G 81 S	2.86	-
G 132 S (Densus)	3.37	-
XL 220	3.88	-
Trial mean	10.88	-
Mean of 5 more infected hybrids	19.73	-

Source: Waquil et al., 1996a

formula, as cited by Tanaka (1990). In the trial planted in December, using either disease incidence or severity, the results showed large variability among the hybrids. The less susceptible hybrids to MRFV were AGX 5012 and AGX 9332.

Evaluation of CSS on Brazilian hybrids was carried out during the late season planting (Waquil et al. 1996b) by recording individual plant symptoms at the grain filling stage. The average infection of the 30 evaluated hybrids was 31.46%. The average of five other infected hybrids by the CSS was 56.89%; however, some hybrids like C 444, P 3041, AG 519, C 701, and BR 206, had fewer than 15% of the plants express symptoms (Table 3). Comparing the incidence of mollicutes on susceptible maize planted in November and December, 1995, and January, March and May, 1996, Folegatti and Lopes (1997), observed a gradual increase from 24% on the first planting to 46% on the fourth planting date. In resistant hybrids they observed no more than a 10% incidence.

Table 3: Adults and eggs mean number/plot of *Dalbulus maidis* and the percentage of plants with symptoms of CSS (corn stunt spiroplasma) in the National Net Trial of maize hybrids recommended for late planting ("Safrinha-2" in Brazil), CNPMS/EMBRAPA, 1996.

Hybrids[1]	Adults mean number	Eggs mean number	CSS (% incidence)
C 444	7.67	50.00	11.11
P 3041	12.67	32.33	12.22
AG 519	13.00	32.33	12.23
C 701	14.33	66.67	14.44
BR 206	9.33	43.67	14.45
Trial mean	12.32	51.52	31.46
Mean of 5 lines most infected by CSS	22.07	83.47	56.89
Coefficient of variation (%)	26.62	19.68	22.14

[1]Only those 5 hybrids with minimum CSS incidence are listed.
Source: Waquil and Oliveira (1996)

Evaluation of Brazilian Maize Hybrids to *Dalbulus maidis*

A collection of 42 hybrids recommended for late season planting, known as "safrinha" in Brazil, was evaluated in the field for the incidence of adults and eggs. The adults were collected using the plastic bag method by sampling 10 plants/plot. The number of adults per plant was recorded in the laboratory and the number of eggs per plant was counted by dissecting of the first leaves of the plant under steriomicroscope. This revealed significant differences among the hybrids for both variables. The lowest numbers of adults were found on hybrids PX 1373-A, Z 8501, AG 122, G 150-C, and Z 8568. However, the lowest numbers of eggs were found on hybrids PX 1273-A, CO 822992, AL-Manduri, C 615, P 3041, and AG 519 (Table 4) (Waquil et al., 1976b). From all 43 evaluated hybrids, the most infested had an average of 10.0 eggs/plant and the least infested 2.5 eggs/plant. There was no correlation between these two variables, which indicates independent resistant mechanisms for feeding/shelter and oviposition.

Although the *Sorghum bicolor* is not listed as a host for *D. maidis*, some adults have been collected from sorghum fields in Sete Lagoas, MG. Laboratory studies of sorghum with artificial infestation by leafhopper resulted in almost 100% adult mortality. Evaluating the incidence of adults and eggs of *D. maidis* in the Sorghum National Trial for 2 years, in which maize and sorghum were grown in the trials side by side, a 10 fold lower density on sorghum than on maize was observed. Using the same methodology described to evaluate maize, the data for adults and eggs incidence from sorghum trials showed significant differences among the 42 sorghum hybrids studied. Using either variable, the Duncan' Multiple Range Test ($P<0.05$) separated the hybrid means into 4 groups. The least infested ones are listed on Table 5 (Waquil et al., 1997b).

Control of *Dalbulus maidis*

There are many strategies available to manage the virus and mollicutes complex in maize fields. Pathogen resistant plants have been the most successful method used world wide for many crops and diseases. However, no maize immunity to phytoplasma and spiroplasma has yet been found (Jellum and Kuhn, 1970). Under certain circumstances the vector can be controlled to reduce plant damage, using many alternative and complementary methods. Cultural, biological, and chemical controls are generally the most common

Table 4: List of maize hybrids, from the Net National Late Planting Trial ("Safrinha"), with a minimum incidence of *D. maidis* adults and eggs, CNPMS/EMBRAPA, 1996.

Hybrids	Adult number	Egg number
Minimum adult incidence		
PX 1273-A	3.00	22.67
Z 8501	5.67	100.00
AG 122	6.33	40.33
G 150-C	6.67	36.33
Z 8568	7.00	74.67
Minimum egg incidence		
PX 1373-A	3.00	22.67
CO 8222992	11.33	29.33
AL – Manduri	9.00	31.00
C 615	14.33	32.00
P 3041	12.67	32.33
Trail mean of 42 hybrids	12.32	51.52
Mean of the 5 most highly infested hybrids	22.07	83.47
Coefficient of variation (%)	26.62	19.68

Source: Waquil et al., 1996b

Table 5: List of sorghum hybrids from the National Net Trial of Grain Sorghum with a minimum incidence (adults and eggs mean number/plot) of *D. maidis*, CNPMS/EMBRAPA, 1995.

Hybrids	Adults mean number	Eggs mean number
Lower adult incidence		
F 903	0.00	8.67
DK 47	0.67	5.00
C51	0.67	1.00
CMSXS 376	1.00	2.67
A 6304	1.00	7.33
73 E2	1.00	5.33
AGN 8050	1.30	2.33
Lower egg incidence		
BR 300	2.00	0.33
C 51	0.67	1.00
AG 1016	4.00	1.67
CMSXS 213	7.33	1.67
CMSXS 375	2.67	1.67
Trail mean of 25 hybrids	2.10	4.96
Mean of the 5 most highly infested hybrids	7.33	11.00

Source: Waquil et al., 1997b

methods used. Cultural strategies, such as the elimination of volunteer maize plants from the previous planting, the concentration of the planting dates to a short period of time, and the avoidance of continuous planting dates throughout the seasons, can reduce the increase of the vector population and thus the spread of disease. Reduction of Tungo virus transmission by *Nephotettix virescens* (Homoptera: Cicadellidae) was reported by Saxena et al., (1987) using Neem cake-treatment on rice seedlings. Biological controls also have a strong potential. In addition, many predators and microorganisms such as fungi can be used, and egg parasites, notably *Anagrus breviphragma* Soyka, have been found in Piracicaba, SP (Oliveira et al., 1997) and in Sete Lagoas, MG (Santana et al., 1997). *Oligosita* sp. Has also been found in Piracicaba (Oliveira and Lopes 1997b). Chemical control should be used as an emergency method, and information about appropriate pesticides will have to be researched before any specific recommendations can be made. The information available suggests that soil treatment with carbofuran can reduce disease incidence by 70% and increase grain yields by 300% (Pitre, 1967, and 1968). The efficiency of carbofuran on vectors was also reported by All et al. (1981). For spraying, the best results were obtained with oxydemeton methyl and acephate, although they had short residual action (Tsai et al., 1990). In Brazil, under green house conditions, a residual effect of up to 59 days after planting was obtained with aldicarb and imidacloprid used as soil and seed treatment, respectively (Waquil and Viana, 1996). Other chemicals, including carbofuran and thiodicarb, had a shorter plant protection period, and fipronyl and terbufos were not efficient (Table 6). Aldicarb and imidacloprid also had a 100% rate of efficiency protecting maize plants against MRFV infection and corn leaf aphid infestation throughout the entire vegetative stage (Table 7).

Table 6: Residual effect of soil (Sl) and seed (Sd) treatment efficiency on the control of corn leafhopper, *D. maidis,* in maize, CNPMS/EMBRAPA, 1995.

