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INTRODUCTION

Interpretive Summary of Proceedings

These Proceedings present a wealth of information on virus and mycoplasmalike organismal (MLO) diseases of maize (Zea mays L.) worldwide. Subjects covered include: the viruses and MLO’s; their vectors and hosts; symptomatology; identification, including serology; geographical distribution and economic importance of the diseases; and disease etiology, epidemiology, and control. These Proceedings report numerous significant findings from which we have selected those we believe to be of greatest interest. An interpretive summary of these findings and a discussion of some of their attendant issues are presented in the following. (For this introduction, author names not followed by year of publication refer to the manuscripts in these Proceedings.)

The importance of these findings, particularly as they relate to increased maize production, may be realized in light of projections by the U.S. Agency for International Development (USAID) on human population increase and the increases in agricultural output required to meet the needs of the world’s rapidly expanding population (Smith). The importance of maize in meeting the future needs for food is expressed in Dr. Norman E. Borlaug’s estimate that, “in the decades ahead, maize is destined to become the most important cereal crop and this crop will be the salvation of increasing millions of people by the year 2000” (Havener). It is estimated (Smith) that a modest reduction in crop losses due to virus diseases worldwide would result in a dramatic increase in maize output.

An important value realized from the Colloquium and Workshop and these Proceedings was that they have allowed us to share our findings through personal contact, to expand our understanding of maize virus diseases, to define the problems concerning these diseases, and to make arrangements for cooperative plans for future work to investigate these problems (Sect.).

Importance of Maize Dwarf Mosaic Worldwide

Unquestionably, the most widely distributed and important virus disease of maize is maize dwarf mosaic (Ammar, Autrey, Castillo, Conti, Exconde, Greber, Klinkong and Sutabutra, Kitajim and Costa, Lastra and Carballo, Lockhart and Elyamani, Sharma and Payak, Signoret, Teyssandier et al., Tosic, Uyemoto, von Wechmar, Zhu et al.). In spite of this obvious importance and considerable research, beginning with the discovery of the virus in Italy in the late 1930’s (Conti), our information is inadequate for resolving several fundamental issues. Among these are the relationships between the many isolates and strains of the maize dwarf mosaic virus (MDMV), including those of sugarcane mosaic virus (SCMV) to which MDMV is related. A tentative grouping of many of these strains and isolates (Tosic, Tosic and Ford), based on host preferences and symptomatology among selected sorghum [Sorghum bicolor (L.) Moench] genotypes, has been proposed in a preliminary study of these relationships (Tosic and Ford). The suitability of these groups needs to be tested by study of relationships based on other viral properties, particularly serology.

Another issue concerning these strains and isolates is the inconsistency or non-uniformity in their nomenclature. A synonymy is presented (Tosic) which promises to eliminate some of the confusion in the literature wherein different virus names have been used for the same virus. However, this nomenclatural problem would best be resolved by a consensus among researchers to use a standard, universally accepted nomenclature.

Important subjects of inquiry currently attracting considerable interest and research effort are the epidemiology (Knoke et al., Madden et al.) and genetics of resistance (Scott) of maize dwarf mosaic. The extensive review (Knoke et al.) of information on MDMV aphid vectors provides fundamental information needed for understanding the vector’s role in the epidemiology of the disease. This review also covers similar information relevant to the epidemiology of maize chlorotic dwarf, a disease which frequently is found juxtaposed to maize dwarf mosaic in the U.S.

Identity of Rhabdoviruses Infecting Maize Worldwide

The identities of the rhabdoviruses infecting maize worldwide (Autrey, Castillo, Greber, Kitajima and Costa, Jones, Lastra and Carballo, Lockhart and Elyamani, Sharma and Payak, Signoret) have not always been clearly resolved. Maize mosaic virus (MMV) is presumed to be the principal rhabdovirus of maize worldwide (Autrey, Castillo, Kitajima and Costa, Jones, Lastra and Carballo, Sharma and Payak). However, MMV is apparently limited in occurrence to tropical and subtropical regions where moisture is adequate for maize cultivation. Since MMV’s host range is limited to maize and only a few other plant species, survival of the virus between seasons is very restricted, mainly limited to where maize is planted continuously.

Another factor restricting MMV’s geographical distribution is its planthopper vector, Peregrinus maidis (Ashmead), which is also limited to the tropics and subtropics for year-round survival. Although P. maidis is migratory and is found in temperate climates during the growing season, it cannot survive freezing temperatures.

Occurrences of rhabdoviruses infecting maize other than in the tropics or subtropics (Gordon, unpublished; Signoret) suggest that viruses other than MMV are involved. Further, recently several rhabdoviruses found infecting maize in the subtropics have been shown to be distinct viruses unrelated to MMV (Greber, Lockhart and Elyamani). In some instances these distinct viruses have been extensively characterized and named as new viruses (Greber). In other cases character-
izations have been insufficient to warrant new names (Lockhart and Elyamani, Signoreti). In some cases, it is questionable whether rhabdoviruses of maize designated as MMV are correctly named (Gordon, unpublished).

Status of the Etiologies of Some Maize Virus Diseases

Several presumed virus-caused diseases have yet to have their etiologies demonstrated. Prominent among these are maize stripe (Gingery), maize mottle chlorotic stunt (Rossel and Thottappilly), and maize chlorotic dwarf (Gordon, Knoke, and Nault, unpublished). Resolution of the etiology of maize stripe seems close at hand and research on this subject has led to the description of a new group of plant viruses (Gingery). Likewise, the etiology of maize mottle chlorotic stunt seems virtually resolved (Rossel and Thottappilly). However, the etiology of maize chlorotic dwarf is unresolved and for some time has been an intractable problem (Gordon, Knoke, and Nault, unpublished), causing considerable consternation for maize breeders concerned with breeding resistance to the disease (Scott).

One reason for the consternation is that maize chlorotic dwarf virus (MCDV) infection is associated with two types of symptoms, a mild and a severe type. Corn breeders in breeding for resistance to the disease are faced with questions of whether both types are caused by MCDV and whether resistance should be bred to both types (Scott). Maize mottle chlorotic stunt shows a similar dual symptomology which clearly seems attributable to differences in genotype (Rossel and Thottappilly) and not in disease etiology as suggested for maize chlorotic dwarf (Scott). Resistance has been bred into maize to both the mild and severe symptoms of maize mottle chlorotic stunt (Rossel and Thottappilly).

Other less prominent diseases, presumably virus caused, have unresolved etiologies or virus identifications. These diseases are enumerated by several authors (Ammar, Autrey, Exconde, Johnston, Jones, Klinkong and Sutabutra, Lockhart and Elyamani, Louie et al., Sharma and Payak, Signoreti, von Wechmar). Study of the etiology of maize stripe involved identification of the morphology of maize stripe virus (MStpV), and these studies have provided conflicting evidence (Autrey, Gingery, Greber, Jones, Lasstra and Carballo). However, it now seems apparent that MStpV has a slender filamentous particle unique among viruses except for rice stripe virus (Gingery), rice grassy stunt virus (H. Hibino, personal communication), and rice hoja blanca virus (Morales and Niessen, 1983). A puzzling aspect of maize stripe is its dramatic increase in importance very recently in Venezuela, displacing maize mosaic as the most important maize virus disease (Lasstra and Carballo). In fact, maize mosaic has virtually disappeared from areas of Venezuela where only recently it was the most prevalent maize disease. This apparent displacement of maize mosaic by maize stripe is unexplained.

In an unusual turn of events regarding the etiology of a maize virus disease, maize wallaby ear disease, frequently believed to be caused by a virus and even "demonstrated" as such in the literature, has been shown to be incited by the feeding of Cicadulina leafhoppers (Greber). No virus has been implicated in the disease. Maize leaf gall in the Philippines, a disease which has gradually increased in importance over many decades, resembles maize wallaby ear disease (Exconde) and to a lesser degree maize rough dwarf. The agent of maize leaf gall is transmitted by a Deltocephaline leafhopper, a species of Cicadulina associated with maize wallaby ear disease, whereas maize rough dwarf virus (MRDV) is transmitted by Delphacid planthoppers.

In light of the findings for maize wallaby ear disease, it seems possible that maize leaf gall might be incited by leafhopper feeding, especially since a Cicadulina sp. is involved in this disease. Possibly the same or different pathogens have gone undetected in both leaf gall and wallaby ear disease. Diseases resembling maize wallaby ear also have been reported from Egypt (Ammar) and India (Sharma and Payak). For Egypt the occurrence of maize rough dwarf seems more likely than wallaby ear disease, since MRDV occurs in other Mediterranean countries (Conti) and MRDV vectors occur in Egypt (Ammar). However, the Cicadulina sp. which induces wallaby ear disease also occurs in Egypt (Ammar), keeping open the possibility that maize wallaby ear is present.

New Maize Virus Diseases

Several authors have reported the occurrence of unidentified virus or viruslike diseases which may be manifestations of diseases known elsewhere or of diseases not previously described. One virus very recently described is maize subtle mosaic virus (MSMV) (Louie et al.). The virus is transmitted mechanically and through the soil and has a flexuous rod particle of indeterminant length. Serologically the virus reacts with MDMV-A antisera in some assays but not others (Louie et al.), leaving the question of its relationship to MDMV unresolved. For the moment, MSMV appears to differ enough from all known strains of MDMV and other maize viruses to be considered a distinct virus. So far it has been found only in the U.S.

Another new maize virus, as yet unnamed, occurs in Thailand (Klinkong and Sutabutra). It has an isometric particle (27 nm in diam), is mechanically transmitted, and causes mosaic and severe stunting. A unique feature of this virus among maize viruses is that it reacts strongly with antisera to rose mosaic and prune necrotic ringspot viruses. It also reacts serologically, but less strongly, with MCMV antisera, and not at all with antisera to brome mosaic virus (BMV) and several other Graminiae-infesting viruses with isometric particles. What may be a similar virus has been reported from South Africa (von Wechmar). This latter virus has an isometric particle, is seed-transmitted, and also does not react with BMV antisera.

Several authors in studies of infected maize from Africa have reported isometric, viruslike particles (40-45 nm in diam) from infected tissue (Ammar, Autrey, Jones, Rossel and Thottappilly). One such study in-
volved maize mottle chlorotic stunt virus (MMCSV) which is transmitted by Cicadulina triangula Storey. While the mottle phase of the disease, as seen in African adapted maize, is not a new disease, the severe phase (chlorotic stunt), seen in exotic maize genotypes, is new. This disease may also occur in Zimbabwe (Johnston) and other East African countries (Rossel, personal communication) and in Nigeria (Rossel and Thottappilly).

Other reports of 40-45 nm diam isometric particles relate to MSV, and/or maize line virus (MLV) as originally described by Kulkarni (Ammar, Autrey, Jones) or a recently named disease, maize chlorotic stripe (Autrey). However, more recent tests show the former to be a manifestation of maize stripe (Autrey, Gingly, and Jones, personal communication). Since MSV has been shown to be associated with a filamentous rather than an isometric particle (Gingly) and MLV with MMV, a rhabdovirus (Autrey), these isometric particles may be of a new virus distinct from others characterized from Africa. This virus is possibly transmitted by P. maidis or a related Delphacid species (Ammar, Autrey). However, at this time the identity of this (or these) isometric particle(s) is unknown and this uncertainty causes some confusion.

Virus Diseases of Recent Increased Importance

Since the 1976 International Maize Virus Disease Colloquium and Workshop, several maize virus and MLO diseases have expanded geographically and achieved greater importance. Mention has already been made of the dramatic increase in incidence of maize stripe with a corresponding decrease in maize mosaic in Venezuela (Lastra and Carballo).

Maize chlorotic mottle virus at the time of the 1976 Colloquium and Workshop was known only in Peru where it caused significant crop loss in maize (Castillo). Since then it has become a major maize virus in two U.S. states (Eberhart, Uyemoto) and has been reported from Argentina (Teyssandier et al.) and Mexico (Gordon, unpublished). A serologically related virus has been reported from Thailand (Klinkong and Sutabutra). In the U.S., it has been associated principally with strain B of MDMV in synergistic interactions causing the corn lethal necrosis disease (Uyemoto), a major concern to commercial maize breeders (Eberhart) and growers.

The recent demonstration of MRDV in Argentina (Teyssandier et al.) and the report of its occurrence in China (Conti; J. H. Tsai, personal communication) is evidence of its recent increased importance as a maize pathogen worldwide. In Argentina MRDV has become the most damaging maize virus (Teyssandier et al.) and in Italy it has recently become again an important pathogen due to changes in maize cultural practices (Conti).

Maize white line mosaic virus (MWLMV) is another virus which has increased in importance on maize since 1976. In 1976 it was known only from France where it was called "Nanisme et anneaux foliaires du Mais" (maize dwarf ringspot) (Signoret). Since then it has been detected in maize during one season (1978) in Italy (Conti) and in the U.S. every year since 1979 (Louie et al.). For the U.S., MWLMV has been reported from eight northeastern and north central states where in some it has notably decreased yields. MWLMV is the only non-mechanically transmitted maize virus which is soil-borne and for which no vector has been demonstrated (Louie et al.). The lack of a known vector and the inability mechanically to transmit MWLMV have prevented demonstration of Koch’s postulates for the virus (Louie et al.).

Barley yellow dwarf virus (BYDV) is another virus which has increased in prominence among maize-infecting viruses in recent years. It has been reported from maize in France (Signoret), Italy (Conti), Morocco (Lockhart and Elyamani), and the U.S. (Gordon, unpublished). In the epidemiology of BYDV, maize may serve principally as an alternate host between seasons during which susceptible grains [barley (Hordeum vulgare L.), oats (Avena sativa L.), and wheat (Triticum aestivum L.)] are cultivated, rather than as an economic host in which BYDV causes major crop loss.

The corn stunt spiroplasma (CSS) is yet another maize pathogen that has been found occurring in new areas since 1976, having been recently identified in the U.S. states of California and Florida (Davis, personal communication; Davis and Lee). Previously it had been identified only in Texas, Louisiana, and possibly Mississippi. Although there had been numerous references to corn stunt occurring in many southern U.S. states, most reports lacked proof that CSS was involved (Gordon and Nault, 1977). The original claim that the spiroplasma isolated from maize with a corn stuntlike disease in California was not CSS has apparently been successfully challenged (Davis and Lee). CSS appears to have been the most likely pathogen for disease occurrences in 1981 and 1982 (Davis and Lee). Thus, CSS still appears to be the only spiroplasma infecting maize in nature.

Although of minor importance, both BMV and barley stripe mosaic virus (BSMV) were reported to have been identified recently naturally infecting maize in South Africa (von Wechmar). Natural infection of maize by BSMV has not been reported previously, although maize is a well-known experimental host of the virus. Another noteworthy finding was that BMV was transmitted by aphids (von Wechmar); aphids were previously unknown as vectors of the virus.

Maize Streak Virus

The importance of maize streak, well known in sub-sahara Africa for many years, is attested by the number of participants presenting findings relating to the disease or to maize streak virus (MSV) (Ammar, Autrey, Johnston, Ndewga, Rossel and Thottappilly, von Wechmar, von Wechmar and Milne). Among the noteworthy reports was the description of a new method of MSV purification which yielded a four-fold increase in purified virions (von Wechmar and Milne). This improvement in yield may be important to researchers.
interested in MSV as a geminivirus and as a potential eucaryotic cloning vector. Noteworthy for epidemiological studies was the detection of MSV in single leafhopper vectors by the enzyme-linked immunosorbent assay (ELISA) (von Wechmar and Milne).

Vectors of Maize Viruses and MLO's

The number of vector species of the principal maize viruses and MLO's is relatively small and various authors in these Proceedings present findings for most of them. These vectors and the viruses or MLO's they transmit are: *P. maidis*, vector of MMV and MStpV (Nault); *Dalbulus* spp., vectors of maize rayado fino virus (MRVF), CSS, and maize bushy stunt mycoplasma (MBSM) (Gámez, Nault); *Cicadulina* spp., vectors of MSV, CSS (experimental), and MMCSV (Markham and Alivizatos, Rossel and Thottappilly); *Graminella nigrifrons* (Forbes), vector of MCDV (Knoke et al.); *Rhopalosiphum* spp., plus numerous other aphid species, vectors of MDMV (Knoke et al.); *Laodelphax striatellus* (Fallen), vector of MRDV (Conti); and *Diabrotica* spp., vectors of MCMV (Krysan and Branson, Uyemoto). Particularly noteworthy is the report of the experimental transmission of CSS by *Cicadulina mbila* (Naude), the African corn leafhopper, as well as two other leafhopper species also found on continents of the Eastern Hemisphere (Markham and Alivizatos) where CSS is not known to occur. Since CSS susceptible hosts occur in moist tropics and subtropics of the Eastern Hemisphere, these findings suggest that CSS could become disseminated in these areas if introduced.

Epidemiology of Maize Virus Diseases

The epidemiologies of MRFV (Gámez), MCMV (Uyemoto), and MDMV (Knoke et al., Madden et al.) represent three contrasting types differentiated primarily by means of virus survival between maize crops, vector life cycles, and virus-vector relationships. Among these, only that of MRFV appears sufficiently understood to account for known disease occurrences within fields and over broad geographical regions. However, a complete statistical analysis of these epidemiologies (e.g., as initiated by Madden et al.) needs to be done for each of them and for other major maize virus diseases in order for us to have a precise understanding of each epidemiology and to be able to predict the intensity of disease occurrence (e.g., as in Madden et al.). Earlier, the epidemiology of maize rough dwarf had been extensively studied (Conti) and this information has permitted insight into how changes in maize cultural practices in Italy have brought about a recent resurgence of the disease in the Piedmont region (Conti).

The close relatives of maize, the teosinte and gama-grasses (*Tripsacum* spp.), play a vital role in the epidemiology of several maize virus and MLO diseases in the tropics and subtropics of the Western Hemisphere (Doebly, Nault). Specifically, these relatives serve as hosts for these pathogens and/or their vectors.

Among the important alternate hosts of maize viruses, johnsongrass (*Sorghum halepense* (L.) Pers.), host of MDMV and MCDV (Knoke et al.), and itchgrass (*Rottboellia exaltata* L.), host of MMV and MStpV (Autrey, Nault), are particularly significant weed grass species. Recently, downy chess (*Bromus tectorum* L.) has been implicated as an overwintering host of the B strain of MDMV in the Great Plains of the U.S. (Uyemoto) and in northwestern China (Zhu et al.). These reports provide an answer to the long-standing question of how MDMV-B survives between seasons to become the source of the virus for primary infections of maize.

In the previous section on virus diseases of recent increased importance, mention was made of several pathogens (MCMV, MRDV, MWLMV, and CSS) which had recently expanded geographically beyond natural geographical and geophysical barriers. The means by which these pathogens achieved these expansions are unknown. However, potential means include transmission of virus through seed (Damsteegt) or by means of vectors carried beyond these barriers (Damsteegt, Nault). MDMV (Tosic), already widely distributed as previously noted in the section on its importance, and possibly MWLMV (Louie et al.) are seed transmitted. Most of the leafhopper and planthopper transmitted viruses and MLO's are persistent in their vectors and if the latter were transported by man, the pathogen could be introduced into new geographical regions.

Soil transmission as demonstrated for MCMV (Uyemoto), MWLMV (Louie et al.), and MSMV (Louie et al.) may provide another way to transverse the barriers (Damsteegt). Infected tissue of MCMV, an unusually stable virus which is transmissible by vector beetles from debris, could be a means of dissemination for this virus. Speculations (Nault) on the means of dissemination of MMV and MStpV between continents provide detailed accounts of how these pathogens might have become distributed worldwide in the past.

Control of Maize Virus Diseases

Recommendations on control of maize virus and MLO diseases have emphasized planting of resistant genotypes (All, Eberhart, Havener, Scott). The efficacy of other control strategies has been demonstrated for MDMV and MCDV (All) and for MCMV (Uyemoto). For the control of MDMV and MCDV, an integrated pest management concept has been employed (All). While control of maize dwarf mosaic and maize chlorotic dwarf have been achieved by an integrated program of six or seven measures, economic realities have limited control practices recommended to growers to use of resistant hybrids and early planting (All). For MCMV, crop rotation is the recommended practice (Uyemoto).

Maize improvement and Breeding for Resistance

Maize breeding for virus disease resistance has been done against a background of considerable effort to improve maize production through breeding programs involving many agronomic and insect-resistance factors (Duvick). These efforts have met with considerable demonstrable progress (Duvick). Breeding programs
for resistance to maize viruses and MLO's highlighted in these Proceedings have been for resistance to MDMV (Eberhart, Scott), MCDV (Eberhart, Findley, Scott), and MCMV (Eberhart) in the U.S.; for CSS in the developing countries of the Western Hemisphere through the CIMMYT program (Havener); and for MSV and MMCSV in the developing countries of Africa through CIMMYT and IITA programs (Havener, Rossel and Thottappilly). National programs in developing countries are also contributing to resistance breeding as described for the programs in Kenya on MSV resistance (Ndegwa).

Current breeding programs have utilized only a relatively small portion of the genetic variability which exists in maize (Goodman). This is true even of CIMMYT's program which has a relatively broad genetic base (Havener). Further, the genetic diversity in teosinte has been utilized very sparingly in maize genotype improvement (Doebley). Currently, contrary to this trend, MCDV resistance genes from Zea diploperennis (Eberhart et al.) are being incorporated into maize genotypes for improvement of resistance (Findley et al.). Further potential sources of MDMV resistance, so far not utilized worldwide, are the old Australian genotypes which are highly resistant or immune to isolates of MDMV strain A in that country (Greber).

Cooperative Work on Various Aspects of Maize Virus and MLO Diseases

Work on maize genotype improvement within the developing countries by CIMMYT (Havener) and IITA (Rossel and Thottappilly) and within the U.S. between government and company scientists (Eberhart, Scott) are prime examples of cooperative efforts which have been highly successful in providing resistant genotypes to maize growers. Another example of international cooperation to deal with maize virus diseases involves virus identifications by scientists in developing countries working cooperatively with scientists in the developing countries (Castillo, Jones). The work in Peru (Castillo) is particularly noteworthy in that through this cooperation the major virus and MLO pathogens of maize were identified in a relatively short time (less than 1 year), whereas such identifications generally take considerably more time (many years) when cooperation is not pursued.

Serological techniques have been particularly important in identifying these pathogens (Castillo, Jones). The presentation of a variety of serological methods useful for making these identifications (von Wechmar et al.) should serve workers lacking experience with such techniques in attempting to use them for pathogen identification. Antisera have been prepared to most of the maize viruses and to CSS, but supplies are restricted and allow for only limited testing (Gordon, unpublished).

International Working Group on Maize Virus Diseases

To further communication and cooperation among scientists working on maize virus diseases, an International Working Group on Maize Virus Diseases (IWGMVD) was established at the 1982 International Maize Virus Disease Colloquium and Workshop. Membership was extended to those active in maize virus disease research and scientists with international responsibilities for maize and its diseases.

Functions of the IWGMVD are: 1) to publish an annual newsletter; 2) to publish proceedings of international meetings of the group, such as the 1982 Colloquium and Workshop; 3) to foster cooperative projects, to provide assistance to scientists in developing projects, and to provide assistance to scientists in developing countries for dealing with maize virus diseases; 4) to assist in the publication of "A List of References: Maize Virus and Mycoplasma Diseases," published under the auspices of The Ohio State University (OSU), Ohio Agricultural Research and Development Center (OARDC); 5) to schedule and hold meetings of the group at regular intervals; and 6) to make available the education, training, and research opportunities workers need to realize research duties and interests and to gain professional development.

To provide leadership to this group, a six-member executive committee was elected by the participants at the Colloquium and Workshop. The members of the executive committee are: Drs. D. T. Gordon (USA), chairperson; R. Gámez (Costa Rica), vice chairperson; L. R. Nault (USA), secretary; and M. Conti (Italy), R. S. Greber (Australia), and H. W. Rossel (Nigeria-IITA), advisory committee. In addition, Dr. L. J. C. Autrey (Mauritius) was appointed editor of the group's newsletter.

At a meeting of the executive committee, four subject matter committees were formed and chairpersons were designated to deal with issues related to: 1) maize virus nomenclature, Dr. L. J. C. Autrey, chairperson; 2) maize virus detection, identification, and relationships, Dr. E. W. Kitajima (Brazil), chairperson; 3) maize virus vectors and epidemiology, Dr. L. R. Nault, chairperson; and 4) maize virus disease crop loss and resistance (chairperson to be appointed). Membership of these committees has been designated.
The Maize Virus Information Service

As mentioned in the preceding section, the OSU-OARDC Maize Virus Information Service (MAVIS) publishes annually a bibliography entitled "A List of References: Maize Virus and Mycoplasma Diseases." The publication contains a list of pertinent articles, a key word index, and an author list. The present policy on annual distribution of the publication is that it is free to any scientist, institution, or library requesting it. For assembling this list of references, R. M. Ritter, the editor of the publication, reviews 59 research and 5 abstracting journals for relevant articles. He prepares the key word list from original articles or, if not available, from abstracts of the articles. The quality of the publication and especially the key word list depends on the authors of relevant publications supplying copies to the editor. Authors are encouraged to send relevant articles which are not in the MAVIS collection.

ACKNOWLEDGMENTS

The success of the second International Maize Virus Disease Colloquium and Workshop was due to the efforts of many people and the financial support of several organizations and institutions. The editors wish to acknowledge those individuals and the organizations and institutions which contributed to this success.

First, we thank the participants for their splendid cooperation and their notable scholarly contributions as evidenced by their papers in these Proceedings. Second, we acknowledge The Ohio State University, Ohio Agricultural Research and Development Center, as the host institution and in particular Dr. Charles Johnston, the institution’s liaison person. We also acknowledge the Agricultural Research Service of the U.S. Department of Agriculture for the contributions of its scientists, Drs. W. R. Findley, R. E. Gingery, and R. Louie, in planning and implementing plans for the Colloquium and Workshop.

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LITERATURE CITED


The Role of USDA-Agricultural Research Service

K. F. Schertz

USDA-ARS, National Research Program Leader, Corn, Sorghum and Grain Millet.

ABSTRACT


The U.S. Department of Agriculture has been operating since 1862 to conduct agricultural research, through its Agricultural Research Service (ARS), on problems of national concern. Approximately 2700 ARS scientists are stationed at 145 locations. Of these, 30 scientists at 13 locations conduct research on maize (Zea mays) productivity and include researchers dealing with maize virus diseases. ARS scientists are working with university and experiment station scientists, such as those here at the Ohio Agricultural Research and Development Center, to study maize virus diseases and provide means of increasing maize yields by reducing disease loss.

U.S. Department of Agriculture-Agricultural Research Service (USDA-ARS) Administrator Kinney had intended to address this colloquium. He asked me to commend you for holding this colloquium and to assure you of the importance that he places on the research that you are conducting on virus diseases of maize (Zea mays L.).

Your colloquium is important! Your colloquium is important to you as scientists because it provides an opportunity to share results of your research and your plans for the future. From this exchange you will be able to sharpen your objectives for further research. Of particular significance, this colloquium provides an opportunity to develop cooperative studies.

Secondly, this colloquium and your research are important to world agriculture and to all of us as consumers. Maize is one of the most important crops in the world. It is the principal feed crop in many countries, a major food crop in some, and in many the main commodity of trade. In the USA it is the leading feed grain crop.

World-wide production in 1981 was 402 million metric tons. In the USA maize was harvested from 74.6 million acres [30.2 million hectares (ha)]. The USA record average yield of 110 bushels per acre (123 quintals per ha) resulted in a total production of 8.2 billion bushels (208 million metric tons).

Control of diseases was an important factor in the production obtained. It is imperative in a crop as important as maize that losses due to diseases, including viral, be kept to a minimum. Your research to that end is essential and is also vital to establishing principles and concepts regarding viruses and viral diseases.

Maize is the base on which many other enterprises are built. Livestock and poultry industries are dependent on feed grains, of which maize is the major grain. Grain processing is an important industry heavily involved with maize, as are marketing and transportation industries.

Thirdly, this colloquium is important to the organizations you represent. The information and stimulation you receive this week will be reflected in the vitality of your research and that of your organizations. I assure you that for these same reasons this conference is important to the USDA. It is important to USDA-ARS as a partner in agricultural research.

The USDA had its origin in 1862 at the urging of President Lincoln and in a law signed by him. The Department was organized for the express purpose of conducting agricultural research and has done so ever since. Although research has changed during the past 120 yr, the USDA, through its Agricultural Research Service (ARS), still has a responsibility for research on problems of national concern. Today more emphasis is placed on fundamental research, but always with the purpose of assuring an adequate supply of high quality food and fiber.

The ARS has responsibility for research within USDA, and the Cooperative State Research Service works with and provides funds to the State Agricultural Experiment Stations for research by state scientists. ARS uses a line-staff approach to the administration of research and is organized with four regional administrators with four or more area/center directors within each region. National research program leaders are part of Administrator Kinney's staff and work on program matters with line administrators at all levels and with scientists.

An important responsibility of ARS is to assure national needs and provide leadership in organizing research to address those needs. ARS is now involved in such a study and is developing a Strategic Plan of research. The major objectives of research identified in that plan are: a) soil and water conservation, b) plant productivity, c) animal productivity, d) commodity conversion and delivery, e) adequate human nutrition, and f) integration of systems.
The budget of ARS is approximately $425 million. ARS has scientists at 145 locations, with the number at any one location varying from 1 to about 250; the Beltsville Agricultural Research Center, Maryland, is the largest. ARS pathologists are at more than half of these locations.

Budgets and laboratories, however, do not conduct research; scientists conduct research. Of the 2700 scientists, 230 are conducting research in plant pathology. Of that number, 70 are identified directly with the basic plant pathology program with a budget of $9.5 million. Others are identified with other programs, one of which is the maize productivity program which is conducted at 13 locations, by 30 scientists, with a budget of $3 million. Included are studies of pathology, entomology, selection methodology, biochemistry, genetics, and recombinant DNA.

The USDA is a partner in research with the state agricultural experiment stations, private companies, and agencies in other countries. Cooperation with the states is through the Cooperative State Research Service, grant programs, and especially by scientists of the states and ARS working together, frequently at the same location.

Partnership with industry is important also. Many companies cooperate in research by sharing information, providing grants, and conducting related research. Information and improved germplasm are available to all companies, and training in special techniques is provided to their scientists. Another area of cooperation is in the identification of research needs; the recent corn priorities study is an example. A committee representing state agricultural experiment stations, private companies, the National Corn Growers Association, and the USDA just completed a study of research needs. This study, funded by the USDA, identified needs and proposed research to address them. Of particular interest to you should be the statement regarding research on maize diseases. It reads, "Disease resistance is the most widely used means of control for the major corn diseases. Objective: To investigate the role of pathogens which are or have potential of being major causes of disease losses in corn." Then a list of approaches was included. I urge you to read this report of the National Corn Research Priorities Study and to help me with your suggestions regarding its implementation.

Let us consider some examples of the virus research of scientists in ARS. The maize virus team of ARS—Ohio Agricultural Research and Development Center (OARDC) scientists exemplifies the worth of cooperative research. ARS scientists Findley, Gingery, Knoke, and Louie work with OARDC scientists Bradfute, Dollinger, Gordon, Madden, and Nault to address problems in maize virus disease research. Included are studies on the incidence and inheritance of resistance, the identification of viruses, the transfer of resistance to maize from other species, vectors, biochemistry, epidemiology, systems analysis, and many other important studies. We are proud of these scientists and their accomplishments and are glad to be partners in this research.

ARS conducts research on viral diseases at other locations, including Mississippi, Missouri, and Texas, where the scientists are making contributions to the better understanding of these diseases of maize.

Our scientists work on other diseases of maize as well. Included are studies of downy mildew in Texas; rust in Mississippi; leaf blight in Indiana and Texas; stalk rots in Iowa; and Aspergillus in Georgia, Mississippi, North Carolina, and Missouri.

I appreciate this opportunity to share my thoughts with you. Let me emphasize the main points.

- We recognize the importance of this colloquium and the research that you scientists are conducting.
- Maize is such an important crop that we must continue a strong research effort to prevent losses by pathogens, including viruses.
- ARS is committed as a partner in this and related research.
- Success in this research depends on you scientists, your knowledge, your skill, and your innovation. Our purpose is to help you achieve that success.
CIMMYT's Maize Improvement Research Program

Robert D. Havener

Director General, International Maize and Wheat Improvement Center (CIMMYT), El Batan, Mexico.

ABSTRACT


CIMMYT, the Spanish acronym for the International Maize and Wheat Improvement Center, is a nonprofit autonomous agricultural research institution dedicated to supporting and complementing the research and production efforts of developing countries on maize (Zea mays) and wheat (Triticum aestivum). CIMMYT strives to develop broadly adapted germplasm with superior yield performance and dependability of yield across various production environments. Maize currently ranks third in production among the major cereal crops worldwide. More than 70 countries, including 58 developing countries, grow over 100,000 ha of maize annually.

CIMMYT's principal maize improvement program emphasizes the development and improvement of broad-based gene pools and populations for development of superior varieties in both normal and quality protein materials. Open-pollinated varieties are generally the end product of CIMMYT's program. CIMMYT's gene pools are mass reservoirs of genes which have a broad genetic constitution. A modified half-sib method of selection is used by CIMMYT for the recombination and improvement of gene pools, whereas full-sib family selection is used for improvement of advanced populations. International testing plays a major role in the selection and refinement of improved materials. The development of these materials involves four stages beginning with the development and improvement of broad-based gene pools for different specified areas of the world. These pools are further improved and refined to produce upgraded populations. International testing of these populations leads to the development of a few highly improved experimental varieties from which superior experimental or elite varieties are selected. These varieties are for use by farmers and to date 566 have been identified by CIMMYT. Of these, 70 have been released by 22 national programs during 1977-81.

Specifically, CIMMYT has improved maize in grain efficiency (tropical maize), tolerance to moisture stress, and resistance to insects and diseases. For the latter, improvement in resistance is being achieved for downy mildew (Peronosclerospora spp.) in southeast Asia, corn stunt in Mexico and Central and South America, and maize streak virus in Africa. CIMMYT seeks to establish collaborative ties with scientists in various centers of research excellence to benefit its primary research objective of solving applied, production-oriented problems in the developing countries.