	Days after planting[1]					
Treatments	**12**	**14**	**17**	**22**	**32**	**59**
aldicarb (Sl T)	93.8 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
imidacloprid (Sd T)	68.8 a	100.0 a	100.0 a	100.0 a	100.0 a	66.7 b
carbofuran 5G (Sl T)	65.0 a	100.0 a	100.0 a	96.4 a	69.1 b	71.1 b
carbofuran (Sd T)	28.8 b	96.9 a	97.6 a	79.5 b	66.0 b	40.0 c
thiodicarb (Sd T)	10.0 b	95.4 b	96.4 a	18.1 c	55.7 b	44.4 c
terbufos (Sl T)	10.0 b	30.8 c	38.8 b	15.7 c	41.2 b	17.7 d
fipronyl (Sd T)	10.0 b	30.8 c	23.5 b	3.6 d	2.1 c	26.7 d

[1]Means, in each column, with the same letter are not significantly different by Duncans' multiple range test (P<0.05).
Source: Waquil and Viana 1996.

Table 7: MRFV (Maize "rayado fino" virus) and corn leaf aphid incidence in maize in which the seed or soil was treated with insecticides, CNPMS/EMBRAPA, 1995.

Treatments	**Dose**	**Maize "rayado fino" virus(%)[1]**	**Corn leaf aphid(%)[1]**
aldicarb (Sl)	25 kg/ha	0	0
imidacloprid (Sd)	1 kg/100 kg	0	0
carbofuran 5G (Sl)	25 kg/ha	0	17
carbofuran (Sd)	2 ℓ/100 kg	33	0
thiodicarb (Sd)	2 ℓ/100 kg	40	33
terbufos (Sl)	25 kg/ha	20	67
fipronyl (Sd)	2 kg/100 kg	17	50
Check	-	66	50

[1]Percentage of plants with disease symptoms or insect colony.
Source: Waquil and Viana 1996.

References

ALL, J.N., KUHN, C.W. and JELLUM, M.D. 1981. Control strategies for vectors of virus and viruslike pathogens of maize and sorghum. In: Virus and Viruslike Diseases of Maize in the United States. Southern Cooperative Series Bulletin, 247, Ohio Agric. Research and Dev. Center, Wooster, Ohio. Pp. 127-131.

BRUNT, A., CRABTREE, K., and GIBBS, A. 1990. Virus of topical plants. Wallingford: CAB International, 1st ed.

BUSHING, R. W. , and BURTON, V. E. 1974. Leafhopper damage to silage corn in California. J. Econ. Entomol. 67: 656-658.

COSTA, A.S., KITAJIMA, E.W., and ARRUDA, S.C. 1971. Moléstias de Virus e de Micoplasma do milho em São Paulo. Rev. Soc. Bras. Fitopatologia. 4:39-41.

DAMSTEEGT, V.D. 1981. Exotic virus and viruslike diseases of maize. In: GORDON, D.T., KNOKE, J.K., and SCOTT, G.E. eds. Virus and virus like disease of maize in the United States. Southern Cooperative Series Bulletin 247. Ohio Agric. Res. And Dev. Center, Wooster, Ohio, USA. Pp. 110-123.

DOUGLAS, W.A., WHITCOMB, W.A., HEPNER, L.W., KIRK, V.M., and DAVIS, R. 1966. Some Cicadellidae (Homoptra) collected from corn in the Southeastern United States. Ann. Entomol. Soc. Am. 59:393-396.

FLECHTMANN, C.H.W. and SANTANA, D.L.Q. 1997. A preliminary note on mites on corn in Brazil with redescriptions of *Catarhinus tricholaenae* and *Oligonycus zeae* (Acari: Diptilomiopidae, Tetranychidae). Systematic and Applied Acarology 2: 189-194.

FOLEGATTI, M.E.G. and LOPES, J.R.S. 1997. Incidência de enfezamento do milho e população do vetor *Dalbulus maidis* (Hemiptera: Cicadellidae) em cinco épocas de plantio em Piracicaba, SP. In: Anais do 16°Congresso Brasileiro de Entomologia, 2 a 7 de março de 1997, Salvador, Bahia, Brasil. 361pp. (Resumos).

GORDON, D.T., BRADFUTE, O. E., GINGERY, R.E., KNOKE, J.K., LOUIE, R., NAULT, R.L., and SCOTT, G.E. 1981. Introduction: History, geographical distribution, pathogen characteristics, and economic importance. In: GORDON, D.T.; KNOKE, J.K. and SCOTT, G.E. eds. Virus and viruslike disease of maize in the United States. Southern Cooperative Series Bulletin 247. Ohio Agric. Res. And Dev. Center, Wooster, Ohio, USA. P. 1-12.

JELLUM, M.D. and KHUN, C.W. 1970. Evaluation for resistance to corn stunt and maize dwarf mosaic diseases in corn. Univ. of Georgia Coll. Of Agr. Exp. Sta. Res. Rep. 82. 37pp.

LEAO VEIGA, A.F.S. 1977. O Parasitismo de Ovos de Cigarrinha do Milho – *Peregrinus maidis* (Ashemed) por *Anagrus flaveolus* Waterhouse, 1913., (Hym:Mymaridae) em Pernambuco Brasil. In: Anais do 4º Congresso Brasileiro de Entomologia, realizado de 6 a 11 de fevereiro de 1977, SEB,. Goiânia, GO. 96pp. (Resumos).

MARÇON, A., KAEPPLER, S.M., and JENSEM, S.G. 1997. Genetic variability among maize inbred lines for resistance of the high plains virus – wheat streak mosaic virus complex. Plant Dis. 81:195-198

MENDES, L.O.T. 1938. Observações sobre alguns insetos coletados sobre algodoeiro durante os anos de 1936 e1937. Boletim técnico do Instituto Agronômico 45:1-5.

NAULT, L.R. and KNOKE, J.K. 1981. Maize Vectors. In: GORDON, D:T.; KNOKE, J.K. and SCOTT, G.E. eds. Virus and Viruslike Disease of Maize in the United States. Southern Cooperative Series Bulletin 247. Ohio Agric. Res. And Dev. Center, Wooster, Ohio, USA. Pp. 77-84.

OLIVEIRA, C.M. and LOPES, J.R.S. 1997a. Polimorfism entre populações de *Dalbulus maidis* (Hemiptera: Cicadellidae) no Brasil. In: Anais do 16°Congresso Brasileiro de Entomologia, 2 a 7 de março de 1997, Salvador, Bahia, Brasil. 209pp. (Resumos).

OLIVEIRA, C.M. and LOPES, J.R.S. 1997b. Parasitoides de ovos da cigarrinha-do-milho *Dalbulus maidis* (Hemiptera: Cicadellidae) em Piracicaba, SP. In: Anais do 16°Congresso Brasileiro de Entomologia, 2 a 7 de março de 1997, Salvador, Bahia, Brasil. 123pp. (Resumos).

OMAN, P.W. 1948. Distribution of *Baudulus maidis* (DeLong and Wolcott). Proceedings of the Entomological Society of Washington 50:34.

ORTEGA, A. 1987. Insect Pest of Maize: A guide for field identification, Mexico, D.F.; CIMMYT.

PITRE, H.N. 1967. Control of Corn Stunt Disease, systemic Aid in Control of Vector. Mississippi Farm Res. 30:1-5.

PITRE, H.N. 1968. Systemic Insecticides for Control of the black-faced leafhopper Graminella nigrifrons, and Effect on Corn Stunt Disease. J. Econ. Entomol. 61:765-768.

SANTANA, D.L.Q., WAQUIL, J.M., OLIVEIRA, E. 1997. Ocorrência de *Anagrus breviphragma* Soyka (Hym.: Mymaridae) parasitando ovos de *Dalbulus maidis* no Brasil. In: Anais do 16°Congresso Brasileiro de Entomologia, 2 a 7 de março de 1997, Salvador, Bahia, Brasil. 101pp. (Resumos).

SAXENA, R. C., KHAN, Z.R., and BAJET, N.B. 1987. Reduction of Tungo virus transmission by *Nephotettix virescens* (Homoptera: Cicadellidae) in Neem Cake-treated Rice Seedlings. J. of Econ. Entomol. 80:1079-1082.