I am happy indeed for the opportunity to address this gathering of maize scientists from around the world and others concerned with maize improvement and production. I am sure that most of you have heard of CIMMYT; quite a few of you might know CIMMYT reasonably well. Some of you have visited our programs and others are active collaborators. Yet for the benefit of those who may not know CIMMYT well, I will first talk briefly about CIMMYT, and then about our maize research program, emphasizing our efforts to improve disease resistance.

THE CIMMYT MANDATE AND PROGRAM OF WORK

The International Maize and Wheat Improvement Center, known by its Spanish acronym, CIMMYT, is a nonprofit, autonomous agricultural research institution dedicated to supporting and complementing the research and production efforts of developing countries in two of the most important cereal crops: maize (Zea mays L.) and wheat (Triticum aestivum. L.) [also including barley (Hordeum vulgare L.) and triticale (x Triticosecale Wittmack)]. CIMMYT is part of a global network of 13 international agricultural research centers supported by the Consultative Group on International Agriculture Research (CGIAR). A group of donors (some 55 in number) mobilize financial support for these centers. These donors include individual governments, both from developed and developing countries, international agencies, and private foundations, with most support coming from public sector organizations.

CIMMYT’s mandate requires it to complement, support, and strengthen the research and production efforts of developing countries where maize and wheat are, or are likely to become, economically important crops. These efforts are directed toward assisting the developing countries to increase the quantity, dependability, and quality of food and feed. Our mandate is global and involves virtually every maize and wheat
producing country in the world. Currently there are 53 countries with more than 100,000 hectares (ha) planted to maize and 27 countries with more than 100,000 ha planted to wheat in the developing world. The total number of countries currently cooperating with CIMMYT in both maize and wheat research and production efforts is well over 100.

CIMMYT's primary research thrust is to develop new and superior germplasm of maize, wheat, barley, and triticale with broad adaptation and the potential to produce high and dependable yields over a wide range of production conditions. A number of program activities have been designed to support and strengthen the capacities of the national maize and wheat research and production programs in the developing world. These activities fall into the following categories: a) development and distribution of improved germplasm, b) staff training and development programs, c) procedures for crop improvement and production research, d) information services, and e) consultation assistance.

CIMMYT's human resource base consists of 68 senior international staff members and 14 post-doctorals and associate scientists. Forty-six staff and associated scientists are located at headquarters in Mexico. In addition to responsibilities assigned to them at base, they also work around the world with national maize and wheat program scientists. Twenty-two staff members are outposted in various national and regional programs throughout the developing world.

CIMMYT'S PHILOSOPHY IN GERMPLASM DEVELOPMENT AND DISTRIBUTION

Because of the nature of reproduction in maize and wheat, the specific crop improvement procedures followed by CIMMYT scientists are different from each other. However, both programs strive to develop broadly adapted germplasm with superior yield performance and dependability of yield across various production environments. This is achieved through a large-scale multilocational testing and selection network in which national collaborators play a vital and full partnership role. National programs use these materials as direct releases or in their breeding programs, where they focus on further selection for specific production requirements to develop varieties with the best adaptation to specific local conditions.

CIMMYT neither names nor releases varieties. This is the responsibility of cooperating national programs which are free to use the germplasm best suited to their program and release it with an appropriate name depending upon their variety release and registration rules and regulations. We only ask that they keep us informed of the parentage of such releases.

CIMMYT believes in the free and essentially unrestricted exchange of germplasm with any bonafide scientist or research organization. It does, however, follow certain guidelines and priorities since seed requests usually far exceed the available supply. The first priority is given to our collaborators in national research programs and universities in developing countries. The second priority goes to public institutions in developed countries and the third to private organizations.

Through the advent and development of international testing networks, CIMMYT has succeeded in bringing together the research work of thousands of scientists and hundreds of organizations worldwide. This has led to an acceleration of research efforts and has resulted in the release of hundreds of high-yielding maize and wheat varieties with broad adaptation and yield dependability in developing and developed countries.

IMPORTANT OF MAIZE IN THE DECADES AHEAD

I shall now turn to a discussion of CIMMYT's maize program, the subject crop of this symposium. Maize currently ranks third in production among the major cereal crops, closely following wheat and rice (Oryza sativa L.) in terms of global importance. In the period 1978-80, an average of 380 million tons of maize were produced annually on 120 million ha. This represented nearly a quarter of world cereal production. Further data on maize production and consumption are presented in World Maize Facts and Trends, 1981, CIMMYT, El Batan, Mexico.

Today more than 70 countries, including 53 developing countries, grow over 100,000 ha of maize annually. This makes maize the world's most widely distributed major crop. Botanically, the maize plant is unfit to survive in nature without man's assistance. Yet this species has capacity to adapt to the widest array of environments of any cereal grain. From its center of origin in the tropics of Mexico and Central America, it has now spread to almost all tropical areas of the world as an economic crop. It also has spread to temperate areas as far as 65th latitude and to tropical highlands over 3000 m in altitude. The story of maize improvement in the USA is a classical example of crop improvement and achievement through plant breeding. This success story has not been repeated in the tropics, but I see many reasons to believe that maize production in the tropics is on the road toward higher levels of productivity.

I agree with Nobel Laureate wheat breeder Dr. Norman E. Borlaug when he says that, in the decades ahead, maize is destined to become the most important cereal crop and this crop will be the salvation of increasing millions of people by the year 2000. World maize utilization trends also underscore its increasing importance. While per capita human consumption of maize has been roughly constant in developing countries in recent decades, high rates of population growth in several countries where maize is a staple food have necessitated increased maize production or imports, or both. With gains in per capita incomes, direct human consumption can be expected to decline in some countries. However, the demand for maize as a feed grain will continue to expand rapidly as increasing numbers of consumers with higher incomes seek more livestock products. Per capita grain use for livestock production in developing countries is still at low levels, although it
is expanding rapidly. Most of the expansion to date is occurring in middle-income countries where meat consumption is on the rise.

CIMMYT'S MAIZE IMPROVEMENT PROGRAM—FROM GERMPLASM BANK TO THE FARMER

CIMMYT's maize research and production program is primarily oriented towards the needs and problems of developing country producers. This program is designed to: a) provide an overall strategy that can effectively serve the world's different maize-growing areas which have varying levels of capability; b) serve as a mechanism for continuous development and improvement of maize germplasm to meet current and future needs; c) provide a smooth and efficient delivery system to, from, and between national programs; and d) meet the needs for exploratory and innovative maize research.

The primary program emphasis is on the development and improvement of broad-based gene pools and populations leading to the development of superior varieties in both normal and quality protein materials. It is a multistage process with a continuous and systematic flow of germplasm from CIMMYT's germplasm assembly line to the farmer's field, with national programs participating as full cooperators. Further details are presented by Paliwal and Sprague (1982).

CIMMYT believes that the development and improvement of broad-based gene pools should be the backbone of every aggressive maize breeding program. This approach is the best insurance against genetic vulnerability due to a narrow gene base, as it continuously provides new sources of superior germplasm for current and future breeding efforts.

Keeping in view the seed production and distribution circumstances of many national programs in developing countries, we emphasize open-pollinated varieties as the end product of our efforts. Nevertheless, where the requisite infrastructure has been, or can be, developed to sustain such a program, CIMMYT assists the national programs in hybrid development.

There are four main stages in our system: a) development and improvement of broad-based gene pools for different specified areas of the world; b) continuous improvement and refinement of populations with upgraded materials from the corresponding pool; c) international testing for selection of superior and broadly adapted families for continued improvement of maize populations as well as selection of most superior families for development of a few experimental varieties; and d) selection of superior experimental varieties and their use by the farmers.

International testing plays a major role in the selection and refinement of improved materials. The testing begins as soon as the populations are considered to be sufficiently advanced to be useful to the national programs whose scientists may use these materials as they see fit. In some cases, promising experimental varieties can be put to commercial production immediately. National programs also may further select and refine the materials to suit their conditions or incorporate the materials at various stages in their breeding programs. Some national programs may utilize the materials in a hybrid program. Although the emphasis is on intrapopulation improvement with open-pollinated varieties as the end product, information is collected and made available to national programs on the heterotic response among various populations for interpopulation improvement and for the development of hybrids.

DEVELOPMENT OF BROAD-BASED GENE POOLS AS GERMPLASM RESOURCES

Most maize breeding programs around the world are working with rather narrow genetic bases, despite the fact that they may be handling a large number of materials. An important activity of CIMMYT's maize program is to develop broad-based gene pools as functional germplasm resources. Genetic diversity and variability are basic requirements for any successful population improvement program.

CIMMYT's gene pools are mass reservoirs of genes which have a broad genetic constitution. They are formed by the genetic mixing of several diverse varieties, varietal crosses, and hybrids with similar climatic adaptation, maturity, grain color, and texture. These gene pools are designed to meet the climatic requirements of tropical highlands, tropical lowlands, and subtropical zones. These pools are further classified on the basis of maturity (early, intermediate, and late), grain color (white and yellow), and grain texture (flint, dent, and flouncy). Of the 29 gene pools currently handled by CIMMYT, 9 are meant for tropical highland, 12 for tropical lowland, and 8 for subtropical zones.

The maize germplasm bank at CIMMYT has more than 13,000 accessions from 46 countries. It is a service unit for resident as well as collaborating national scientists. We maintain and catalog the germplasm collections and renew stocks as needed. Periodically, about 300 collections are evaluated systematically at two or more locations with appropriate pools as checks. The best-performing materials are grouped according to their adaptation, maturity, grain color, and texture. In the following season, the selected bank collections are incorporated into the appropriate pools as female rows only. Observations can be made for the combining ability of the pools x collections and, if needed, the can be carried to the F2. Based on the performance of topcrosses, the families from these crosses are later merged with the main body of the pool.

Improvement of gene pools. A modified half-sib method of selection is used for the recombination and improvement of gene pools. The size of a pool is maintained at approximately 500 families. This number is manageable and maintains a high level of genetic variability. The 500 families making up a gene pool are planted in a ratio of two female: one male row. The pollinator is a balanced seed mixture of superior families. For traits expressed before flowering, selection pressure is also exercised by detasseling undesirable plants in the pollinator rows.
Each pool is grown at more than one site. Superior families are identified at each location by a team of scientists from various disciplines. Yield potential, height, maturity, lodging, insect and disease response, and uniformity are taken into account at appropriate stages of plant development. Depending on their intended use in various locations, the pools are subjected to different insect and disease pressures. These include sugarcane borer (Diatraea saccharalis (Fabricius)), southwestern corn borer (D. grandiosella Dyar), fall armyworm (Spodoptera frugiperda (J. E. Smith)), corn earworm (Heliothis zea (Boddie)), ear rots (Fusarium roseum Link, F. moniliforme Sheld.); and stalk rots (F. moniliforme).

In addition to serving as pollinator for the female rows, we have used the male rows of each pool for improving resistance to both ear and stalk rots. This is done by selecting 500 agronomically desirable plants around flowering time. The selected plants are artificially inoculated with stalk and ear rots. In ear rots, the spore concentration is five times greater than the one used in the normal inoculation. The ears saved at harvest are included in the same pool to further upgrade the level of ear and stalk rot resistance.

Mild selection is applied within each pool to minimize the depletion of attributes or genes necessary for further advancement at a later stage. Lower selection intensity also provides better chances and opportunities for recombination among linked genes. With higher selection intensity, too many materials with useful genes would probably be discarded. Among-family selection pressure is about 50-60% and selection pressure within families is about 6-18%.

At harvest, the selected ears are classified as male (pollen source for the following cycle) and female ears. Approximately 70% of the ears are selected for use as male pollinators. In the following cycle, all selected ears (both male and female) enter as separate individual female families in the half-sib crossing block, with the male rows planted as a balanced male composite made up only of selected male ears.

Development of new sources of genetic variability—new temperate gene pools. Most of the breeding programs in Europe and the USA, where the highest maize yields are obtained, are handling materials with a relatively narrow genetic base. Some maize researchers in these countries have become concerned with the dangers of genetic erosion. To help alleviate this problem, CIMMYT has developed four new gene pools for the temperate areas. These have wide genetic diversity which will facilitate the introduction of exotic tropical germplasm in temperate-based materials. This, in turn, will serve to transfer superior characters from the temperate germplasm into the tropical materials.

The first of these pools was initiated in 1976 as a joint effort between the University of Hohenheim, Federal Republic of Germany, and CIMMYT. The CIMMYT-German gene pool consists mainly of a mixture of lowland and highland tropical maize with very little temperate material. The second gene pool is based on materials from the U.S. Corn Belt and the third pool is based on U.S. Corn Belt plus tropical lowland and highland materials. The fourth pool primarily includes maize materials from Europe. After thorough recombination, these pools are being subjected to multilocal testing, evaluation, and selection, followed by recombination of selected families at CIMMYT.

Several institutions in the USA and Europe are cooperating in this venture and pools have reached a stage where they are now being explored for possible use by various programs. CIMMYT has also started a program for incorporation of the temperate germplasm into the floury materials of the Andean region, which traditionally have a very narrow genetic base.

Improvement of maize populations. Refined and improved gene pools, as well as other improved materials which reach a stage of refinement where most national programs can benefit, are further improved as advanced populations. These populations undergo a higher selection intensity as well as international testing in cooperation with national programs. CIMMYT is currently handling 27 such maize populations—24 normal maize and 3 quality protein maize populations carrying the opaque-2 gene.

The full-sib family selection scheme is used for improvement of these populations. In each population, 250 reciprocal full-sibs are developed and evaluated in international progeny test trials (IPTT's), both in the northern and southern hemispheres. Approximately 80-100 of the best full-sib families are selected on the basis of across-location data for the next improvement cycle. Since the retrieval of data takes about 1 yr, one cycle of selection is completed every 2 yr. The intervening period between the two cycles is utilized to improve the population for the most deficient traits.

The steps involved in this population improvement program are shown in Fig. 1. A selection intensity of 30-35% is used for each population. As the program has evolved, the germplasm in these populations has improved through selection, by partial replacement through incorporation of outstanding half-sib families, and/or by complete substitution of the population. New populations are created by using the best 200-300 half-sib families from the gene pools. The incorporation process normally includes: a) development of full-sibs, b) evaluation in IPTT's of the families tentatively designated for the population, and c) selection of superior families for final incorporation into the respective population(s).

International testing system. CIMMYT's international maize testing program is a cooperative effort between CIMMYT's maize scientists and national program staff. It consists of three levels of testing: a) IPTT's, b) Experimental Variety Trials (EVT's), c) Elite Variety Trials (ELVT's).

The focal point of this international effort is the IPTT's, composed of 250 full-sib families from an advanced population and six check varieties (Fig. 2). The material is arranged in a 16 x 16 simple lattice that is grown by cooperating national programs and appropriate yield trial data are recorded. In most cases, the IPTT is jointly reviewed by CIMMYT and national...
STEP 1   SEASON A
GERMLASM IMPROVEMENT

PROGENY REGENERATION NURSERY
Plant ± 300 half sibs to generate 250 F.S. families for IPTT

PROGENY TRIALS (250 F.S. + 6 NATIONAL CHECKS)

Loc. 1  Loc. 2  Loc. 3  Loc. 4  Loc. 5  Loc. 6

WITHIN-FAMILY IMPROVEMENT
1. Make selfs or within-family sibs in 100 across-site selected F.S. families
2. Save 3 sibs or selfs from each selected family

FAMILY IMPROVEMENT AND RECOMBINATION
1. Plant 240-300 sub-families from ± 80-100 selected F.S. families
2. Select one best sub-family from each selected F.S. family
3. Select best plants from each selected sub-family and bulk pollinate
4. Save ± 300 H.S. ears

PROGENY REGENERATION NURSERY
Plant ± 300 H.S. to generate 250 F.S. families for second cycle of improvement

Fig. 1. Population improvement breeding sequence scheme. Season A refers to CIMMYT’s November-April crop breeding cycle; Season B to its May-October crop breeding cycle; F.S. families to full-sib families; and IPTT to international progeny test trials.

PROGENY REGENERATION
Produce 250 full sibs in each population

PROGENY TRIALS (250 F.S. + 6 NATIONAL CHECKS)
Evaluate in 6 different locations

WITHIN-FAMILY IMPROVEMENT

EXPERIMENTAL VARIETY FORMATION
Recombine 10 superior families from each location and 10 superior across locations to develop site specific and across site experimental varieties.

ADVANCING F1 GENERATION TO F2
Bulk pollinate to advance F1-F2

PROGENY REGENERATION

EXPERIMENTAL VARIETY TRIALS
Evaluate at 30-50 locations

FAMILY IMPROVEMENT AND RECOMBINATION

ELITE EXPERIMENTAL VARIETY TRIALS
Evaluate at 60-80 locations

PROGENY TRIALS

Fig. 2. Steps in population improvement and experimental variety development and evaluation. F.S. refers to full-sibs.
program staff at least once during the growing season. This visual evaluation may reveal that certain progenies have demonstrated superior performance in both replicates. Such observations can be noted and, if subsequent data confirm that the progenies are superior, the national cooperator can request CIMMYT to recombine genetically the selected progenies to produce an experimental variety. In addition, the superior 10 families from each IPTT testing site and 10 best across-site families are also identified from the IPTT data and used to develop experimental varieties.