TANAKA, M.A.S. 1990. Patogenicidade e transmissão por sementes do agente causal da ramulose do algodoeiro. Piracicaba, SP. 111pp. (Tese de Doutorado)

TSAI, J.H., 1988. Bionomics of Dalbulus maidis (DeLong and Wolcott), a Vector of Mollicutes and Virus (Homoptra:Cicadellidae). In: Mycoplasma Diseases of Crops: Basic and Applied Aspects, Ed. Maramorosch, S. P and Raychaudhuri, S.P. Springer-Verlag, New York, USA, p.209-221

TSAI, J.J., STEINBERG, B., and FALK, B.W. 1990. Effectiveness and Residual Effects of Seven Insecticides on Dalbulus maidis (Homoptera: Cicadellidae) and Peregrinus maidis (Homoptera:Delphacidae). J. Entomol. Sci. 25:106-111.

WAQUIL, J.M. 1988. Levantamento de danos da cigarrinha-do-milho *Dalbulus maidis* (DeLong and Wolcott) (Homoptera:Cicadellidae) In: Anais XVII Congresso Nacional de Milho e Sorgo, Departamento de Genética, ESALQ/USP, Piracicaba, SP. 63pp. (Resumos).

WAQUIL, J.M. 1992. Avaliação do terbufós em comparação com outros produtos no controle de pragas na cultura do milho. In: Relatório Técnico Anual do CNPMS/EMBRAPA-1988-1991. Sete Lagoas, MG. 65pp.

WAQUIL, J.M. and FERNANDES, F.T., 1994. Flutuação Populacional da Cigarrinha do Milho *Dalbulus maidis,* no CNPMS. Relatório Técnico Anual do CNPMS/EMBRAPA, 1992-1993, Sete Lagoas, MG. 68pp.

WAQUIL, J.M., OLIVEIRA, E., PINTO, N.F.J.A., FERNANDES, F.T. and CORRÊA, L.A. 1996a. Efeito na produção e incidência de viroses em híbridos comerciais de milho. Fitopatol. Bras., 21:460-463.

WAQUIL, J.M., VIANA, P.A. 1996. Eficiência do tratamento do solo ou da semente no controle de *Dalbulus maidis* no milho. In: Anais do XXI Congresso Nacional de Milho e Sorgo, 7 a 12 de julho de 1996, Londrina, PR., Brasil. 319pp. (Resumos).

WAQUIL, J.M., OLIVEIRA, E. and FERNANDES. F.T. 1996b. Incidência de *Dalbulus maidis* e enfezamento pálido no ensaio nacional de milho safrinha. In: Anais do XXI Congresso Nacional de Milho e Sorgo, 7 a 12 de julho de 1996, Londrina, PR., Brasil. Pp.318 (Resumos).

WAQUIL, J.M. 1997. Amostragem e abundância de cigarrinhas e danos de *Dalbulus maidis* (DeLong and Wolcott) (Homoptera: Cicadellidae) em plântulas de milho. An. Soc. Entomol. Brasil 26:27-33.

WAQUIL, J.M., VIANA, P.A., SANTOS, J.P. 1997a. Biologia da cigarrinha-do-milho, *Dalbulus maidis* (Delong and Wolcott) (Heteroptera: Cicadellidae). In: Anais do 16° Congresso Brasileiro de Entomologia, 2 a 7 de março de 1997, Salvador, Bahia, Brasil. 79pp. (Resumos).

WAQUIL, J.M., VIANA, P.A., SANTOS, J.P. 1997b. Incidência da cigarrinha-do-milho, *Dalbulus maidis* (Delong and Wolcott) (Heteroptera: Cicadellidae) no ensaio nacional de sorgo granífero. In: Anais do 16° Congresso Brasileiro de Entomologia, 2 a 7 de março de 1997, Salvador, Bahia, Brasil. 330pp. (Resumos).

The Potential Collaborative Role of CIAT in Promoting Maize Virology Research in Latin America

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The Virology Research Unit (VRU) was created in 1988 to conduct research on the viral diseases that affect the adaptation, yield potential, nutritional quality, and utilization of the plant germplasm developed by CIAT's genetic or natural resources programs in collaboration with national agricultural research institutions (NARIs). One of the main objectives of the VRU has been to help plant breeders to identify and select agronomically superior plant germplasm possessing resistance to plant viruses. These activities include the search for virus resistant plant germplasm for hybridization purposes, the study of the genetic interaction between viruses and the plant species they attack, and the screening of segregating populations to select promising virus-resistant plant genotypes.

To fulfil this mission, the VRU has a general virology research laboratory with the necessary equipment to detect and isolate plant viruses, a transmission and scanning electron microscopy facility, an immunology laboratory with the capacity to produce polyclonal and monoclonal antibodies, and a molecular virology laboratory to isolate, clone, amplify (PCR), characterize (sequencing) viral genomes, and utilize recombinant DNA technology, including genetic engineering. Additionally, the VRU has adequate glasshouse, screenhouse, and growthroom space, and insect rearing facilities for virus vector studies. The VRU is staffed by two full-time plant virologists with doctoral degrees, four research associates at the Master degree or equivalent specialist level, three research assistants at the B.Sc. level, and four support staff with technical or secretarial duties.

In the past, the human and material resources of the VRU have been committed mainly to supporting the research activities of the four CIAT commodity programs (beans, cassava, rice, and tropical forages), but the new ecoregional mission of CIAT and the systemwide initiatives fostering the integration of research activities within the CGIAR, have opened new possibilities for collaboration among international agricultural research centers (IARCs). In the case of CIMMYT and CIAT, this new philosophy is contributing to further strengthen a long standing record

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of collaborative activities that began in the 70's, when CIMMYT established a regional program at CIAT.

Since the initiation of the maize regional project at CIAT, CIMMYT scientists have expressed their concern for the high incidence of viruses and phytoplasmas observed in their maize trials in Colombia and other Latin American countries (Williams et al., 1976). The research conducted by the VRU on viruses of tropical forage grasses confirms their observations. Table 1 shows the results of a limited survey of maize, sorghum, and johnsongrass plants at CIAT-Palmira, which were tested as potential sources of a potyvirus found in the tropical forage grass, *Brachiaria*.

It is evident from these assays that the ecology of the viral diseases of tropical grasses may be intimately linked to the epidemiology of maize viruses in Latin America. The results obtained in these surveys indicate that different potyviruses can infect maize and *Sorghum* spp., and that the commercial monoclonal antibody specific for potyviruses does not detect all of the potyviruses present in maize, sorghum, or johnsongrass. In subsequent experiments (data not shown), neither the set of grass differentials nor the commercial antisera available for the sugarcane mosaic virus (SCMV)-subgroup of potyviruses, could be used to unequivocally identify the *Brachiaria* potyvirus. Moreover, the recent revision of the SCMV sub-group by Shukla and co-workers (1992) demonstrated the need for molecular (nucleotide sequencing) or biochemical (HPLC) methods to differentiate between the four major species: SCMV, maize dwarf mosaic virus (MDMV), johnsongrass mosaic virus (JGMV), and sorghum mosaic virus (SrMV). Partial sequencing of the 3' UTR of the *Brachiaria* potyvirus, done at the VRU, conclusively demonstrated that this virus is a strain of JGMV.

Table 1: Serological detection of potyviruses infecting brachiaria, johnsongrass, maize and sorghum at CIAT-Palmira, Colombia.

	Sample	Antisera[1]		
No.	Plant	JGMV	GGMV	PTY1
1	brachiaria	+	+	-
2	"	+	+	-
3	"	+	+	-
4	"	+	+	-
5	"	+	+	-
6	"	+	+	-
7	Johnsongrass	-	+	-
8	"	-	-	+
9	"	-	-	+
10	"	-	-	+
11	"	-	+	-
12	"	-	+/-	+
13	maize	+	-	+
14	"	-	-	+
15	"	-	-	+
16	"	-	-	+
17	"	-	-	+
18	"	+	-	+
19	sorghum	-	-	+
20	"	-	-	+
21	"	-	-	+
22	"	-	-	+
23	"	-	+	-
24	"	-	-	+

8. ELISAs performed with antisera prepared to a *Brachiaria* strain of johnsongrass mosaic virus (JGMV), guineagrass mosaic virus (GGMV), and with the anti-potyvirus monoclonal antibody (PTY1) marketed by AGDIA, Inc.

The importance of maize as a food staple worldwide needs no elaboration. Latin American cultures have depended on the maize crop since pre-Columbian times. Viruses and phytoplasmas have been recognized as major biotic constraints to maize cultivation wherever this crop is grown in the world. In the United States, maize virus characterization projects initiated in 1965 and concluded in 1981 (Gordon et al., 1981), produced a comprehensive study of the viruses and phytoplasmas that cause maize diseases in that country, laying the foundation for all subsequent maize improvement efforts in North America. The Ohio Agricultural Research and Development Center (OARDC) was the leading research group responsible for the development of the U.S. based projects, and it still constitutes the most active group of scientists conducting research on the viral diseases of maize in the Americas.

In 1982, an international working group on maize virus diseases was established during the International Maize Virus Disease Colloquium and Workshop held in Wooster, Ohio (Gordon et al., 1983). This group sought "to foster cooperative projects, [and] to provide assistance to scientists in developing countries for dealing with maize virus diseases." Latin American participants included pathologists from Argentina, Brazil, Costa Rica, Peru and Venezuela. The limited number of participating countries from Latin America has been a constant since the first International Maize Virus Disease Colloquium and Workshop held in 1976. At present, and as reflected in the attendance at this meeting, only Argentina and Brazil represent the interests of Latin American NARIs in maize virology.

To date, CIMMYT has emphasized genetic improvement for disease resistance through the selection of promising breeding maize lines in "hot spots" and/or under artificial infestation. In Latin America, CIMMYT has focused its attention on the selection of maize genotypes exhibiting field resistance to corn stunt. CIMMYT breeders and pathologists are aware of the lack of knowledge about viral diseases in maize that limit the yield potential of cultivars in Latin America, and they would like to widen the scope of their activities in the area of maize virology. However, given the financial constraints that afflict IARCs, and in response to the CGIAR request for collaborative system-wide research activities among IARCs, it is proposed here that the facilities of CIAT's VRU should be made available to pathologists and breeders from CIMMYT and their national program collaborators in support of their virus and crop improvement research activities in Latin America.

Research projects could then be developed to address specific viral disease problems encountered by national program scientists, or to support maize improvement efforts by CIMMYT plant breeders and pathologists. The diagnostic, virus characterization, and maize improvement work conducted at CIAT would be coordinated by CIMMYT scientists through technical staff stationed in Colombia under the supervision of CIAT's virologists.

This proposal can be modified to meet other needs, but the main objective remains the utilization of a fully equipped and modern plant virology facility located in the center of the Americas that will support the activities of NARIs and CIMMYT's scientists in their efforts to control the viral diseases that affect maize production in Latin America.

References

GORDON, D.T., KNOKE, J.K. AND SCOTT, G.E. 1981. Virus and viruslike diseases of maize in the United States. *Ohio Agric. Res. Dev. Cent.*, Wooster. 210 p.

GORDON, D.T., KNOKE, J.K., NAULT, L.R., AND RITTER, R.M. 1983. *International maize virus disease colloquium and workshop: Proceedings*. 261 p.

SHUKLA, D.D., FRENKEL, M.J., MCKERN, N.M., JILKA, J., TOSIC, M., AND FORD, R.E. 1992. Present status of the sugarcane mosaic subgroup of potyviruses. In: *Potyvirus Taxonomy*. Barnett, O.W. (Ed.), pp 363-376. Springer-Verlag, New York.

WILLIAMS, L.E., GORDON, D.T. AND NAULT, L.R. 1976. International maize virus disease colloquium and workshop:*Proceedings. Ohio Agric. Res. Dev. Cent.*, Wooster. 145 p.

Disease Management in Maize

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Introduction

The advent of integrated pest management (IPM) opened new doors for workers dealing with biotic constraints to broaden their approaches to integrated crop management (ICM.) By definition, integrated disease management (IDM) has stressed integration, but it was based on the management of host resistance genes rather than on pesticides. With the advent of the microchip and advances in satellite geopositioning technologies, precise crop management systems are now possible. There are rational reasons for crop management, but these reasons must be tempered in such a manner as to ensure both a reasonable quantity of production and the sound stewardship of resources as well. Future production must be environmentally sound. Prophets of doom contend that the current system of agriculture, irrespective of location, cannot be sustained. There are finite resources and ultimately these will be strained to their limits. Consequently, agricultural programs and policies must be directed toward the most responsible practices that support and protect the environment, biodiversity, and other related factors.

Many of us in the USA have been influenced by Aldo Leopold's (1949) simple yet powerful book *A Sand County Almanac*. Leopold accepts that agriculture as part of the biotic community. Indeed, agriculture has been the basis for all civilizations, past and present, directly or indirectly. It represents *who* and *what* we are. Consequently, the comprehensive understanding of the biotics of agriculture will lead to a better understanding of the ecosystem of which we are part.

The Maize Model

Maize is one of a few important grasses that humanity has cultivated for centuries to provide food and a considerable number of industrial products. What makes maize unique is that from the beginning it was a cultivated crop (Galinat, 1977). While the origins of maize remain an enigma, the fact that it cannot survive without crop management is not disputed. Following the development of double cross hybrids and more recently, sin-

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gle cross hybrids maize production has dramatically increased in the USA (Fig. 1). The dramatic increases have resulted from genetic gains, superior performance from greater stands, increases in supplemental fertility levels, better pest and disease management, and weed control (Botrell, 1979; Duvick, 1994; Troyer and Rosenbrook, 1983).

The application of the principles of integrated disease management require that disease management be considered as only part of the holistic view of plant heath and thus as only part of overall crop management.Crop management then becomes an integral component of the comprehensive ecosystems of the growing region (Bolkan and Reinert, 1994).

Principles of Integrated Disease Management

Many of these principles have been used or adapted by plant pathologists from the earliest attempts to control disease. Apple (1977) and more recently Fry (1982) provided outlines and perspectives for an approach to integrated disease management. The recent program offered by Line and Cu (1993) is remarkably similar in principle. The principles used here are those suggested by Apple (1977).

Define the diseases to be managed

The first goal is to know the problem(s). This requires accurate diagnosis, but obtaining a correct diagnosis is often challenging. Pests, herbicide interactions and residue, plant nutrition, environmental stress, and below ground damage could confuse the diagnostician. While rusts, smuts, downy mildews, and most foliar pathogens are easily determined, interactions with virus and virus-like agents with many abiotic factors often present a challenge to anyone but an experienced diagnostician. Consequently, there is often a constant need for accurate diagnosis on a field by field basis. Missed diagnoses mean additional losses and inappropriate control strategies. Even with a correct diagnosis, the disease could be too far advanced for intervention during the current season. The list of diseases to be entered into this esoteric scheme needs to be clearly defined. For example:

Foliar diseases

Rusts and Phaeosphaeria spot may require intervention prior to selection of the cultivar. Rust resistance has been controlled through quantitative resistance for an extended period of time (Bailey et al., 1987). Host resistance is required for both common and southern rust. Selection of the cultivar takes place prior to planting. The decision concerning the type of resistance, qualitative or quantitative, would be determined by the availability of material, the durability of the resistance, and the identification of the reaction of the genotypes. This, of course, becomes an important objective of the program. It is not uncommon to see or read the recommendation “Grow resistant cultivars.” Unfortunately, it is difficult to obtain reliable information.