Development of experimental varieties. A selection intensity of 4% is used in the selection of families for the development of experimental varieties. Experimental varieties are developed both on the basis of site-specific and across-site progeny test data. Since the best fraction of each population is used to form the experimental variety, it is expected that the experimental varieties will show considerably higher performance as compared to the population mean. In addition to those characters such as yield and grain type that establish a variety, uniformity for maturity and plant and ear height are important considerations in the selection of the 10 best families so that the resultant variety is uniform in appearance.

Each experimental variety then goes into a second-order seed increase to build up a sufficient quantity of seed for the experimental variety trials. These experimental varieties then enter the next stage of the international testing system and are dispatched to cooperators who request them. After data from the EVT's have been returned to CIMMYT for analysis, the superior performing experimental varieties (EV's) are selected. These EV's are used to prepare the ELVT which is again distributed to cooperators upon request. An ELVT is run in the same manner as the EVT. The ELVT represents the last stage of CIMMYT's international variety testing. It is now up to national cooperators to either utilize the variety for production purposes or breeding stock for national improvement programs.

USE OF CIMMYT GERMPLASM

The current scheme of population improvement through international testing leading to the development of open-pollinated varieties was started at CIMMYT in 1974. By 1981, a total of 566 experimental varieties was developed. Out of these, 156 were identified as elite varieties. We have received reports that 70% have been released by 22 national programs during the period 1977-81. Approximately 25% of these releases were in the form of hybrids. The germplasm developed through CIMMYT's international cooperative efforts is now being used by almost every important maize-growing country in the world.

IMPROVING MAIZE PERFORMANCE

Improving grain efficiency of tropical maize. The tropical maize plant traditionally has a low yield potential, in large part because of a low grain-to-stover ratio. CIMMYT has attempted to increase the grain efficiency of tropical maize by using various techniques. One of these was a long-term recurrent selection program for the reduction of plant height in a widely adapted tropical maize population, Tuxpeño 1. Major emphasis was on plant height, and only routine selection was made for other characteristics normal to a plant improvement program. There was a large and nearly linear reduction in plant height (resulting from reduction in the total number of nodes as well as the mean internode length below the ear) and a linear increase in grain yield at an approximate rate of 2.9% per cycle (when different cycles were grown at their optimum density). The harvest index also increased linearly. Seventeen cycles of selection resulted in a reduction in plant height of 117 cm, an increase in yield of 2.18 t/ha at optimum plant density, an 8-day reduction in days to 50% silking, and an increase in harvest index from 0.30 to 0.47. This study not only demonstrates that it is possible to reduce plant height of tropical maize (which reduces lodging and makes it more responsive to better management) without using major dwarfing genes, but that yield efficiency also can be improved through modification of the harvest index.

Improving yield stability. The materials selected through international testing are high yielding with good agronomic characters, lower in height, earlier in maturity, and also have good tolerance to stress situations such as moisture stress. This is illustrated by the comparison under non-stress and stress water regimes of the original and latest cycle of population 21, Tuxpeño 1, which is the basis of the international testing program (Table 1).

Results of Table 1 show that faster progress for drought selection can be made using a multiple selection index and that selection under non-limiting moisture conditions does not adversely affect the performance of the material under stress conditions. They also confirm that multilocation testing and the selection of families on the basis of across-site performance can enhance performance under moisture stress without detriment to their performance under more favorable or non-stress situations.

Insect resistance. Insect damage is another important yield-limiting factor in most developing countries. Effective and judicious use of insecticides appears to be an easy solution to the problem, but our experience in the tropics has shown that such is not the case. Breeding for resistance and/or tolerance to insects has been a continuing concern of CIMMYT's maize breeders. In 1974, CIMMYT set up an insect-rearing laboratory to produce enough insect larvae to infest artificially and screen our maize pools and populations for reaction to prevalent and important insects. This laboratory produces millions of larvae of fall armyworm, corn earworm, sugarcane borer, and southwestern corn borer. Techniques have been perfected for the application of these larvae to thousands of maize progenies through a small dispenser called a "Bazooka" with which one can apply a specified number of larvae per plant with a variation of ± 10-15%. This simple technique now has been adopted worldwide by maize scientists engaged in the breeding for resistance to insects.
TABLE I. Effect of selection for various characters in Tuxpeño on grain yield under irrigation and stress conditions (Tehuitzapan, 1981).

<table>
<thead>
<tr>
<th>Character and selection</th>
<th>Cycle and selection</th>
<th>Grain yield (kg/ha)</th>
<th>Yield gains per cycle (percent of original)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Irrigation</td>
<td>Stress</td>
</tr>
<tr>
<td>Drought resistance</td>
<td>0</td>
<td>5859</td>
<td>1224</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>6179</td>
<td>1572</td>
</tr>
<tr>
<td>EV*</td>
<td></td>
<td>6331</td>
<td>1647*</td>
</tr>
<tr>
<td>International progeny test (IPTT 21) (Ca)</td>
<td>0</td>
<td>5608</td>
<td>1213</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>6458</td>
<td>1315</td>
</tr>
<tr>
<td>LSD P.05</td>
<td></td>
<td>899</td>
<td>433</td>
</tr>
<tr>
<td>CV%</td>
<td></td>
<td>11.7</td>
<td>23.8</td>
</tr>
</tbody>
</table>

a EV denotes experimental variety tested at 4% selection pressure.

An example of international cooperation in the development of disease resistance is provided by CIMMYT’s collaborative research for downy mildew (Peronosclerospora spp.), corn stunt, and maize streak virus (MSV) diseases which cannot be effectively handled in Mexico. In 1974, a collaborative research project for development of resistance to each of these diseases was initiated with national programs for screening germplasm in endemic areas. A shuttle breeding approach was followed. The collaborating countries were Thailand and the Philippines for downy mildew, El Salvador and Nicaragua for corn stunt, and Tanzania and Zaire for MSV. Alternate cycles of selection were carried out in “hot spot” areas of the collaborating countries to select agronomically desirable and disease-resistant plants. The resistant selections were then recombined and further selected for desirable agronomic characters the following season in Mexico.

By 1980, four cycles of selection had been completed in each population and it was becoming apparent that an adequate level of field resistance had been attained for corn stunt and downy mildew diseases (Table 2). A further improvement program through international testing for yield and adaptability was introduced at this point. Full-sibs were developed from downy mildew and corn stunt resistant populations and the progeny

TABLE 2. Progress in improving resistance to downy mildew and corn stunt diseases in collaborative research.

<table>
<thead>
<tr>
<th>Population tested to disease</th>
<th>Cycle of selection</th>
<th>Disease (%)</th>
<th>Yield (kg/ha)</th>
<th>Plant ht (cm)</th>
<th>Days to 50% silk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Downy mildew: (Thailand)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Tropical Intermediate White Flint</td>
<td>C_e</td>
<td>41.4</td>
<td>3836</td>
<td>185</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>C_t</td>
<td>0</td>
<td>4404</td>
<td>160</td>
<td>66</td>
</tr>
<tr>
<td>2. Tropical Yellow Flint-Dent</td>
<td>C_e</td>
<td>67.4</td>
<td>2684</td>
<td>199</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>C_t</td>
<td>2.6</td>
<td>4490</td>
<td>183</td>
<td>66</td>
</tr>
<tr>
<td>Stunt disease: (El Salvador)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Tropical Intermediate White Flint</td>
<td>C_e</td>
<td>30.6</td>
<td>3337</td>
<td>206</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>C_t</td>
<td>21.8</td>
<td>4120</td>
<td>200</td>
<td>49</td>
</tr>
</tbody>
</table>
trials were sent for evaluation to countries where these diseases had a good chance of expression under natural conditions.

Using the information from the trials, 12 stunt-resistant experimental varieties were assembled in 1980 into a variety trial and sent to 18 locations in Central America and Mexico. At most locations, the stunt-resistant varieties performed better than the local checks included in the trial. Performance of some of these varieties is shown in Table 3. Similar trials with downy mildew-resistant varieties were planted in 1982 and data for these trials will be available in 1983.

**Work on maize streak resistance in La Posta (population 43) and selected experimental varieties.** The advanced population La Posta (population 43), which has given rise to very good varieties for several countries of Africa, is being handled by our West Africa regional program in cooperation with the International Institute of Tropical Agriculture (IITA) to improve its resistance to MSV. The population will continue to undergo recurrent selection for yield and other agronomic characters through international progeny testing with emphasis on streak resistance through within-family improvement.

There are several reasons for transferring the work on streak resistance to the West Africa regional program based at IITA. Our earlier work had shown that the natural incidence of MSV was not reliable enough to make good selections. IITA has developed reliable techniques for rearing African maize leafhoppers (*Cicadulina* spp.), vectors of MSV. Using their techniques, it is possible to infect large numbers of families in tests for MSV tolerance. With suitable insect-rearing facilities available for the vector, several West African areas provide good testing sites for MSV as well as to improve agronomic characters, so that improvement of both can be done simultaneously.

In 1980, 250 full-sib families from La Posta were infested in the screenhouse and evaluated for MSV resistance. These plants were selfed and their progenies were used in the next cycle of recombination of selected families. This recurrent selection scheme for MSV resistance will continue to capitalize on the polygenic resistance present in the materials for several cycles.

In addition to these efforts within La Posta, selected full-sib families of this population were crossed with a streak resistance source, TZSR-W-1, a full-season white population developed at IITA with good levels of resistance. These crosses were advanced to F2 under MSV pressure in 1981 and the F2 generation was planted and screened for resistance or tolerance to the virus. The first backcross to the population La Posta was made by bulking pollen of selected plants and pollinating segregants highly tolerant to MSV in the (La Posta x TZSR-W-1)-F2.

Experimental varieties from nine different advanced populations, which have shown good performance in Africa and have a high level of resistance to most tropical diseases except MSV, are undergoing conversion to streak resistance through backcrossing. The initial crosses with other streak resistant donors were made in 1980.

The best performing experimental variety from the latest cycle of each of these populations is used as "recurrent" parent. In this manner, the program takes advantage of the continuous progress being made in the recurrent selection of each of the parental populations. After each backcross, the BCn-F1 generation is advanced to BCn-F2 under artificial screening for streak resistance. Resistant segregants are used as female parents for the next backcross generation. Selection for desirable plant and grain type and maturity group of the recurrent parents is practiced in each generation (Fig. 3).

This cooperative program for streak resistance is making good progress, although it is still too early to measure the gains made in resistance. It is, however, clear that the technique of artificial infestation is reliable, that good selection pressure can be exerted, and that the differences among families and plants within families for tolerance/resistance to MSV are quite marked. We are hopeful that within the next few years we shall be able to provide the African farmers with high-yielding, stunt-resistant germplasm with various grain types and maturities.

### Table 3. Performance of some corn stunt-resistant varieties against best local check in 10 locations.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Country</th>
<th>Best stunt-resistant (kg/ha)</th>
<th>Best check (kg/ha)</th>
<th>Percent best check</th>
</tr>
</thead>
<tbody>
<tr>
<td>Santa Rosa 8073</td>
<td>Mexico</td>
<td>4230</td>
<td>3525</td>
<td>120</td>
</tr>
<tr>
<td>Porrillo 8073</td>
<td>Mexico</td>
<td>3517</td>
<td>2420</td>
<td>145</td>
</tr>
<tr>
<td>Santa Rosa 8073</td>
<td>Panama</td>
<td>4556</td>
<td>3973</td>
<td>114</td>
</tr>
<tr>
<td>Santa Rosa 8073</td>
<td>Panama</td>
<td>4193</td>
<td>3138</td>
<td>133</td>
</tr>
<tr>
<td>Cuyuta 8073</td>
<td>Panama</td>
<td>4860</td>
<td>3773</td>
<td>128</td>
</tr>
<tr>
<td>Santa Rosa 8073</td>
<td>Nicaragua</td>
<td>4933</td>
<td>3316</td>
<td>148</td>
</tr>
<tr>
<td>Titalizapan 8073</td>
<td>Nicaragua</td>
<td>5775</td>
<td>4188</td>
<td>137</td>
</tr>
<tr>
<td>Santa Rosa 8073</td>
<td>Nicaragua</td>
<td>4093</td>
<td>3243</td>
<td>126</td>
</tr>
<tr>
<td>Santa Rosa 8073</td>
<td>Guatemala</td>
<td>5787</td>
<td>4606</td>
<td>125</td>
</tr>
<tr>
<td>Santa Rosa 8076</td>
<td>Guatemala</td>
<td>2808</td>
<td>1599</td>
<td>175</td>
</tr>
</tbody>
</table>
NEED FOR COLLABORATIVE RESEARCH

CIMMYT works in a global setting interacting with hundreds of institutions and thousands of scientists. CIMMYT's improvement programs have direct linkages with national program scientists throughout the developing world and much of the industrialized world. We also have a relationship within the CGIAR community of donors and with other international agricultural research centers. There is still a need for more research linkages and I would like to take a few minutes to talk about our interest in establishing more ties with various centers of excellence. These potentialities could be of mutual benefit to CIMMYT and to the cooperating institutions.

CIMMYT will continue to direct its primary research efforts towards the solution of applied, production-oriented problems of widespread applicability for strengthening national research and production programs in developing countries. Our applied research effort, however, will sometimes be limited by more complex problems that require the application of specialized research techniques, equipment, or knowledge for their solution. CIMMYT believes that this type of research is best undertaken in collaboration with scientists in centers of research excellence found in the universities and public and private research laboratories in both the developed and developing countries of the world.

CIMMYT has a range of collaborative research activities which now complement our other core research programs in a number of ways. First, such projects provide a mechanism for linking the more basic types of research under way at CIMMYT with similar work being conducted at other institutions, for example in wide-cross research. Second, the development of working links with institutions conducting basic research provides CIMMYT with an effective mechanism for monitoring the developments in areas related to our own research and helps to increase our awareness of important new scientific areas; for example, genetic engineering at the molecular biology level.

In the future, CIMMYT intends to probe more aggressively the rapidly changing scientific-biological frontiers related to our crops. This will be done largely through collaborative, mission-oriented basic research projects. We also will continue to explore new research fields by attempting to attract staff in a variety of disciplines from other more basic research institutions. Such collaboration, however, must strengthen rather than dilute CIMMYT's applied, mission-oriented research programs. Indeed, we must always keep sharply in focus that our work, to warrant the investment, must result in increased yields in this generation as well as in the decades to come.

LITERATURE CITED

A.I.D. Agricultural Research Programs

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ABSTRACT


Projections indicate populations will double in Africa and Latin America between 1975 and 2000. In Asia and Oceania, the most populous region of the world, a 60% increase is anticipated during this period. Most countries in these regions will have to improve greatly their agricultural production capabilities to meet only minimal nutritional needs of their people by the year 2000. Consequently, the U.S. Agency for International Development (A.I.D.) and other bilateral and international assistance organizations have placed major emphasis on improving food production capabilities of developing countries through agricultural research and development activities. Improving crop production through control of plant pests is a significant part of this research effort.

A.I.D.’s Bureau for Science and Technology works closely with the regional bureaus in Washington, D.C., which in turn work with A.I.D. missions worldwide to develop agricultural research projects relevant to needs of host countries. Through this working partnership, agricultural research projects are implemented that will have a long range impact on increasing food production capabilities of the developing world.

WORLD POPULATION GROWTH AND FOOD PRODUCTION

Before I discuss the agricultural research programs of the United States Agency for International Development (A.I.D.), I would like to briefly talk about population growth projections that point out the need to increase substantially world agricultural productivity in the next decades. In this regard, our capacity to reduce crop losses caused by plant pests and pathogens such as maize viruses will be an important factor in stabilizing and ultimately in increasing food availability worldwide.

A report commissioned during the administration of U.S. President Jimmy Carter, entitled “Global 2000”, gives us some insight into these population trends and corresponding agricultural production needs in the year 2000 as compared to 1975 (Table 1). These data are projections and thus are not precise. However, they are considered to be well-informed estimates of general population trends that will be realized by the year 2000.

The developing countries in Africa, Asia, and Latin America will have far greater demands placed on their agricultural production capabilities because of rapidly expanding populations than in the developed countries of North America and Western Europe, as well as Australia, Japan, and New Zealand where the population growth is not as rapid. Not only will many countries of Africa, particularly sub-Saharan Africa, Asia, and Latin America, need to increase dramatically their food production capabilities, but they will need to produce this food on very little more land. Land under cultivation is projected to increase only 4% by 2000, primarily because most good agricultural land is

<table>
<thead>
<tr>
<th>Area</th>
<th>Population 1975</th>
<th>Population 2000</th>
<th>Increase by 2000 (%)</th>
<th>Average annual increase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>World</td>
<td>4090</td>
<td>6346</td>
<td>55</td>
<td>1.8</td>
</tr>
<tr>
<td>More developed regions</td>
<td>1131</td>
<td>1323</td>
<td>17</td>
<td>0.6</td>
</tr>
<tr>
<td>Less developed regions</td>
<td>2959</td>
<td>5023</td>
<td>70</td>
<td>2.1</td>
</tr>
<tr>
<td>Major Regions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Africa</td>
<td>399</td>
<td>814</td>
<td>104</td>
<td>2.9</td>
</tr>
<tr>
<td>Asia/Oceania</td>
<td>2274</td>
<td>3630</td>
<td>60</td>
<td>1.9</td>
</tr>
<tr>
<td>Latin America</td>
<td>325</td>
<td>677</td>
<td>96</td>
<td>2.7</td>
</tr>
<tr>
<td>Developed*</td>
<td>708</td>
<td>809</td>
<td>14</td>
<td>0.5</td>
</tr>
</tbody>
</table>

* In millions.