Virus and virus-like diseases

Diagnosis carries a historical message. Quite simply, it implies that the disease needs to be known well in advance of the management tactic. Since the viruses attacking maize are vectored, the knowledge of the habits and habitats of these vectors are also required. As a former student of insect vector agents, I can say that identifying the vector and its relation to the disease is as significant as the causal agent itself. In a dynamic disease management program both the vector and agent must be anticipated. What has happened under these conditions in the past will presumably occur again under the same conditions; therefore, we can anticipate what will happen in the future. As challenging as identifying the agent is, it is even more challenging, in the author’s opinion, to develop an appropriate control strategy. The development of host plant resistance to maize streak in many ways represents the interdisciplinary approach required for eventual disease management. Evaluation required a new paradigm of controlling the vector, the virus acquisition, infestation, evaluation of germplasm, and nursery design. Combining, rearing, transporting, and uniformly infesting maize plants with the vector permitted breeders to rapidly make progress in host resistance.

Ranking of disease problems

This is important for several reasons. Normally, researchers tend to find a niche and remain within that problem area until they have accomplished their goals (some even longer). Growers, however, tend to find and face new problems each year and not too infrequently face several types of problems within a single growing season. In Texas, it is common to have both northern corn leaf blight, followed by southern rust, followed by a major insect infestation and drought. While growers have good memories, they tend to think of today’s problem as the one that needs the greatest attention and

requires a solution—now! The balance between addressing the immediate needs of growers and the need to formulate a long-range focused research agenda cannot be overemphasized. It is difficult to believe that problems facing growers in Brazil are any different from those confronting growers in the USA or elsewhere. Formulating a comprehensive plan that encompasses most, if not all, of the important problems will establish a benchmark for future decisions regarding resource management. Ranking cannot be based on economic factors alone. Certainly, those areas where there may be the greatest return on the investment dollar are among the most important factors to be considered. Problems that can be addressed with the resources and tools available should enter into the decision making process as well. Naturally, the interest of the individual investigators also affects where the research is directed. The interests and training of individuals also limits their contribution.

No one individual should have the responsibility of envisioning all of the research activities. Judgments need to be made by a group composed of growers and multidisicpline research teams, who act in concert with policy and decision makers. Once the list has been made and priorities established, the list needs to be reviewed, perhaps annually and at least every 5 years to make changes and, of course, to chart progress. Oddly enough, sorghum downy mildew of maize in Texas has not caused significant damage for the past 20 years. Integrated disease management has worked so successfully that it is virtually impossible to find resources to continue working with the pathogen (Odvody et al., 1983). This includes support for the evaluation of genetic resistance, development of probes for diagnosis, etc. Ironically, a good epidemic appears to be in order every so often. But as growers know only too well, problems other than downy mildew prevail. Currently, we are not emphasizing research on downy mildew but have shifted much energy to other challenging disease problems such as *Aspergillus* ear rot.

Define the management unit

Different management units will require different approaches. Many disease problems are reduced when there is host diversity, intercropping, intrafield diversity, rotation, etc. When disease problems must be managed within an intensively, monocropped area of genetically homogeneous cultivars, the situation becomes much more challenging. The management unit may be as extensive as the national program or as focused as a single field. Normally, it would include a defined area more or less consistent with the most important growing areas and would be based on the agroecosystem for that region. As with any planning activity, this must be defined. Units may have several details in common but more frequently the differences among locations are the most important. The environments differ, the rotations differ, the growing season from south to north and east to west differs.

Develop a management strategy

Fundamentally, there are only two disease management strategies: those which reduce the initial inoculum and those which reduce the spread of the pathogen within affected areas. Each disease could have one or more control tactics that affect either of these strategies. The management strategy needs to consider both the tools available and those that could be developed. At this time it becomes important to integrate the work within a comprehensive crop management system. Far too often good ideas for disease control fail because of interference with agronomic or entomological actions. A management strategy for the current decade must be comprehensive (Schreiber et al., 1987).

Establish economic thresholds

This is not to suggest that plant diseases can be managed like pest infestations. Growers of maize rarely arrest epidemics with pesticides. Most of these management decisions are made prior to planting. Economics are important because relations between symptoms and loss are not always obvious. Data from reactions of maize hybrids in the USA suggests that some cultivars with severe symptoms have modest to no apparent yield loss whereas others with modest symptoms have major production affects. Kaufman et al. (1994) have determined that maize dwarf mosaic virus caused severe symptoms in some maize hybrids but that yields were essentially normal. Other examples included maize cultivars with modest symptoms but extremely severe yield reductions. Early information becomes the most valuable tool that a grower may have. Within an integrated system the actual economic benefits need to be determined. Interestingly, this can be challenging when a single weed could generate seed for an economically significant infestation next season. For a pathogen, one spore, or in the case of ergot in Brazil, a single sclerotium, may have been responsible for the introduction. Models determining the timing of application of pesticides in maize are known (Bowen and Peterson, 1989). An important aspect of Bowen and Peterson's model was the determination that increasing levels of resistance required less fungicide.

Develop monitoring techniques

Whether there are economic thresholds or not, there is a constant need to monitor disease progress. This information can be used to model the disease and to incorporate the types of changes needed for additional controls in the future. There is no substitute for a good general understanding of the rate of the epidemic and the factors that affect that rate. Disease management could mean many things, from a difference of harvesting at a different stage of growth, to that of varying plant populations, to changing fertility practice. An extraordinary affect of disease has been in the management of collateral hosts in maize such as johnson grass. Movement of aphids from johnson grass affects the population of vectors in maize and could increase the amount of virus infection. Judgments must be made between the importance of the disease in relation to the timing of the application of herbicide.

Evolve descriptive and predictive models

Ultimately the accumulation of these data on disease management needs to be compiled in an accessible, user friendly system that aids the development of comprehensive management programs. For disease management this means providing data on the vulnerability of each cultivar to the possible problems in each area and the host reaction to each (Kaufman et al., 1994). Growers need additional data on the yield potential and the risks associated with the growing of a particular hybrid. With these data, growers are in a much better position to define the risks that they will confront during the cropping season.

Integrated Crop Management

Currently, several integrated crop management programs have evolved out of earlier IPM programs (e.g., Cook and Vesseth, 1991; Schreiber et al., 1987). Note that currently there are several crop health management manuals developed or being developed by APS Press. Most are prepared with the idea of providing growers with the information to make management decisions, often fully integrated for each stage of growth. IPM science has not been implemented to the desired level in practical agriculture because crop protection strategies have not been fully implemented into crop production systems. Current crop protection practices for pests like insects and weeds generally rely on remedial control through the use of chemical pesticides. Consequently, IPM for most pests of most crops is based on pesticides rather than on biologically/ecologically compatible management tactics.

There is a serious need to change from the mentality of remedial pest control using chemical pesticides to that of planned preventive tactics that are environmentally safe. Chemical pesticides are often ecologically disruptive, whereas many non-chemical pest management tactics are ecologically compatible and highly sustainable. An escalating awareness of pesticide effects on the environment and human health, as well as a need for increased crop production efficiency, dictate a greater role for alternative, non-chemical management tactics for crop protection—and the judicious use of pesticides based on immediate need. Non-chemical pest management tactics are preventative in nature and thus must be a planned part of the total crop production system. Also, IPM has failed to be a fully pest integrated approach addressing insects, pathogens, and weeds.

Production practices for agriculture are expected to be significantly impacted by regulations designed to protect the health of humans and ecosystems. This will certainly be true in the US because only 2% percent of the population are involved in agriculture. Frankly, where there is only one well, people will secure the water before the maize fields. In the USA, it is clear that the discharges of soil erosion, nutrients, and pesticides from agricultural cropland must be dramatically reduced. Progress has been made and we believe that it is gaining momentum, driven in part by an increasing demand for organically grown crops. It is unrealistic, however, to believe that this alone will alter the conditions for agriculture.

What is being proposed here is a need for integrated disease management, but it needs to be developed within the framework of a comprehensive crop management model.