* Includes North America, Western Europe, Australia, New Zealand, and Japan.
already in use. In 1970 the food from 1 hectare (ha) of land supported 2.7 people. By 2000 this same ha must produce sufficient food to support 4.0 people, an increase of more than 48% per ha.

These projections present a tremendous challenge and opportunity to those of us involved in plant protection research. Estimates indicate that plant pests including insects, weeds, and pathogens reduce production of basic food crops worldwide 20-40% annually. In this regard, as a hypothetical example let us assume that maize virus diseases cause an annual 1% reduction in world maize (Zea mays L.) production. FAO statistics estimated world production of maize at nearly 400 million metric tons in 1980. Thus, if maize virus research efforts could contribute to cutting these losses in half (0.5%), this would represent an annual increase of approximately 2 million metric tons in world maize production. An increase of this magnitude would equal the 1980 production output of the Central American countries of Belize, Costa Rica, El Salvador, Guatemala, Nicaragua, and Panama combined.

Thus, the need to increase agricultural productivity through research and development is vitally important to current and future generations and is why A.I.D. and many other bilateral and international organizations are heavily involved in improving agricultural research capabilities in developing countries. In fact, the U.S. government in creating A.I.D. in 1961 provided a

| Table 2. International Agricultural Research Centers (IARC's) of the Consultative Group on International Agricultural Research (CGIAR). |
|---------------------------------|-------------------------------------------------|
| **IARC names and locations**    | **Principal research programs**                  |
| International Center for Tropical Agriculture (CIAT) Cali, Colombia | Cassava, field beans, rice, tropical pastures |
| International Center for Maize and Wheat Improvement (CIMMYT) Mexico D. F., Mexico | Maize, wheat |
| International Potato Center (CIP) Lima, Peru | Potatoes |
| International Board for Plant Genetic Resources (IBPGR) Rome, Italy | Collection, evaluation, utilization of genetic resources of important species |
| International Center for Agriculture Research in the Dry Areas (ICARDA) Beirut, Lebanon | Farming systems, cereals, food legumes (broad beans, lentils, chickpeas), forage crops |
| International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) Hyderabad, India | Chickpeas, pigeonpeas, pearl millet, sorghum, groundnuts, farming systems |
| International Livestock Center for Africa (ILCA) Addis Ababa, Ethiopia | Livestock production systems |
| International Laboratory for Research on Animal Diseases (ILRAD) Nairobi, Kenya | Trypanosomiasis, Theileriosis |
| International Rice Research Institute (IRRI) Los Banos, Philippines | Rice |
| International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria | Farming systems, maize, rice, roots and tubers (sweet potatoes, cassava, yams), food legumes (cowpeas, lima beans, soybeans) |
| West Africa Rice Development Association (WARDA) Monrovia, Liberia | Rice |
| International Service for National Agricultural Research (ISNAR) The Hague, Netherlands | Provides assistance to developing countries to plan and manage research effectively |
method to establish partnerships with developing countries in the broad field of agricultural processes related to crop production and to rural income generation.

A.I.D. ORGANIZATION AND AGRICULTURAL RESEARCH PROGRAMS

A.I.D. is divided into a number of offices and bureaus located in Washington, D.C. Four regional bureaus (Africa, Asia, Latin America and the Caribbean, and Near East) coordinate the work of nearly 60 A.I.D. missions located around the world. The Bureau for Science and Technology, also located in Washington, D.C., is organized to supply technical input into the major interests of the regional bureaus and their associated missions. Consequently, regional bureaus and the Bureau for Science and Technology work to assure that agricultural research and other development projects reflect mission and host country needs. The Bureau for Science and Technology maintains a permanent technical staff. However, the Bureau's principal sources of technical expertise for agricultural research projects are U.S. land-grant and 1890 universities and institutions.

A.I.D.-funded agricultural research activities can be divided into four broad categories: a) The International Agricultural Research Centers (IARC's) — A.I.D. is one of the principal donors in the Consultative Group on International Agricultural Research (CGIAR); b) Collaborative Research Support Programs (CRSP's) with U.S. colleges and universities working in cooperation with developing country institutions; c) other projects and contracts with U.S. institutions; and d) individual mission/host country projects.

The CGIAR-IARC research and training activities deal with crops and livestock that encompass three-quarters of the food supply to developing countries. Table 2 lists the CGIAR-IARC's and their major research areas. A.I.D. also assists in the financial support of other non-CGIAR centers, including the Asian Vegetable Research and Development Center (AVRDC) in Taiwan, the International Fertilizer Development Center (IFDC) in Alabama-USA, and the International Soybean Program (INTSOY) in Illinois-USA.

The CRSP's are a mechanism set up under Title XII of the amended Foreign Assistance Act which provides "...support for long-term collaborative university research (in developing countries to the maximum extent possible) on food production, distribution, storage, marketing, and consumption." Four CRSP's have been established. The subject matter areas and the management entities of these CRSP's are: small ruminants, University of California-Davis; sorghum/millet, University of Nebraska; beans and cowpeas, Michigan State University; and soil management, North Carolina State University. Two additional CRSP's will be underway soon: one with the University of Georgia concerning peanuts and another with Oregon State University concerning pond dynamics. In addition, the University of Maryland has been awarded a planning grant to investigate the feasibility of undertaking a CRSP in fisheries management.

Other research projects with U.S. institutions provide a linkage through regional bureaus and missions to furnish technical assistance, training, and important research capabilities in developing countries. Examples of these research project areas are: irrigation water use, biological nitrogen fixation, control of vertebrate pests, tissue culture, seed industry development, food grain storage and marketing, and pest management. Projects in pest management include weed control, control of barley (Hordeum vulgare L.) diseases, pest management and related environmental protection, postharvest food losses, and root-knot nematodes.

The regional bureaus and associated missions worldwide place major emphasis on agricultural improvement efforts in developing countries, and consequently fund a large number of projects in agricultural research and development. It would be impractical to enumerate all of these projects here since they number in the hundreds. However, some of the new or planned projects that may be of interest to the participants at this colloquium, because of their pest management aspects, are: a) Central America coffee rust control project; b) Jordan Valley Agriculture Services Project, with a major pest management control component; c) Association of South East Asian Nations (ASEAN) Plant Quarantine Training Project, headquartered in Kuala Lumpur, Malaysia, to serve all ASEAN countries; and d) Africa — bases of plant resistance to insect attack.
The Taxonomy and Evolution of *Tripsacum* and Teosinte, the Closest Relatives of Maize

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ABSTRACT


Together the genera *Tripsacum* and *Zea* form the subtribe Tripsacinae of the tribe Andropogoneae of the family Gramineae. These genera share in common a number of morphological features, including the highly specialized cupulate fruitcase. *Tripsacum* contains 13 or more perennial species which can be divided into sections *Tripsacum* and *Fasciculata*. Section *Tripsacum* has a larger number of more distinct species which have a much broader geographic range. *Zea* contains two perennial and two annual species which divide into two distinct groups, sections *Luxuriantes* and *Zea*. Section *Luxuriantes* is the more primitive section with two perennial and one annual species, all of which show considerable similarity to some tripsacums. Section *Zea* contains a single species with three subspecies: a) *Z. mays* spp. *mays* (maize or Indian corn); b) *Z. mays* spp. *parviglumis* (a small-spikeleted annual teosinte of middle elevation in southwestern Mexico and western Guatemala); and c) *Z. mays* spp. *mexicana* (a large-spikeleted annual teosinte of high elevation in central and northern Mexico). The morphological, biochemical, and genetic evidence suggests that one of these two wild subspecies is the direct ancestor of cultivated maize.

The genus *Zea* contains the cultigen, maize (*Z. mays* L. spp. *mays*), and its wild relatives of Mexico and Central America, the teosintes. The genus *Tripsacum* contains 13 or more species of wild grasses which occur from temperate North America (Massachusetts) to Paraguay in South America. Together these two genera form the subtribe Tripsacinae of the tribe Andropogoneae of the family Gramineae (Clayton, 1973). The Tripsacinae, like other members of the Andropogoneae (such as sorghum), have their spikelets arranged in pairs, with one member of the pair being sessile and the other borne on a slender pedicel. The spikelet pairs are distichously arranged along the branches of the inflorescence. The Tripsacinae are distinguished from the other Andropogoneae by the following: a) Their male and female spikelets are born in separate inflorescences (as in *Zea*) or separate parts of the same inflorescence (as in *Tripsacum*); b) In the female inflorescence, the pedicellate member of the spikelet pair is completely suppressed (Figs. 1 and 2). [Maize is an exception to this rule, as the pedicellate spikelets of its female inflorescence (ear) are fertile and produce grains]. c) The grain is invested by a cupulate fruitcase which is comprised of the deeply excavated rachis internode and the outer glume of the sessile spikelet (Figs. 1 and 2). (Again, maize is an exception in that its grains are not enclosed in cupulate fruitcases but are instead naked.)

*Tripsacum* has been recognized as closely related to *Zea* because of the morphological features described above and because it is known to cross with maize and produce viable but mostly infertile hybrids (Galinat, 1961, 1962; Mangelsdorf and Reeves, 1931, 1939). Further, biochemical data have shown that these two genera are more closely related to one another than to other members of the Andropogoneae (Khavkin et al., 1979; Smith and Lester, 1980). The fact that both taxa are native to the New World with their centers of diversity in southern Mexico supports the conclusion that they are closely related (Figs. 3-6).

Because of their affinity to maize, *Tripsacum* and teosinte have been the subjects of numerous biosystematic and evolutionary studies. Much of the work on *Tripsacum* was stimulated by Mangelsdorf and Reeves (1959), who suggested that introgression of *Tripsacum* germplasm into *Zea mays* was responsible for some of the present-day variation in the cultigen. However, it was not until 1967 with the publication of Wilkes' thesis (Teosinte: the closest relative to maize) and the resurrection of the hypothesis that teosinte was the direct ancestor of maize by Beadle (1972), deWet and Harlan (1972), Galinat (1971), and Iltis (1971) that the teosintes were subjected to careful taxonomic study.

In this paper I will summarize much of the taxonomic and evolutionary information available for both
Fig. 1. Inflorescence structure of *Tripsacum dactyloides* (Weatherwax, 1935: Fig. 7). On the left, a cluster of female teosinte spikes as commonly found within the leaf sheaths of the main stem of plants grown in dense competition; thus, the cluster is lateral with respect to this main stem.

Fig. 2. Pistillate inflorescences of maize and teosinte (Weatherwax, 1955: Fig. 9).
Tripsacum and the teosintes. Understanding the taxonomy and evolution of the teosintes is of obvious importance. The teosintes belong to the same genus as maize (some even to the same species) and one of the teosintes is the direct ancestor of maize. Further, they all cross with maize to produce fertile hybrids, making them potentially useful sources of germplasm for maize improvement. Understanding the taxonomy and evolution of Tripsacum is of less agronomic interest. Tripsacum was not directly or even indirectly involved in the origin of maize and there is no evidence that it has contributed substantially to maize diversity (cf. deWet and Harlan, 1978; Galinat, 1977). Further, Tripsacum maize hybrids are essentially sterile, making it difficult to use them in maize improvement. However, insect pests and a virus disease associated with Tripsacum have become problems on maize, and a thorough understanding of these diseases and insects requires some basic knowledge of Tripsacum (Nault and DeLong, 1980; Nault et al., 1980).

**THE GENUS TRIPSACUM**

Despite the relatively small number of species in this genus and that several botanists have studied it in varying depths over the past 50 yr, Tripsacum remains a taxonomically difficult group. The reasons for this include the following. First, the genus contains diploids, triploids, tetraploids, and even higher ploidy levels (N=18); some species have several of these. Second, some species of Tripsacum are apparently facultative apomicts which can give rise to minutely but consistently differentiated forms (deWet et al., 1981; Farquharson, 1955). Finally, natural hybridization between species may have produced intermediates which blur species boundaries (Randolph, 1970). Nevertheless, because of the efforts of Hitchcock (1906), Cutler and Anderson (1941), Randolph (1970), and deWet, Harlan and their associates (deWet et al., 1976; deWet et al., 1981), all of whom have studied aspects of Tripsacum taxonomy, a reasonably clear picture of the genus can be drawn.

All species of Tripsacum are perennial and all but one (T. zopilotense Hernández and Randolph) produce stout rhizomes. They are vegetatively robust plants ranging from 1-5 m in height with leaves 1-10 cm wide. Their inflorescences are terminal on the stems and branches. When the branches of the main stems fail to elongate, their inflorescences remain partially enclosed by the leaf sheaths. The inflorescence is composed of 1 to 100 spikelet branches bearing pistillate spikelets below and staminate ones above (Fig. 1). Each pistillate spikelet contains both a fertile floret (flower) and a suppressed one; these are enclosed together in the cupulate fruitcase. The indurate fruitcases are either rectangular or trapezoidal in outline, and the horizontal abscission layers between them allow the inflorescence to disarticulate so the fruitcases can be dispersed. The

![Fig. 3. Distribution of Tripsacum dactyloides and T. floridanum in the United States and the Caribbean.](image-url)
Fig. 4A. Distribution of *Tripsacum* in Mexico and Central America. Section *Tripsacum*.

Fig. 4B. Distribution of *Tripsacum* in Mexico and Central America. Section *Fasciculata*. 
staminate spikelet contains two fertile florets, each with three fully functional anthers. The outer glumes of the male spikelets generally have 10 to 20 nerves; the two major lateral nerves are elaborated into wings near the apex of the spikelet.

*Tripsacum* chromosomes are frequently marked by terminal heterochromatic regions called knobs, although some species apparently lack these structures (Chaganti, 1965; Longley, 1941; Ting, 1960). Chromosome knobs are found in other genera of the Andropogoneae; however, they are most common in *Tripsacum* and *Zea* (Galinat, 1977).

In 1906, Hitchcock divided the genus into sections *Dactyloides* and *Fasciculata*. Changes in the rules of nomenclature now require that section *Dactyloides* be called section *Tripsacum*; however, the taxonomy of the plants has not changed and this sectional division remains useful today.

**Section *Tripsacum***. The key taxonomic traits of this section are: a) the pedicellate spikelet of the spikelet pair sessile or nearly so; b) outer glume of staminate spikelets coriaceous; and c) branches of inflorescences few in number (usually 1 to 10), digitately arranged, stiff and straight, and ascending or weakly arched.

This section contains nine species which cover the entire geographic range of the genus.

*Tripsacum dactyloides* L. As applied by most workers, this name refers to an extremely diverse group of plants ranging from Massachusetts in North America to Colombia and Venezuela in South America, as well as the Caribbean Islands (deWet et al., 1981). The plants range from small to robust, being from 1-3.5 m tall and having leaves from 0.5-5.0 cm wide. The inflorescence may have from 1 to 10 branches and the male spikelets vary from 5-9 cm in length. The species includes diploids, triploids, and tetraploids, as well as higher

![Fig. 5. Distribution of *Tripsacum* in South America.](image)
Fig. 6. Distribution of teosinte (Doebley and Ilis, 1980: Fig. 29).
ploidy levels. Some members of this species are facultative apomicts (Farquharson, 1955). Newell and deWet (1974) studied cytological and morphological variation in *T. dactyloides* of the United States, but found no way in which this portion of the species could be divided taxonomically. Further, taxonomic research will undoubtedly result in splitting this “species” into several smaller, more reasonably defined groups.

Both the diploid and tetraploid forms of *T. dactyloides* have been crossed to maize. The morphology, cytology, and potential agronomic uses of these hybrids and their derivatives have been studied extensively (Cohen, 1982; deWet et al., 1978; Stalker, et al., 1977).

*Tripsacum floridanum* Porter ex Vasey. This is a slender plant with culms rarely more than 1 m tall and leaf blades less than 1 cm in width. The inflorescence is composed of one to three branches and has male spikelets similar to those of *T. dactyloides*. The two species appear to be closely related both morphologically and cytologically (Tantiravahi, 1968). The species is diploid and occurs in the Pinelands of southern Florida and in Cuba. It is of interest that this species crosses relatively easily with maize as compared to some other *Tripsacum* (Galinat, 1961, 1962).

*Tripsacum zopilotense* Hernandez and Randolph. This slender, diploid species, unlike other *Tripsacum*, lacks rhizomes. Its leaves are at most 2 cm wide and its culms rarely exceed 1.5 m in height. The inflorescence is composed of a single spike which bears relatively small male spikelets (3-7 mm long). This species was described from specimens collected in the Zopilote Canyon in Guerrero, Mexico (Hernandez and Randolph, 1950). Similar narrow-leaved, rhizomeless *Tripsacum* from localities in Guerrero, Jalisco, and Chiapas have also been classified as *T. zopilotense* (Randolph, 1970).

*Tripsacum bravum* Gray. This moderately robust species has culms from 1-2 m in height and leaves from 3-5 cm in width. Like *T. australis* Cutler and Anderson, its basal leaf sheaths are tomentose, especially along the midrib. Each of its numerous inflorescences consists of a single (rarely two or three) spike. It is unique among the *Tripsacum* in having few (three to six) nerves on the outer glume of its male spikelets. The species is diploid and known only from the moist woods and shady roadside escarpments of Valle de Bravo, Mexico, Mexico (deWet et al., 1976).

*Tripsacum andersonii* Gray. This species, generally known as “Guatemala grass”, is cultivated and occurs feral in both Meso and South America. It is the most robust of all *Tripsacum* species, with culms up to 5 m tall and leaves up to 10 cm wide. It forms dense clumps and spreads by means of its robust stolons. Its fruitcases (4-6 mm wide and 7-8 mm long) and male spikelets (7-9 mm long) are among the largest in the genus. Unlike male spikelets of other species of section *Tripsacum*, one member of its pairs is borne on a short pedicel (1-3 mm). It grows throughout much of Latin America from Peru to Guatemala.

*Tripsacum andersonii* is unique within the genus in two respects. First, it is sterile, reproducing only by vegetative means. Second, its chromosome number (2N = 64) is unknown among other *Tripsacum* (Levings et al. 1976). Curiously, a specimen from Costa Rica (Pohl and Davidsee 11411) of this species was determined to have 2N = 72, the unusual tetraploid number in the genus. Because of this unusual chromosome number, the species has been interpreted as a *Zea-Tripsacum* hybrid with three sets of *Tripsacum* chromosomes (3 x 18) plus one set of *Zea* chromosomes (N = 10) (deWet and Harlan, 1978; deWet et al., 1981).

*Tripsacum latifolium* Hitchcock. This species is particularly robust, forming large clumps and having culms up to 5 m tall and leaves up to 7 cm wide. In habit, it resembles *T. andersonii* and at times these two species have been confused. This similarity led deWet and Harlan (1978) to hypothesize that *T. latifolium* is the *Tripsacum* parent of *T. andersonii* (the supposed *Zea-Tripsacum* hybrid). *T. latifolium* is distinguished from *T. andersonii* and most other *Tripsacum* by its unusually small male spikelets (4-6 mm long). In some specimens, the spikelets are nearly as broad as long and superficially resemble those of the genus *Paspalum*. The species is tetraploid, and it occurs in the mesic woods of Guatemala, Belize, and Honduras. A specimen from Costa Rica has also been identified as *T. latifolium*; however, as illustrated by Pohl (1980), it has a distinctly pedicelled spikelet, a character not found in *T. latifolium*.

*Tripsacum australis* Cutler and Anderson. This robust species has culms up to 4 m in height and leaves up to 6 cm in width. It is distinguished by its densely tomentose basal leaf sheaths. The species is diploid and reproduces sexually. It grows in the moist open woodlands of Venezuela, Colombia, Brazil, Bolivia, and Paraguay (Cutler and Anderson, 1941; cf. deWet et al., 1981).

*Tripsacum peruvianum* deWet and Timothy. This is an erect (1-2.5 m tall), moderately robust species with leaves 3-4.5 cm wide. It is distinguished by its strongly hirsute leaf sheaths. The inflorescence has three to five branches. The species occurs along shady stream banks in Peru and Ecuador. It is a gametophytic apomict (2N = 72, 90, 108) (deWet et al., 1981).

*Tripsacum cundinamarcae* deWet and Timothy. This robust species has culms 3-4 m tall and leaves 4-6 cm wide. The inflorescence is composed of three to nine spikes having small spikelets (6-7 mm long). The species is distinguished by its glabrous, glaucous leaf sheaths and blades. This diploid species is found on river banks of the Department of Cundinamarca, Colombia (deWet et al., 1981).

Section Fasciculata Hitchcock. The key taxonomic traits of this section are: a) inflorescences with 10-100 or more lax spike-like branches, with the lower ones fascicled; b) one member of each spikelet pair borne on a slender pedicle; and c) outer glume of the staminate spikelet membranaceous.

This section contains four species which are restricted to Mexico and Central America.

*Tripsacum maizar* Hernandez and Randolph. This is a particularly robust species with thick culms (4 cm in diam) up to 4.5 m in height and leaves which range
from 7-10 cm in width. Its basal leaf sheaths are densely hirsute, while its coriaceous leaf blades are essentially glabrous. The terminal inflorescence has from 18-50 or more spikelike branches, with spikelets from 5-8 mm long. The species is diploid and occurs from Costa Rica (?) to Guatemala to Nayarit, Mexico. Except for differences in general robustness, ploidy, and pubescence, *T. maizar* remarkably similar to *T. pilosum*.

**Tripsacum pilosum** Scribner and Merrill. This is a robust tetraploid with culms up to 3 m tall and leaves from 3-6 cm wide. The lower leaf sheaths are densely hispid and the blades are pilose. The inflorescence has 2 to 15 spikelike branches which have relatively large spikelets (7-9 mm long). This species is broadly distributed, ranging from Honduras to Zacatecas, Mexico.

**Tripsacum lanceolatum** Rupe. ex Fourn. This is a narrow-leaved (1-3 cm wide), small to moderately robust species (1-2 m tall), with hispid lower leaf sheaths. The inflorescence has relatively few (one to nine) spikelike branches, the lowest number for section *Fasciculata*. The species occurs on the moist escarpments and stream banks of western Mexico (Jalisco) north to Arizona (deWet and Harlan, 1978). The species is tetraploid.

**Tripsacum laxum** Nash. This is a robust species up to 3.5 m tall with leaves up to 8 cm wide. Its inflorescence has 5 to 20 long and slender spikelike branches. It is distinguished from other tripsacums of section *Fasciculata* by its usually glabrous leaf sheaths and blades. The species is diploid and occurs from southern Mexico to Guatemala.

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*Fig. 7. Branched and unbranched plants of Zea luxurians* (Weatherwax, 1955: Fig. 6).
THE GENUS ZEA

Zea contains only four species and the relationships among these are well understood. The genus lacks the cytological complexities of Tripsacum; three of its species are diploid ($N = 10$) and one is tetraploid. Although each of the wild species hybridizes in nature with cultivated maize, backcrosses are infrequent, and there is no evidence of introgression into the wild taxa by the cultivar (Doebley, 1980; cf. Wilkes, 1972).

Zea contains both annuals and perennials, but only the annuals are robust maize-like plants. While most maize varieties are strictly monopodial, the teosintes when grown in the open or in cultivation tend to produce a lateral branch at each node as well as 1 to 10 (or more) basal tillers. However, in Mexico and Guatemala, the teosintes generally grow in strong competition with the surrounding vegetation; thus tillering is rare and branches are either absent or produced only along the upper third of the main stem. In teosinte a tassel crowns the apex of both the culms and their branches (Fig. 7), while the female spikes are produced either singly or in clusters within the leaf sheaths. Thus, the female spikes are lateral in position with respect to the main stem and its primary branches (Fig. 2). In maize the female spike (ear) is usually terminal on a highly shortened, primary, lateral branch, a position usually occupied by a tassel in an open grown teosinte (cf. Iltis, 1979, 1981). Also in maize, tassels are restricted to the apex of the main stem and do not occur on lateral branches as in teosinte.

In teosinte each individual female spike is surrounded by a spathe (a modified leaf sheath) that protects it from being damaged before it matures. The spikes are composed of 4 to 12 or rarely more distichously arranged cupulate fruitcases which are exceedingly indurate and polished on the exterior. Abscission layers between the fruitcases allow them to separate from one another at maturity so they can be individually dispersed.

The male inflorescence (tassel) has up to 100 or more spikelike branches along which the spikelet pairs are distichously arranged. The central spike of the maize tassel is an exception to this rule of distichy in that its spikelet pairs, like those of the maize ear, are polystichously arranged (Fig. 8).

Like Tripsacum, Zea can be divided into two natural sections.

Section Luxuriantes Doebley and Iltis. The key taxonomic traits of this section are: a) tassels with few branches, usually less than 15; b) male spikelets with stiff, firm glumes, the outer one flat on the back and having two strong lateral nerves which elaborate into wings spicily; c) fruitcases trapezoidal; and d) chromosome knobs strictly terminal.

This section contains two perennial and one annual species.

Zea diploperennis Iltis, Doebley and Guzman. This is a robust, clump-forming, perennial species that forms both short, tuberous and cordlike rhizomes with short internodes. The plants are from 1-2.5 m tall with leaves up to 5 cm wide. The male inflorescence has 2 to

Fig. 8. Staminate inflorescence of maize (Weatherwax, 1955: Fig. 10).
13 branches and the outer glumes of its spikelets possess well-developed lateral wings. The species is rare, restricted to the Sierra de Manantlan, Jalisco, Mexico (Iltis et al., 1979).

**Zea perennis** (Hitchcock) Reeves and Mangelsdorf. This species closely resembles *Z. diploperennis* but differs in several key characters. Notably, the plants are less robust, about 1.5 m tall with leaves from 1-3 cm wide. It lacks the short, thick, tuberous rhizomes of *Z. diploperennis*, but produces an abundance of longer, collars-like rhizomes with much longer internodes. These rhizomes spread aggressively so that the species grows in thick, dense mats. The morphology of the inflorescence is very nearly identical to *Z. diploperennis*, although *Z. perennis* has fewer (one to eight) tassel branches. *Z. perennis* is the only tetraploid in the genus. It has a narrow distribution and is known only from the vicinity of Ciudad Guzman, Jalisco, Mexico.

**Zea luxurians** (Durieu and Ascherson) Bird. This is a robust annual species that grows up to 3.5 m tall. It resembles the perennials in many respects, including its trapezoidal fruitcases, terminal chromosome knobs, and few tassel branches. It resembles the other annual teosintes in its almost strictly monopodial habit. It is distinguished from all *Zea* species by the numerous (15 to 25) closely spaced nerves on the outer glume of its spikelets. These spikes, which disarticulate from race *diploperennis*, occur in Michoacan, Guanajuato, eastern Jalisco, and Mexico (the Palsas River drainage), as well as central and, to a much lesser extent, in northern Mexico.

The similarity in tassel morphology between vars. *huhueuenganensis* and *parviglumis* is not entirely reflected in their biochemistry and cytology. Isoenzymatically, there are larger differences between these two varieties than one would predict baseu on morphology (Doebley, M. M. Goodman, and C. W. Stuber, unpublished). The same is true for their chromosome knobs (Kato, 1976). However, these two varieties are quite similar in their cytoplasm DNA's (Timothy et al., 1979) and seed proteins (Mastenbroek et al., 1981). These varieties might best be recognized as separate subspecies, if it were not for their similar inflorescence morphologies, cytoplasm genomes, and seed proteins.

**Zea mays** L. ssp. *mexicana* (Schrader) Iltis. In the wild, this is a robust (up to 3.5 m tall), usually unillered plant with a weak root system. Its tassel has relatively few (1 to 30) branches which bear large spikelets (6.5-10.5 mm long). The fruitcases are generally large, ranging from 56-104 mg in weight. The leaf sheaths are often dark red in color and densely pubescent. It grows at relatively high elevations (1750-2500 m), mostly in central and, to a much lesser extent, in northern Mexico.

The subspecies is divided into three weakly differentiated races. Race *Chalco* has the reddest, most pubescent leaf sheaths and is restricted to the Valley of Mexico and its slopes. The plants of race *Nobogame* are generally somewhat depauperate and flower earlier than others of *Z. mays* ssp. *mexicana*. This race, the most northern of all teosintes, is restricted to the Nobogame Valley of Chihuahua. Race *Central Plateau* has some depauperate populations which resemble race *Nobogame* and other robust populations indistinguishable from race *Chalco* (Wilkes, 1967). Race *Central Plateau* occurs in Michoacan, Guanajuato, eastern Jalisco, and Durango.

**Zea mays** L. ssp. *mays*. The essential botanical difference between maize and the annual teosintes of Mexico are confined to the inflorescences. While the central spike of the teosinte tassel is slender and distichous, the central spike of the maize tassel is substantially thickened and polystatic (Fig. 8). The female inflorescences of maize and teosinte are especially dissimilar. As mentioned previously, the teosinte female spike is composed of 4 to 12 distichously arranged, highly indurate cupulate fruitcases, each containing a single fertile spikelet. These spikes, which disarticulate at maturity, are lateral to the mainstem and its primary branches. The maize female spike (ear) is polystitute, with from 8 to 26 or more rows of grain, these firmly attached to a massive central axis (cob) and thus incapable of dispersing. The equivalents of the teosinte cupulate fruitcases in the maize ear are the hidden, indurate invaginations in the cobs known as cupules.
The entire maize spike is completely surrounded by a series of enlarged leaf sheaths (husks) which originate on the telescoped primary branch (shank) and protect the developing ear from pests and predators. The ear is terminal on a lateral branch (Fig. 2).

THE PHYLOGENY OF THE TRIPSACINAE

In this section, I will discuss the evolutionary relationships among the tripasacums and teosintes. To begin, one can safely conclude that of the two genera, *Tripsacum* is the older and more primitive. Support of this statement comes from several independent lines of investigation.

First, in considering the inflorescence, one must recall that the inflorescences of most Andropogoneae contain at least some bisexual spikelets. This is the primitive condition. In *Tripsacum*, the spikelets are not bisexual but rather may be either female proximately or male distally; these two types of spikelets occur together in the same inflorescence. In this sexual dimorphism, one witnesses an advancement over the typical Andropogonaceae pattern. In *Zea* there is further evolutionary change in that not only are the spikelets unisexual but the male and female spikelets are segregated into separate inflorescences. In this sense then, *Tripsacum*, with its male and female spikelets in the same inflorescence, more closely resembles other Andropogoneae, suggesting that it is less specialized than *Zea*.

Similarly, if the structure of the cupulate fruitcase is considered, it will be recognized that the *Tripsacum* fruitcase much more closely resembles the rachis internode (the structure from which it evolved) than does the fruitcase of teosinte. The *Tripsacum* fruitcase is often rectangular in outline (cylindric in shape), the same as the rachis internode. Although it shows considerable induration, it never approaches the extreme induration of the teosinte fruitcase. In this sense then, the fruitcase of *Tripsacum* more closely resembles the rectangular, non-indurate rachis internode, its precursor.

In seasonal growth form, *Zea* also appears more specialized. Although the overwhelming majority of Andropogoneae are perennial (suggesting this to be the primitive condition), a few genera include some annual species. In this regard, *Zea*, which includes two annuals, must be judged more specialized than *Tripsacum*, which has only perennials.

The biogeography of *Tripsacum* and *Zea* also hints that *Tripsacum*, with its much broader, natural distribution, is the older genus. Similarly, the larger number of species in *Tripsacum* suggests a longer evolutionary history for this genus.

Except for the basic division of *Tripsacum* into sections *Fasiculata* and *Tripsacum*, little is known of the evolutionary relationships within the genus. Both of these sections have a combination of specialized and primitive characteristics. Morphologically, the stiff, ascending inflorescence branches and the brittle, chartaceous, and flattened outer glumes of section *Tripsacum* resemble quite closely certain species of other genera of the Andropogoneae, including *Andropogon* and *Rottboellia*. However, one apparently advanced morphological trait found in section *Tripsacum* is that the pedicellate male spikelet of each pair is essentially sessile. The male spikelet pairs in section *Fasiculata*, on the other hand, are clearly one pedicellate-one sessile; this is the usual pattern in the Andropogoneae.

In *Zea*, the morphological and other evidence suggests that the teosintes of section *Luxuriantes* are the most primitive taxa in the genus. These teosintes, especially the perennials, hold many morphological features in common with *Tripsacum*, including: a) typically many-nerved, flattened outer glumes with two prominent lateral nerves developed into wings; b) short, flattened internodes in the male portion of the inflorescence; c) trapezoidal fruitcases; and d) perennial rhizomatous root system and in the perennials, a weakly monopodial habit. Cytologically, the chromosomes of section *Luxuriantes*, like those of *Tripsacum*, have many terminal chromosome knobs and no internal ones. Section *Zea*, on the other hand, has internal chromosome knobs which probably represent an evolutionary advancement.

After the initial divergence of sections *Luxuriantes* and *Zea*, diversification continued within each of these two taxa. Within section *Luxuriantes*, *Z. luxurians* probably separated quite early from the perennials. It might have abandoned the perennial habit as an adaptation to disturbed conditions in the dry and highly seasonal environments of southeastern Guatemala and acquired the monopodial form typical of annual pioneers. The divergence between *Z. diploperennis* and *Z. perennis* apparently came somewhat later by means of autoploidy (Shaver, 1962; Galinat, 1971).