It is necessary to understand how maize production fits within the total agroecological system and how this in turn impacts relations between the agroecosystems and the entire environment. According to Cook and Veseth (1991) there are 8 principles of integrated crop management (ICM). These are:

8. Know the production limits of the cropping system.
8. Maintain soil organic matter and soil structure.
8. Rotate crops.
8. Choose well-adapted, pest and disease resistant cultivars.
8. Select high-quality, weed and disease free seed.
8. Minimize environmental and nutritional stresses on the crop.
8. Conserve and enrich populations of beneficial insects and microorganisms.
8. Scout for pests and treat with pesticides as necessary.

Knowing the limits of economic yield is important because if production inputs exceed the levels of economic yield there is a vast waste of resources. If not already known, limits can be established through experiments or from long term records. Naturally, these yields will vary with location because some environments are better than others for production. The law of diminishing returns does apply to agriculture. Far too often resources can be wasted because the expected yield—while attainable—exceeds the economic optimum. Establishing those standards makes it easier to determine the effects of other yield diminishing factors as well.

Maintaining organic matter can be very difficult in the tropics. Agriculturists in parts of west Africa have a major interest in capturing organic matter because of the long term benefits for the soil. But the demand is high for organic matter, which is used for forage, fuel, and in some cases for construction. There is little doubt that organic matter is valuable for maize in Brazil. But has the actual value been determined, not only in the short range but over a period of decades?

Rotation is constantly being reinvented. Not surprisingly, it was well established as an important practice centuries ago. The classic studies at Rothansted need not be referred to in this paper, but the wisdom of rotation cannot be ignored. Comparable studies have undoubtedly been conducted in Brazil as well. One benefit, based on studies in the USA, has been the reduction in loss of soil because of erosion in a rotation as compared with continuous maize. Interruption of survival of soil borne pathogens and survival structures of others are likely benefits. These can be evaluated with careful study. The type of rotation and period of fallow need to be carefully addressed.

Disease resistant and pest resistant cultivars are important. This information needs to be available to growers, and it needs to be obtained from an independent agency working for the public, such as EMBRAPA. The basic objective of an integrated system is the distribution of information that details the available options. The Rice Production Guidelines (Drees, 1996) is such a program. This manual provides growers with the best inputs from a team of production experts for the upcoming season. Essentially, it is a “what to, how to” manual for maximizing efficient rice production in Texas.

Similarly, poor quality seed with low or weak germination has no role to play in modern agricultural production. Normally, the industry provides excellent hybrid seed—this should be expected. Poor quality seed could result in less than appropriate plant stands. Years ago at CIMMYT, workers expected a plant from each seed (G. F. Sprague, personal communication). Standards may not be as high for small seeded and oil seed crops but for maize hybrids this is not the case. Nearly perfect stands can be approached with current technologies.

Stress, particularly environmental stress, can occur at virtually each stage of growth, and there is little that can be done about some of these stresses following establishment of the crop. Again, knowledge of the history of the crop is important in considering the steps needed to limit stress. The planting date cannot always be determined, but when possible it should conform to the most appropriate time for maximizing production. Soil acidity and related soil problems constitute a major stress factor under many conditions in Brazil. In the long run, environmental stresses may be easier to handle than biotic stresses. Biological organisms can and do change rapidly.

Biodiversity and Maize Production

Maize can be cultivated in a number of ways. The North American model calls for the growing of highly efficient, single cross hybrids in high populations on much of the most productive land. Greater and greater emphasis has been placed on the value of monocropping vast hectarages of genetically homozygous, homogeneous maize fields. The result of this has been an extraordinarily productive crop based on extensive inputs such as fertilization, weed management and pest control. Today that same genotype(s) of maize is being improved with enhanced pest resistance through biotechnology, and many other traits that will transform maize are in the R&D pipeline. This model is as genetically narrow today as it was in 1970, during the great southern corn leaf blight epidemic. Furthermore, maize is becoming more and more dependent on management by seed producers, and the costs of seed are mounting (commensurate with increasing yields). Pundits of agriculture in the USA have pointed to the vulnerability of this practice for nearly 3 decades. While the pending catastrophe has not yet appeared, the potential consequences continue. For example, because of extensive flooding and other environmental factors over the past 2 years, stocks of maize and other cereals are relatively low in the US. New Federal agricultural policies suggest more wall to wall planting of maize in the future. Consequently, not only will production increase, but, in the author's opinion, so will erosion and pollution as a result of attempts to maximize production and there will be an increase, in the author's opinion, so will erosion and pollution. Additionally, attempts to maximize production will increase the vulnerability of maize to biotic agents.

Mounting evidence suggests that increases in diseases such as southern corn leaf blight, southern rust, anthracnose, gray leaf spot, aspergillus ear rot, and even Diploidia ear rot are indications of increased dependence on more and more genetically uniform hybrids or monocropping or minimum tillage systems. The continuing increase in the production of maize hybrids (Fig. 1) indicates that there are benefits from this system at least for the present. Yet are there alternative approaches that can be taken to conserve the most valuable genetic resources? It seems almost too obvious that risks are increasing. Many suggest that maintaining biodiversity as a management tool to reduce risk is important (Alexander, 1989; Browning, 1988). Diversity need not mean mixtures of genetically different hybrids, but carefully managed and selected approaches to intra field diversity.

Ethics and Maize Production

Ethics is not the correct word but it is an attention getter. Maize is an important food in Brazil as well as in many other countries of the world. It will become even more important. Currently, more and more individuals are looking at the questions of ecologically stable agriculture. According to Thompson (1995), "anthropocentric answers to such questions predict catastrophe for human populations if biological limits are not respected. Ecocentric answers attribute intrinsic moral value to the integrity of ecosystems, and ecocentrists defend a prima facie moral obligation to preserve them without regard to consequences for human populations."

Our conscience tells us that we may be playing with human lives. Our new management models need to include the alternatives to having a reliable food supply. Somewhere in this vast formula, policy makers need to look at the options that recognize the potential of long range planting. Maize is far too valuable a resource and food to begin looking at alternative crops. Planning means accepting the consequences for the decisions made and promises kept.

References

ALEXANDER, H. M. 1989. Spatial heterogeneity and disease in natural populations. Pp. 144-164. In *Spatial Components of Plant Disease Epidemics*. M. J. Jeger, ed. Prentice Hall, Englewoods Cliffs, New Jersey 07632.

APPLE, J. L. 1977. The theory of plant disease management. Pp. 79-101. In *Plant Pathology an Advanced Treatise*. J. G. Horsefall and A. E. Diamond eds. Academic Press, New York, NY.

BAILEY, B. A. , SCHUH, W., FREDERIKSEN, R. A., BOCKHOLT, A. J., and SMITH, J. D., 1987. Identification of slow rusting resistance to *Puccinia polysora* in maize inbreds and single crosses. *Plant Dis*. 71:518-521.

BOLKAN, H. A., and REINERT, W. R. 1994. Developing and implementing IPM strategies to assist farmers: An industry approach. *Plant Dis*. 78:545-550.

BOTRELL, D. G. 1979. *Guidelines for Integrated Control of Maize Pests. FAO Plant Production and Protection Paper*. FAO, Rome 91 pp.

BOWEN, K. L, and PEDERSON, W. L. 1989. A model of corn growth and disease development for instructional purposes. *Plant Dis*. 73:83-86.

BROWNING, J. A. 1988. Current thinking on the use of diversity to buffer small grains against highly epidemic and variable foliar pathogens: Problems and future prospects. In *Breeding Strategies for Resistance to the Rusts of Wheat*. N. W. Simmonds and S. Rajaranm, eds. CIMMYT Mexico, D. F. CIMMYT.

COOK, R. J, VESETH, R. J. 1991. *Wheat Health Management*. APS Press. St. Paul, MN, USA 152pp.

DREES, B. M. 1996. 1996 *Texas Rice Production Guidelines*. Texas Agricultural Extension Service. D. 1253. Texas A&M University, College Station TX. 63 pp.