Section *Zea* probably diverged from section *Luxuriantes* by developing the annual habit as an adaptation to disturbed conditions and to highly seasonal, arid habitats. It can be observed that perennial species commonly assume the annual habit in adapting to dry, seasonal environments. At some point, section *Zea* underwent diversification, producing the two wild subspecies of *Z. mays*. One of these, *Z. mays* spp. *mexicana*, colonized the higher, colder, drier sites with a shorter growing season and is characterized by large seeds, red hairy sheaths, large male spikelets, and fewer tassel branches as an adaptation to this environment (Doebly, 1980). The other, *Z. mays* spp. *parviglumis*, colonized the lower, warmer, seasonally moister sites with a longer growing season and is characterized by small seeds, glabrous green or dilute red sheaths, small male spikelets, and many tassel branches (Doebly, 1980).

THE ORIGIN OF MAIZE

The origin of maize has beguiled botanists for well over a century. During this time most authors have supported either the hypothesis that maize evolved from a polystichous wild maize (Mangelsdorf, 1974; Randolph, 1976; Wilkes, 1979) or that teosinte is the ancestor of maize (Beadle, 1972; deWet and Harlan, 1972; Galinat, 1971; Iltis, 1971; Iltis and Doebly, 1980; Kato Yamakake, 1976). Since the early 1970's, this tide of opinion has moved in favor of the latter theory; however, the subject remains controversial.
In my own view, the most parsimonious interpretation of the facts is to consider the anomalous polystichy of maize, which is found only in the cultigens and in no other Andropogoneae, as the utilitarian artifact of domestication (Doebley and Iltis, 1980). Specifically, maize appears to be a domesticated form of one of the teosintes of section Zea, differing from these teosintes in none of its essential botanical characteristics. Other available data support this interpretation of morphology. Beadle (1972) has indicated that the number of critical gene differences between maize and teosinte are very small, perhaps only four or five. Timothy et al. (1979) have shown that certain maize lines and Z. mays ssp. mexicana and ssp. parviglumis were not distinguished by either their chloroplast or mitochondrial genomes. A study of isoenzymatic variation of maize and teosinte showed no substantial differences between maize and Z. mays ssp. parviglumis var. parviglumis (Doebley, M. M. Goodman, and C. W. Stuber, unpublished). Cytological studies of certain maize races and teosinte showed no substantial differences between Z. mays ssp. parviglumis var. parviglumis and Z. mays ssp. mays (Kato Yamakake, 1976). Indeed, when size and position of chromosome knobs, presence of B chromosomes, and an abnormal form of the tenth chromosome are all considered, this teosinte and many Mexican maize races are astoundingly similar (McClin-tock et al., 1981). Finally, and perhaps most importantly, maize and the teosintes of section Zea belong to the same species, crossing with complete freedom and producing fully fertile hybrids in most cases (Darlington, 1956; Iltis, 1972).

While many authors have recognized that the biosystematic data point to teosinte as the ancestor of maize, the archaeological evidence apparently does not. The oldest archaeological maize recovered from Tehuacan possesses long, soft glumes, a narrow flexible rachis, and shallow non-indurate cupules (Mangelsdorf, 1974). These are all characteristics which would be difficult to derive from the female teosinte spike. For this reason and others, Mangelsdorf and his colleagues have remained staunchly committed to their theory that maize evolved from a "wild maize" and not from teosinte. Recently, Iltis (1979, 1981) proposed a new theory called the Catastrophic Sexual Transmutation Theory which tries to explain both the biosystematic and archaeological evidence. Iltis suggests that the central spike of the teosinte tassel, which normally terminates a lateral branch in teosinte (and not the teosinte female spike), gave rise to the familiar maize ear. This idea is related to earlier theories of Kellerman (1895), Montgomery (1906), and Weatherwax (1935) in which the maize ear is derived from a male inflorescence but not the male inflorescence of teosinte.

This theory makes good sense morphologically because the maize ear is terminal on a lateral branch, a position that in teosinte is usually occupied by a tassel. Thus, the initial step in the domestication of maize would have been a sex change from male to female of the central spike of the tassel terminating the lateral branch. Iltis believes this change and the subsequent (or concurrent) condensation of the central spike into the maize ear happened rapidly; thus, we have the "Catastrophic Sexual Transmutation Theory."

One of the most intriguing aspects of this theory is its implication for the archaeological specimens from Tehuacan. As described above, they do not appear intermediate between the teosinte female spike and the maize ear. However, under Iltis' theory, they are precisely the intermediates expected. The central spike of the teosinte tassel has a flexible rachis, shallow non-indurate cupules, and spikelets with long, soft glumes, the same as the Tehuacan specimens (cf. Mangelsdorf, 1974). Further, the central tassel spike of teosinte has two functional spikelets per node (cupule), the same as the maize ear but unlike the teosinte ear. So by following Iltis' theory, one need no longer be concerned with the reactivation of a suppressed female spikelet (cf. Beadle, 1972).

Iltis' theory on the origin of the maize ear must now be assessed in relationship to the older theory that the maize ear evolved from the female spike of teosinte (Galinat, 1971). The need for this reassessment provides an opportunity to restudy in detail the developmental morphology of the inflorescences of Zea. The future for research on the ancestry of maize promises to be full of new discoveries and revelations.

**THE USE OF TRIPSSACUM AND TEOSINTE IN MAIZE BREEDING**

Neither *Tripsacum* nor teosinte have been of any importance in improving maize or in broadening its genetic base. They have not generally been used by commercial maize breeders simply because any positive traits they possess are drastically overshadowed by other deleterious traits, including reduction in yield, loss of stalk strength, and undesirable changes in plant architecture such as increases in tiller and ear number. Indeed, with rare exceptions, practicing maize breeders are not only reluctant to work with these wild relatives of maize, but have generally portrayed a reluctance to incorporate even tropical maize races in their breeding programs. For this reason, the chore of tapping these genetic resources has fallen largely on the shoulders of the basic research community. The accomplishments of these scientists demonstrate a certain degree of promise in some areas, most notably in the area of disease and insect resistance. There have been several accomplishments to date.

Bergquist (1981), starting with a maize-*T. dactyloides* hybrid, obtained agronomically useful inbred lines that were resistant to the common rust, *Puccinia sorghi* Schw. Similarly, resistance to northern leaf blight was obtained from *T. floridanum* by Hooker and Perkins (1980). Nault and Findley (1981) are in the process of developing maize resistant to maize chlorotic dwarf virus using *Z. diploperennis* as the source for this resistance.

The wild relatives of maize may be useful in breeding for resistance to other diseases and insects. Branson (1971) and Branson and Guss (1972) have shown that *T. dactyloides*, *T. laxum*, and *T. floridanum* are highly...
resistant to western corn rootworm (Diabrotica virgifera LeConte). Branson (1972) found these same three species to show considerable resistance to the corn leaf aphid [Rhopalosiphum maidis (Fitch)]. Nault et al. (1982) report the perennial teosinte to be resistant or immune to a number of maize viral diseases, including maize chlorotic dwarf, maize chlorotic mottle, maize streak, and maize stripe.

The fact that the perennial species of the Tripsacinae show greater resistance to diseases and insects is of some interest. In general, perennials maintain higher disease and insect resistance because their sedentary and long-lived lifestyle makes them easily locatable by pests which accumulate on them. Annually, which can evade diseases by migrating more regularly from place to place, are not under as strong selection to develop resistance, and thus tend to show greater susceptibility to diseases and predation (Feeny, 1976).

The ability of researchers to transfer disease resistance from Tripsacum and teosinte to maize resides in part in the genetic nature of these traits. If polygenic, the chances of successfully transferring the trait are small and get progressively smaller as the number of genes increases. This is especially true with Tripsacum because its chromosomes exhibit a low degree of homology with those of maize, making it difficult to transfer a single gene, let alone several genes, simultaneously. At the other extreme, there exists considerable hope that Z. diploperennis can make an important contribution to maize improvement, because it is both genetically similar to maize and a disease resistant perennial.

**LITERATURE CITED**


Racial Diversity in Maize

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ABSTRACT


Six major maize (Zea mays) racial groups of worldwide genetic and economic importance are described and enumerated, along with several additional groups of regional importance. The current status of the racial collections and descriptions of the collections housed in the major maize germplasm banks in the Americas are presented. Tentative plans for making these materials available and accessible to researchers are described. An attempt is made to demonstrate the degree to which our usual genetic stocks reflect the available genetic variation among the races. In addition, for four distinct maturity groups, specific agronomically promising collections are enumerated. These collections include a wide array of races.

Although earlier collections of maize (Zea mays L.) had been made by Vavilov and his associates (Kuleshov, 1929, 1950), the Rockefeller Foundation-National Academy of Sciences collections, dating from about 1945 in Mexico to about 1955 in Chile and numbering nearly 12,000 collections, still serve as the basis for current studies (Brown and Goodman, 1977). From these collections, about 250 races were named in a series of monographs describing the maize of Latin America, its uses and history (Brieger et al., 1958; Brown, 1960; Grant et al., 1963; Grobman et al., 1961; Hatheway, 1957; Ramirez et al., 1960; Roberts et al., 1957; Timothy et al., 1961, 1963; Wellhausen et al., 1952, 1957). Since these publications, archaeological studies of maize remains have supplemented studies of currently existing maize varieties and races (Cutler and Blake, 1971; MacNeish, 1964; Mangelsdorf, 1974; Mangelsdorf et al., 1964, 1967). Discussion here will be largely limited to the maize of the New World. While certain parts of Africa, Asia, and especially Europe (Trifunovic, 1978) have become secondary centers of significant variation in maize, by far the greatest variation is represented by the Latin American races (Brown, 1979). Brown estimated that there may be 150 to 180 distinct racial groups worldwide, of which 130 or so are Latin American.

Anderson and Cutler (1942) defined a race as "a group of related individuals with enough characteristics in common to permit their recognition as a group." They described a race as "a group of individuals with a significant number of genes in common, major races having a smaller number in common than do sub-races." Thereafter, Anderson (1943, 1944, 1945, 1946, 1947), Anderson and Brown (1952), Brown (1950, 1953), Brown and Anderson (1947, 1948), Carter and Anderson (1945), and Cutler (1946) further extended the concept of races of maize, described morphological characters thought to be useful in racial studies, and tentatively classified much of the maize of the New World. Wellhausen et al. (1952) published results of a study of an extensive collection of Mexican corn used for a breeding program begun in 1943 in Mexico. This publication was sponsored jointly by the Rockefeller Foundation and the Mexican government and was the first of a series of racial bulletins (cited earlier) which eventually described most of the maize of Latin America.

After the original series of almost 12,000 collections of Latin American maize was completed, preparation of the races of maize reports continued into the early 1960's. Descriptions of the original collections were published in mimeograph form by the Committee on Preservation of Indigenous Strains of Maize (1954, 1955), but distribution of these publications was very limited and copies are rare. Since data for the almost 12,000 collections were presented on only about 600 pages, information on each collection is necessarily brief. However, the listing typically includes the site of each collection, its latitude and altitude, local name of the variety, endosperm type (pop, flint, dent, floury, sweet), and kernel color(s). Although complete data are not available for all collections, listings are remarkably complete in comparison to most such germplasm listings.

Since 1955, more than 3000 additional collections have been made in Latin America (M. Gutierrez G., R. Sevilla, E. Hernandez X., A. Blumen .chein, and W. L. Brown, personal communications), unfortunately with little accompanying published documentation. Some collections were aimed at strengthening representation of certain races, others at replacing lost collections, and still others have filled geographic gaps (collections fre-
requently follow the Pan-American highway system. Some collections have been made specifically to obtain material of immediate interest to breeders.

With few exceptions (Brieger *et al.*, 1958; Brown, 1960; Hatheway, 1957), one important feature of the races of maize reports was listings of specific collection numbers for collections considered most typical of each race. This has provided a rational basis for further racial studies and has allowed systematic sampling of variability present in Latin American maize. In the past 20 yr, some typical collections have been lost (Ortega and Angeles, 1978; Timothy and Goodman, 1979). Others have been replaced or supplemented by more recent (often undocumented) collections. In addition, a few new races have been named, and in some cases collections typical of these races have been identified. Unfortunately, much work done within the last 20 yr has not been published. Exceptions are the newly described races of northwestern Mexico (Hernandez and Alanis, 1970) and a description of agronomically important races from Brazil and adjacent areas by Paterniani and Goodman (1977). A revised report (Rodriguez *et al.*, 1968) on the races of maize in Bolivia unfortunately not only fails to list typical collections but appears to be based upon collections which may no longer exist.

WIDESPREAD RACIAL COMPLEXES

Mexican dents (such as Celaya, Tuxpeño, Vándeo, etc.) and their derivatives are the most agronomically important maize races in widespread use (Fig. 1). Some of the Mexican dents apparently spread to the southeastern part of the United States about the time of European settlement. Later their descendants, the Southern dents, crossed with Northern flints from the northern U.S. to produce the widely used Corn Belt dents (Anderson and Brown, 1952; Brown and Anderson, 1947, 1948). Southern dents were widely introduced into Brazil during the 1869's, if not before, and the Corn Belt dents were widely introduced there near the beginning of the 20th Century (Brieger and Blumenschein, 1966). Lesser introductions of both types have continued. Mexican dents, as well as U.S. and Caribbean types largely derived from Mexican dent germplasm, have been widely used for breeding purposes in Brazil, Chile, and Argentina (Wellhausen, 19/8).

The earliest maize descriptions from the Caribbean appear to be of floury types (Sauer, 1960), but Mexican dents apparently were introduced there by early European colonists. There and along the northern coastal regions of South America, Mexican dents apparently intercrossed with flints from northern and/or eastern South America to form two racial complexes, the Tusóns and the Caribbean flints, which are now widely distributed throughout the tropics and subtropics (Brown, 1960, 1979; Hatheway, 1957; McClintock, 1978). The Tusóns are cylindrical semi-dents, flintier than Mexican dents but similar in ear size and shape. The Caribbean flints (Coastal Tropical Flints, Comúns, Costeños, the northernmost Catetos, etc.) are usually more conical in ear shape than the Tusóns and are even

![Fig. 1. Groups of maize races of world-wide economic importance plus the Andean and Coroico types which are regionally important (adapted from Simmonds, 1976).](image)

30
more flinty, but usually still have some soft starch (Brown and Goodman, 1977).

Other widely important races are the orange-yellow Cateto flints of southern Brazil, Uruguay, and Argentina, including the widespread Cuban Flints, and the long, narrow-eared Northern Flints and Flours from the northern U.S. and southern Canada (Brown, 1979). These two races have spread throughout the temperate regions of the world, wherever flint types of corn have been preferred. They have been particularly important in Europe, Argentina, and Uruguay. There appears to have been essentially continuous variation along the northern and eastern coast of South America from the Caribbean flints of Venezuela and the Guianas extending to the Cateto Sulinos of Argentina and Uruguay before 1900. In recent years Brazilian and Argentine breeding programs have resulted in composites and hybrids, sometimes based in part upon Mexican dent germplasm, and these have tended to displace the Catetos (Paterniani and Goodman, 1977; Wellhausen, 1978).

REGIONAL COMPLEXES

Six racial complexes (the Mexican dents, the Corn Belt dents, the Tuscons, the Caribbean Flints, the Northern Flints and Flours, and the Catetos) have achieved worldwide importance, but several groups are regionally dominant (Goodman, 1978). The long, narrow-eared Indian corns of the U.S. southwest and northwestern Mexico form one such complex. They are typically flouncy, with ears tapering at both ends. Flints are also common there as are the "popcorns", Reventador and Chapalote. This racial group's influence seems to extend as far north as the U.S., where it is represented by Hickory King, and as far south as Guatemala, where it is represented by Dzit-Bacal.

In central highland Mexico, the predominant corn types have conical ears, narrow and droopy leaves, sparsely branched but highly condensed tassels, and very weak roots. The kernels are consistently pointed, but they may be flinty (Palomero Toluqueño, from which "Japanese Hulless" popcorn was apparently derived), floury (Cacahuacintle), or slightly dent (Cónico, Cónico Norteño, Pepitilla). For a discussion of possible relationships between these races and morphologically and cytologically similar races from Guatemala, see McClintock (1978), McClintock et al. (1981), and Wellhausen et al. (1952, 1957).

The most distinctive, widespread corn in Guatemala, the race Olotón, is flinty and late maturing, with large, rounded kernels on long ears positioned high on the tall, thick stalks. It is found at mid-to-high elevations (about 2000 m) in both Guatemala and adjacent portions of Mexico. Morphologically similar races (Montaña, Amagaceño) are found at similar altitudes in Colombia and/or Ecuador.

Along the northern edge of South America and in the Caribbean, a maize with long, narrow, often flexible, ears and deep kernels contrasts with the more widely distributed Tuscons and Coastal Tropical Flints. It is known variously as Canilla (which is usually flinty) or Chandelle (more often dent). The elongate Puyas from Venezuela and Colombia seem to be derived in part from these races. In tropical maize breeding programs, Chandelle has proven to be a good source of prolificacy and low ear placement (Brown, 1979).