DUVICK, D. N. 1994. Intensification of known technology and prospects of breakthroughs in technology and future food supply. Presentation to IFPRI Roundtable on *Population and Food in the Early 21st Century: Meeting Future Food Needs of an Increasing World Population*. Feb. 145-16, 1994. Washington DC.

FRY, W. E. 1982. *Principles of Plant Disease Management*. Academic Press. New York, NY 378 pp.

GALINAT, W. C. 1977. The origin of corn. Pp. 1-47. In *Corn and Corn Improvement*. G. F. Sprague, ed. American Society of Agronomy. Madison, Wisconsin, USA.

KAUFMAN, H. W., FREDERIKSEN, R. A. and KRAUSZ, J. P. 1994. *Disease resistance in corn hybrids*. Texas Agricultural Extension Service B. 5099 6 pp.

LEOPOLD, A. 1949. *A Sand County Almanac*. Oxford University Press. Oxford, GB. 226 pp.

LINE, R. F and. CU, R. M. 1993. *MoreCrop: Managerial options for reasonable economical control of rusts and other pathogens. An expert system for managing wheat diseases in the Pacific Northwest*. Wheat Genetics, Physiology, Quality, and Disease Research Unit, Agricultural Research Service, U. S. Department of Agriculture, Pullman, WA 99164-6430

ODVODY, G. N., FREDERIKSEN, R. A. and CRAIG, J. 1983. The integrated control of downy mildew. In *Proceedings American Seed Trade Association Corn and Sorghum Conference* 38:28-36.

SCHREIBER, M. M. , ABNEY, T. S., and FOSTER, J. E. 1987. Integrated pest management systems: a research approach. *Purdue University Research Bulletin 985*. West Lafayette, Indiana 47907.

THOMPSON, P. B. 1995. *The Spirit of the Soil: Agricultural and Environmental Ethics*. Routledge, NY, NY. 196 pp.

TROYER, A. F. and ROSENBROOK, R. W. 1983. Utility of higher plant densities for corn performance testing. *Crop Sci.* 23:863-867.

Appendix A

Working Group Recommendations

Two working groups were set-up
to address the establishment of research priorities
regarding disease problems and disease management strategies.

Southern Rust, Common Rust, Tropical Rust, and Phaeosphaeria Leaf Spot

Chairperson: *Francisco Xavier Ribeiro do Vale*

Reporters: *Fernando Tavares Fernandes*
Carlos Roberto Casela

Short Term Recommendations

Several institutions have gathered information from research or field observation on losses caused by rusts and phaeosphaeria leaf spot and on maize disease epidemiology. The development by EMBRAPA/CNPMS of a database based on all the available information, including results obtained by the Maize Research Support Group (NAP) was suggested. These data will be available to all people interested in maize.

A maize disease nursery was organized for the purpose of evaluating all commercial cultivars. This nursery will be planted in areas of high disease incidence in order to select resistant genotypes to the major maize diseases.

Research Priorities

During the Workshop, the economic importance of phaeosphaeria leaf spot became evident, and it became equally evident that information is lacking in regard to its epidemiology and management. The three rusts are present in all the major maize production areas of Brazil, which include the States of Minas Gerais, São Paulo, Goiás, and Paraná, and they have the potential to severely reduce maize production in all these areas. The following research priorities were identified:

Host Plant Resistance

Determine the genetics of disease resistance and develop a maize disease evaluation nursery to evaluate the reaction of current commercial hybrids. Following the identification of good sources of genetic resistance this resistance should be incorporated into good agronomic, commercial cultivars. The utilization of molecular biology tools was considered important for the success of this work.

Chemical Control

Applying fungicides is a short term alternative for seed production purposes, and they have been applied in areas of high disease incidence. Additional research is needed on chemical controls for these four diseases in order to use fungicides as efficiently and productively as possible.

Epidemiology

Precise knowledge is needed about the epidemiological factors that affect disease severity and incidence in order to develop control strategies. Studies addressing the influence of abiotic and biotic factors (climate, plant nutrition, cultural practices, crop rotation, etc.) on disease incidence and severity need to be undertaken, as do studies on the survival and dissemination of the major foliar pathogens.

Etiology and host-pathogen interactions

In order to develop more stable and durable resistance to these diseases, the variability of maize rust pathogens and Phaesphaeria maydis must be characterized. The completion of Koch's postulate is required to confirm *P. maydis* as the causal agent of the phaeosphaeria leaf spot.

Crop loss evaluation

Although field observations indicate the destructive potential of these diseases, it is important to measure these losses both qualitatively and quantitatively.

Decisions taken

1. A steering committee was formed to coordinate decisions concerning the development of a cooperative research project and the definition of future strategies on maize disease problems at a national level. Dr. Alvaro Eleutério da Silva was nominated as the coordinator of the steering committee.
2. The short term recommendations for the control of foliar fungal pathogens will be published in specialized publications as well as in newspapers of national circulation in Brazil.
3. The steering committee will meet in Piracicaba (São Paulo) on a date to be set by the coordinator. The purpose of this meeting will be to define the cooperative research projects involving all the interested private and public institutions.

Corn Stunt Spiroplasma, Maize Bushy Stunt Phytoplasma, and Viruses

Chairperson: *Renato O. Resende*

Reporters: *Elizabeth Oliveira*
João R. Spotti

Introduction

Viruses, corn stunt spiroplasma and maize bushy stunt phytoplasma were discussed in regards to: a) the high incidence of these diseases in several countries of Latin America; b) the major aspects of pathogen dissemination, diagnosis, and disease management; and, c) the experience of the group, including virologists, plant pathologists, entomologists, breeders, and other experts on maize crop management.

As a result of these discussions, short term management and research priority recommendations were established. Strategies were offered to implement these recommendations, in which official and private sectors will participate. A cooperative research project, involving all the institutions represented in the working group, was elaborated, and possible sources of funding to support this work were considered.

Research Priority Recommendations

Etiology and production of kits for pathogen detection in plant tissue and insect vectors

It was agreed that studies are needed to identify and/or characterize the causal agents of the corn stunt complex and viruses, which are highly prevalent in maize in certain areas of Brazil. The reports of many participants, who displayed maize leaf samples infected with the corn stunt complex symptoms, but in which no pathogen was detected by diagnostic test kits, clearly demonstrated the need for this research.

Given that the diagnosis of viruses and corn stunt complex diseases based only on symptoms is usually very difficult or even impossible, there was a consensus that the development, adaptation, and/or production of diagnostic kits is urgently needed. The etiology and development of kits for pathogen detection were also declared fundamental for future research on epidemiology and the genetics of resistance.

Considering the facilities and experience available at several institutions, collaborative work coordinated by the EMBRAPA - Maize and Sorghum Research Center (CNPMS) on etiology and diagnostic kits production was suggested as follows:

CNPMS/EMBRAPA:	studies on the etiology, insect vectors and the production of kits for virus and mollicute detection.
UnB:	production of kits for virus detection.
ESALQ:	studies on the etiology of insect vectors through electron microscopy.
INTA:	studies on maize rough virus.
CIAT:	etiological studies using electron microscopy.
USDA:	studies on mollicute pathogens.
University of Nebraska:	studies on the virus' etiology.
CIMMYT:	etiological studies.

It was agreed that all the available diagnostic technology must be made available to both private and official institutions. Also, short courses to demonstrate these technologies should be conducted.

Identifying areas of high virus, corn stunt spiroplasma, and maize bushy stunt phytoplasma incidence.

Information on the incidence of these diseases is scarce, especially in Brazil. It is important then, to develop a monitoring system to establish their geographic distribution and incidence. This work will be developed through the evaluation of the National Maize Cultivar Performance Tests and the observation of susceptible cultivars. This information will identify areas and seasonal periods of higher incidence, as well as the relative importance of each disease. The areas and time of the year to carry out epidemiological assays and germplasm evaluations will also be identified based on this information.

Representatives of the private sector and of the University of Lavras (UFLA) affirmed their willingness to cooperate in this monitoring work under the coordination of EMBRAPA. There was agreement, however, that the methodology for the evaluation of these diseases must be standardized.

Germplasm bank

Efforts to increase the germplasm bank through the introduction of new sources of resistance to these diseases and to insect vectors are needed.

Host plant resistance

The use of resistant cultivars is the most effective way to control plant diseases. There was general agreement that research on maize resistance to viruses, corn stunt spiroplasma, and maize bushy stunt phytoplasma must be part of all breeding programs in both official and private companies.

Epidemiology

For a better understanding of the effects of biotic and abiotic factors on disease outbreaks, epidemiological assays on viruses, corn stunt spiroplasma, and maize bushy stunt phytoplasma must be carried out on susceptible cultivars in different periods of the year and in the major maize production areas. These studies will involve monitoring disease incidence, populations, and the infectivity of insect vectors during the maize crop season.

The importance of several other studies was also mentioned: a) pathogen transmission by unknown insect vectors, b) other pathogens and insect vectors hosts, and c) seed transmission of these pathogens.

Maize crop loss evaluation

Very little information is available on maize production losses due to viruses, corn stunt spiroplasma, and maize bushy stunt phytoplasma. It is important to obtain data on crop losses.

Other important disease management strategies

- maize cultivar resistance evaluations
- effects of the control of insect vectors on the spread of their associated diseases
- effects of crop management on disease incidence.

Short Term Recommendations

1. A high incidence of diseases caused by leafhopper *Dalbulus maidis* transmitted pathogens have been observed in late plantings of maize (after November in Central Brazil) and on the second growing season named "safrinha". This high incidence has been attributed to larger populations of *D. maidis,* normally present in March and April. It is recommended, at least for Central Brazil, to avoid late plantings and keep fields free of maize plants for at least two or three months between growing seasons to reduce pathogen inoculum and *D. maidis* populations.
2. Resistant cultivars should be grown in areas and periods of high disease incidence.
3. The use of insecticides is not recommended to control insect vectors without specialized technical assistance.
4. Support is needed from the official and private sectors to improve diagnostic kits to screen resistant germplasm and to monitor diseases.
5. Training, through short courses, of personnel involved in viruses and corn stunt disease diagnosis will be offered.
6. Finally, it is important to establish an information network to support growers as they seek to manage maize viruses, corn stunt spiroplasma, and maize bushy stunt phytoplasma.

Conclusions and Remarks

A steering committee was established to implement the recommendations of this working group. This committee will always meet during the Maize and Sorghum National Congress at Sete Lagoas (MG), and whenever else it is needed, to report and analyze the development of activities, and if necessary, to define new directions in research development and to consider new recommendations.

Appendix B

Protocol for Diagnosing Maize Viruses, Corn Stunt and Rayado Fino

These tests and MABs were developed as part of the collaborative project between Pioneer Hi-Bred International and CNPMS of Brazil under a project developed under the aegis of ISAAA

Protocol for ELISA

Day Before Assay

1. Prepare wash and sample buffer the day before running the assay. Add contents of one packet to 750 ml of water. Dissolve the reagents. Make volume up to 1 liter with distilled water.
2. Prepare concentrated $MgCl_2$, Substrate Buffer and Coating buffer. Store as indicated.
3. Determine the number of samples to be processed. Design the ELISA sample format.
4. Cut enough carborundum paper for all samples.
5. Add 2-3 ml of Wash Buffer to each tube for sample processing.
6. Dilute the Corn Stunt Antibody to 1:5000/Coating Buffer. Add 200 µl/ well. Label the plate. Seal the plate and store at 4°C.
7. Dilute the Rayado fino Antibody to 1:5000/Coating Buffer. Add 200 µl/ well. Label the plate. Seal the plate and store at 4°C.

Sample Preparation

Grind a section of leaf (2.5 x 5 cm) between a piece of carborundum. Put one piece /tube. Shake the tube for 30 seconds. Let the tube stand to allow the plant material to settle. Process all the samples in the same way.

The samples can be collected in the field and processed in the lab. I advise using fresh material, until stored material can be tested along with fresh material. I do not know what happens to stored material in these detection systems. You will also need to collect negative material or uninfected material. The source of this protein will be VERY important. You MUST have tissue that is NOT INFECTED. You may add as many negative controls as you wish.

Assay Protocol

1. Turn the ELISA plate over and shake out the coating antibody. Wash the plate once with Wash buffer. You may leave the buffer in the plate until you add the samples and controls.
2. Transfer 200 µl of sample to each test well. Run each sample in duplicate. Prepare a dilution of the purified Corn Stunt spiroplasma and the Rayado fino coat protein to give
   1 ug/ml. Prepare 0.5 ml of each protein for each ELISA plate. Add 200 µl of each protein, in duplicate, to each test plate. Incubate the sample and controls on the plate for 2 hours at room temperature. This incubation can be done at 4°C overnight for convenience.
3. Fill plastic wash bottle with wash buffer.
4. At end of sample incubation, turn over the plate and shake out the sample extracts. Wash the plates three times with the Wash Buffer. Be certain to fill the wells completely.

5. Calculate how much diluted conjugate you will need for the number of samples you are testing. For example: 20 ml /ELISA plate or 46 samples in duplicate plus negative control extract and positive control. Dilute the enzyme conjugated antibodies to 1:2000/Wash Buffer. BE CAREFUL NOT TO MIX THE CONJUGATED ANTIBODIES AND NOT TO ADD THE WRONG CONJUGATE TO THE WRONG PLATE.
6. Add 200 µl the diluted conjugate to each test well. Incubate for 2 hours at room temperature.
7. Prepare the substrate. For one plate you will prepare 25 mls or 5 tablets/ 25 mls of Substrate Buffer. It will take a little time for this to go into solution. Incubate at room temperature. Prepare only what you need. You cannot store this solution.
8. Wash the plates as in Step 4.
9. Add 200µl/ well of substrate solution.
10. Incubate the plate for at least 1 hour at room temperature. A yellow color in the positive control wells will tell you that the assay is working and the plate received all the right reagents.

NOTE: We have incubated the plates overnight and found that it is very easy to distinguish the positive controls because the negative samples remained clear or had very little color.

ELISA for the Detection of Corn Stunt and Rayado Fino

Reagents and Buffers

1. *Coating Antibody* – Rabbit and anti-corn stunt and rabbit anti-rayado fino coat protein.
2. Coating Buffer - 0.015M Na_2CO3, 0.035M $NaHCO_3$, pH 9.6. Store at 4°C for one week.
3. *Sample and wash buffer* – Sigma 10 mM Phosphate Buffered Saline packets.
4. 96–well ELISA plates
5. *Carborundum strips* – This is for sample processing.
6. *Alkaline phosphatase –conjugated antibodies*. There are two antibodies. Each antibody is specific for the pathogen. They are the same antibodies as used for coating but have been chemically coupled to enzymes.
7. *Sigma substrate tablets*. (104- 105). The substrate is p-nitrophenylphosphate. Each tablet is 5 mg. Each tablet is dissolved in substrate buffer to give a final concentration of 1 mg/ ml.
8. *0.5M $MgCl_2$* Store at room temperature.
9. *Substrate Buffer:* 10% Diethanolamine, 0.2 mM $MgCl_2$, pH 9.8.
10. Add 50 grams (liquid) to 400ml of H_2O. Adjust pH to 9.8 with concentrated HCl. Add $MgCl_2$ to a final concentration of 0.2 mM. Adjust the volume to 500 ml. Store at 4°C.
11. *Positive Controls* – The positive controls are purified preparations of killed corn stunt spiroplasma and rayado fino virus coat protein.
12. *ELISA design formats*. – This is a copy of the 96 well-plate format.

Additional materials

1. Plastic squeeze bottle for washing plates.
2. Vessel for preparing wash buffer.
3. Multi-channel pipette (This is not necessary but is convenient.)
4. Disposal pipette tips for transferring sample from tube to ELISA plate.
5. Distilled water.
6. Plastic tubes for sample preparation.
7. Plastic plate sealers.

US$ 10.00

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