At mid-to-high elevations in the Andes, there is a great diversity of maize. The variations in aleurone, pericarp, and endosperm colors, in kernel shape and size, and in ear size are particularly striking, as are differences in plant morphology. Much of the variation is encompassed by three racial complexes, the North Andean flints and flours, the Cuzcooid races, and the Central Andean complex. The North Andean flints and flours (Bird and Goodman, 1977) are predominantly conically eared, with large, rounded kernels and moderate and irregular row numbers. Perhaps most typical and widely distributed of these is the race Sabinero (which can be either flint or flour) from northern Peru, Ecuador, Colombia, and Venezuela. Other closely similar races include Cacao and Cabuya from Colombia, Kelito from Ecuador, Pollo from Venezuela and Colombia, and Huacito from Venezuela.

The various Cuzco races are typical of a group of races found in southern Peru and western Bolivia near the center of the influence of the Incas and which apparently arose shortly before European conquests (Goodman and Bird, 1977). This distinctive group is usually floury with conical ears, low row numbers, and often flattened, almost coin-shaped kernels. In extreme cases, such as the race Cuzco Gigante, the kernels can be the size of large lima beans (Phaseolus lunatus L.).

The central Andean complex (Goodman and Bird, 1977) consists of races with rounded to oval ears having high row numbers of floury, often highly colored kernels. Ear size and plant height of these races is inversely correlated with altitude (often exceeding 3500 m) in which the plants are grown. The group encompasses races such as the various Chulpis, Paro, Huayleno, Capia, Shajatu, etc.

Cytological studies by McClintock (1978) and McClintock et al. (1981) suggest that influence of Andean races has extended to much of eastern South America as well. They also suggest that the majority of races of maize which evolved over most of South America were probably effectively geographically isolated from those of Mexico and Central America during much of their evolution.

In the Amazon basin east of the Andes, a single racial complex predominates. Maize cultivation is sparse throughout the area, since the population tends to rely on manioc (Manihot esculenta Crantz) as the staple crop. The typical maize of the region, originally called Coroico by Cutler (1946), is known as Piricinco in Peru, Pojoso in Bolivia, and Entrelacado in Brazil. It is characterized by long, narrow ears, often with a distinctive kernel arrangement which results in very low numbers of kernel rows, especially near the tip of the ear. It often has odd numbers of kernel rows rather than the usual even numbers found in almost all other maize. Galinat (1970) has given a detailed analysis of the structure of the cob of this type of maize.

In addition to the racial groups mentioned here, there
are many additional individual races and small groups of races. Many of these are discussed by Brown and Goodman (1977). Attempts to apply several techniques of numerical taxonomy to racial studies with maize (Bird and Goodman, 1977; Cervantes et al., 1978; Goodman, 1972; Goodman and Bird, 1977; Goodman and Paterniani, 1969) have been reasonably successful; however, caution should be exercised until it is clear that the results of such studies are indeed generally valid. McClintock et al. (1981) have studied the cytological characteristics of a large number of races of maize (summarized by McClintock, 1978); their results should help attain a much better understanding of the relationships among races. In addition, Mangelsdorf (1974) has surveyed the races of maize, not from the point of view of overall racial similarities, but from the viewpoint of “descent in a line from a common progenitor.” He described six lineages, several of which were quite speculative and several others which appeared to merit further investigation. His conclusions about the widespread white, pointed popcorn appear to be in concordance with the data of McClintock et al. (1981). The only other recent attempt at comprehensive coverage of the races of maize is that of Brandolini (1970), who listed races associated with primary and secondary centers of differentiation. Unfortunately, I am not familiar with the Russian book on maize phylogeny, classification, and breeding by Shmaraev (1975), which is not generally available.

**STATUS OF THE COLLECTIONS**

At the present time, the objective of minimizing genetic vulnerability in corn through use of exotic germplasm or of screening the samples which have been collected for resistance to specific viruses is faced with several difficulties. The problems of acquiring, maintaining, and documenting the collections must be solved before any systematic evaluation can be undertaken. Unfortunately, these problems have never been satisfactorily addressed by the USDA or other U.S. agencies.

The major germplasm banks holding accessions of most interest to plant breeders are at the Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT) and the Instituto Nacional de Investigaciones Agrícolas (INIA) in Mexico, at the Instituto Colombiano Agropecuario (ICA) in Colombia, at the Programma Cooperativo de Investigaciones en Maíz (PCIM) in Peru, and at the North Central Plant Introduction Station (NCIPS) in the U.S. The collections housed at each bank and the addresses of the banks are listed in Table 1.

A detailed documentation of the several sets of collections is not available within the National Plant Germplasm System or elsewhere. Thus, it is necessary to consult the various races of maize booklets, choose appropriate races or collections based on those descriptions, send seed requests to the appropriate bank, wait patiently for arrival (which is inversely correlated both with the size of the request and with its importance to the requestor), and then hope that germination is sufficient for the purposes needed.

By and large, the banks have done a good (and largely thankless) job, considering their inadequate funding, facilities, and staffing. However, corn is a cross-pollinating species and populations of at least 100-200 are desirable to maintain the integrity of an individual collection and to minimize genetic drift. With large numbers of collections at each Center, maintenance becomes a formidable task. Maintenance, relative to other research activities, has not been accorded a high priority at any of the Latin American Centers. As a consequence, possibly as many as 20% of the collections are no longer viable. Others will certainly be lost unless the maintenance effort is greatly expanded. Agricultural developments in some areas have been sufficiently rapid that the inviable material within the collections can no longer be re-collected. Such materials are lost forever.

In order to make available to maize breeders, geneticists, plant pathologists, and physiologists the wide array of germplasm represented among the approximately 20,000 Latin American maize collections, immediate action is imperative, and the USDA appears to have recognized the problem. It remains to be seen if any action will follow. Far too many of the collections are represented by a few grams of aged seeds. Very few collections are safeguarded by maintenance at more than one bank. Only a very small percentage of the collections have been entered into the National Plant Germplasm System, partly as a result of the complete lack of any formal provisions for maintenance of day-length sensitive maize in our plant introduction procedures. Even in those cases where duplicate samples have been sent for safeguarding to the National Seed Storage Center at Fort Collins, Colo., the samples often have not been formally entered into the acquisition listings and, as a result, can be (and in the past have been) disposed of during routine housekeeping operations.

The Maize Crop Advisory Committee has urged the USDA to determine the extent to which each bank can be relied upon to increase regionally or locally adapted collections in quantities sufficient for our needs as well as local needs, and to make preliminary arrangements for acquisition of samples in the following order of precedence, whenever possible: a) stocks of questionable viability; b) stocks no longer to be maintained by the appropriate bank; c) stocks which are inadequate for distribution but adequate for maintenance and which cannot be or are unlikely to be maintained by the bank; d) stocks which have limited uses available for distribution; e) stocks for which surplus seed is available; and f) stocks which are scheduled for increase at the appropriate bank within the near future.

Funds need to be made available to augment all these increases, whenever possible, to ensure an adequate seed increase so that a representative surplus of at least 3000 viable kernels can be packaged and shipped to the North Central Plant Introduction Center at Ames, Iowa, for distribution and guaranteed storage.

It is hoped eventually to have all of the maize accessions available from the North Central Plant Introduction Center at Ames, which could coordinate their pro-
<table>
<thead>
<tr>
<th>Bank</th>
<th>Address for seed requests</th>
<th>Telephone</th>
<th>Areas covered</th>
<th>Approx. No. collections</th>
<th>Approx. No. available for distribution</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT)</td>
<td>Director, Maize Germplasm Bank CIMMYT Apdo. Postal 6-641 Londres 40 Mexico 6, D.F. Mexico</td>
<td>905-585-4355</td>
<td>Eastern South America, Mexico, Central America, West Indies</td>
<td>13,000</td>
<td>9,000</td>
<td>Banks of first choice when appropriate region is represented. Many composite and duplicated samples.</td>
</tr>
<tr>
<td>Instituto Nacional de Investigaciones Agricolas (INIA)</td>
<td>Maize Germplasm Bank Dr. Francisco Cardenas INIA-Chapingo Apartado Postal No. 1 Chapingo, Mexico 56230 Mexico</td>
<td>905-585-4555, ext. 5311 or 905-585-6044</td>
<td>Mexico, Guatemala, West Indies</td>
<td>9,000</td>
<td>5,000</td>
<td>Bank has better representation of individual Mexican and Guatamalan collections than does CIMMYT. Sample acquisition is at best difficult, however.</td>
</tr>
<tr>
<td>Instituto Colombiano Agropecuario (ICA)</td>
<td>Dr. Manuel Torregroza C. ICA Apartado Aereo 7984 Bogota Colombia</td>
<td>57-285-5520 or 57-281-4942</td>
<td>Venezuela, Colombia, Ecuador, Bolivia</td>
<td>6,000</td>
<td>5,000</td>
<td>This bank also theoretically houses Peruvian and Chilean collections, but neither are available for distribution.</td>
</tr>
<tr>
<td>Programma Cooperativo de Investigaciones en Maiz (PCIM)</td>
<td>Dr. Antonio Manrique Ch. PCIM Universidad - La Molina Apartado 456 Lima, Peru</td>
<td>51-14-35-2035</td>
<td>Peru</td>
<td>3,000</td>
<td>1,900</td>
<td>The only source for Peruvian collections. Perhaps the most cooperative major bank.</td>
</tr>
<tr>
<td>North Central Plant Introduction Station (NCPIS)</td>
<td>USDA North Central Plant Introduction Station Iowa State University Ames, Iowa 50011</td>
<td>515-294-3265</td>
<td>Temperate World</td>
<td>3,000</td>
<td>3,000</td>
<td>Some 2400 of these are foreign. Most represent early maturing types which can be reproduced in the U.S. The remaining 690 collections represent the only comprehensive source for old U.S. open-pollinated varieties, including U.S. Indian corns.</td>
</tr>
<tr>
<td>National Seed Storage Laboratory (NSSL)</td>
<td>USDA National Seed Storage Laboratory Colorado State University Fort Collins, Colorado 80521</td>
<td>303-484-0402/6418</td>
<td>Eastern South America, Colombia, Chile, Mexico</td>
<td>2,800</td>
<td>0</td>
<td>These are standby collections, 2000 of which are Colombian and those are assigned P.I. numbers. Many of the remainder are inaccessible. The Chilean collections are unique.</td>
</tr>
</tbody>
</table>
cessing, packaging, distribution, and guaranteed storage. In most cases, seed increase will need to be made in southernmost Florida, in Hawaii, or in Puerto Rico, with the time of planting (winter, spring, summer, or fall) depending upon the ecological adaptation of the corn in the area of its origin and determined by altitude, rainfall, and daylength.

Equally important as the acquisition and increase of the collections is the publication of a catalog listing the availability of the collections, the source(s) from which they may be obtained, the costs (if any) per sample, and a brief description of each collection: local name, race name, collection site (latitude, longitude, altitude, name), source (field, market, or institution), kernel type, and color. As such collections are grown in Florida or elsewhere, short-day maturity, plant and ear height, and lodging data should also be included in such a catalog. Whenever possible, comparable data for a commercial single-cross hybrid (e.g., B73 x Mo17 or Pioneer 3369A) adapted to the central U.S. should also be included. Such a catalog needs to be compiled and published at this time for the maize germplasm bank collections for which seeds are currently available in quantities suitable for wide distribution. As collections are increased, the published catalog will need to be updated on an annual or biennial basis.

Once the materials have been increased and processed and their storage guaranteed, they need to be evaluated for a number of traits. Disease and insect resistance are considered to be of highest current concern to maize breeders. However, the acquisition, increase, and safeguarding of the collections must take immediate precedence. Despite the fact that corn is our most important feed grain, the germplasm stocks supposedly available to breeders are difficult to obtain, poorly described, totally uncataloged, and often unavailable. Virtually none of the photoperiod sensitive materials, which comprise the bulk of the important Latin American materials, are available through the U.S. germplasm system. This is, in part, due to the fact that these collections have to be maintained by hand pollination in short-day environments, such as winter nurseries in Florida, Hawaii, or Puerto Rico.

**EVALUATION OF GENETIC DIVERSITY**

At present we have only limited measures of the nature of the germplasm base for maize, even in the U.S. The reports of the Committee on Genetic Vulnerability of Major Crops (1972) and of Sprague (1971), Zuber (1975), and Zuber and Darrah (1980) certainly suggest that the U.S. germplasm base is narrow. Brown (1979) reports that, on a racial basis, those of us in the temperate regions are using only 2% of the germplasm available. On a worldwide scale, we use a somewhat greater percentage; but excluding subsistence farming, it is doubtful that as many as 5% of the races are represented in any significant amount among commercial varieties and hybrids. Perhaps 10% are represented among our breeding stocks, if token amounts of germplasm contained in materials which will never proceed beyond preliminary testing are included.

A less subjective way to appraise the diversity present among breeding stocks, base populations, or commercial hybrids or varieties is by ascertaining the variation present using genetic markers such as isozyme alleles or chromosome knobs. A comprehensive study of racial variation of chromosome knobs has recently been published (McClinock et al., 1981), but comparative data for breeding materials are unlikely to be available soon. However, a similar survey for isozyme alleles is currently underway, with about half of the typical collections of Latin America having been assayed and only a few results published (Goodman et al., 1980a, b, 1981; Goodman and Stuber, 1980; Stuber and Goodman, 1983). In addition, many inbred lines (Goodman and Stuber 1980; Stuber and Goodman, 1983) and commercial hybrids (Cardy and Kannenburg, 1982) have been surveyed using the same techniques. Thus, data are available to compare the potential variation, as observed among the races, with actual variation among widely used inbred lines. Furthermore, since the techniques involved have proved to be very useful commercially for quality control purposes (J. S. C. Smith and D. N. Duvick, personal communication), it is likely that such data will continue to accumulate.

For the present purpose, the data can be summarized very simply. Twenty-three isozyme loci have been studied, and thus for any set of materials at least 23 alleles (one per locus) must be observed. The number of alleles observed in excess of 23 serves as a simple and reasonably adequate measure of the diversity of any given set of material. In Table 2, this measure of diversity is pre-

---

**TABLE 2.** Diversity observed among sets of inbred lines and among Latin American racial stocks of maize.*

<table>
<thead>
<tr>
<th>Number of alleles observed in excess of 23 for 23 isozyme loci</th>
<th>Most popular lines</th>
<th>39 popular lines, 1980</th>
<th>406 American races</th>
</tr>
</thead>
<tbody>
<tr>
<td>1970</td>
<td>15</td>
<td>18</td>
<td>11</td>
</tr>
<tr>
<td>1975</td>
<td>18</td>
<td>11</td>
<td>27</td>
</tr>
<tr>
<td>1980</td>
<td>11</td>
<td>27</td>
<td>57</td>
</tr>
</tbody>
</table>

*a Adapted from Goodman and Stuber, 1980; Stuber and Goodman, 1983.

*b From Sprague (1971), Zuber (1975), and Zuber and Darrah (1980). The most popular lines are those accounting for more than 5,000,000 kg of seed each, approximately 1% of U.S. annual needs. Eleven lines were represented in 1970, 12 in 1975, and 6 in 1980.

*c From Zuber and Darrah (1980). Each line accounts for at least 500,000 kg of seed each, approximately 0.1% of U.S. annual needs.
sented for several sets of inbred lines that have been, or are being, used in the U.S. and for the racial materials described above. It is very apparent that, among the popular U.S. public inbred lines, there is little diversity compared to that found thus far among the races. The percentage varies from slightly more than 5% to about 15%, depending upon which set of popular lines is chosen for comparison. Even the set of 406 lines contains only about one-third that found among the races, and the 406 lines include sweet corn, popcorn, and Canadian flint lines, as well as white and yellow dents.

**SELECTING MATERIALS**

**FOR A BROADER GERMPLASM BASE**

Corresponding to the well known and widely based heterotic response which generally occurs when Reid and Lancaster lines are crossed, several different heterotic combinations have been widely used in Latin America (Wellhausen, 1978). Almost nothing is known, however, about how various Latin American races combine with widely used U.S. or other temperate materials, although the studies being conducted by Stuber (1978, unpublished) should help fill that gap. Since virtually all the races are adapted to short days, when they are grown in temperate regions under long days, flowering, if it occurs, is very late. Only the earliest tropical materials (often inherently low in yield, disease and insect resistance, and stalk quality) manage to flower by mid-season under long days. Thus, to evaluate materials holding any promise for general use, the materials need to be either evaluated in a short-day, neutral environment or crossed onto adapted materials as Stuber (1978) has done.

For many purposes, composite populations from CIMMYT or elsewhere or advanced generations of widely used tropical hybrids might be a logical starting point. However, as Wellhausen (1978) has suggested, use of such composite populations fails to provide much information about actual source identification. In addition, those materials have their own rather narrow germplasm bases.

Ideally, a breeding program builds upon an historical base, identifying successful combinations, re-sampling the useful sources (and their relatives), and taking full advantage of identified heterotic combinations. Furthermore, when specific disease or insect resistance is sought, then the widest possible range of usable materials is wanted, not several hundred samples of closely related materials. Thus, relative performance of the typical collections of the Latin American races when grown under the neutral daylength encountered under autumn conditions in southern Florida is about as valid a measure of their agronomic utility as one can obtain directly from purely Latin American collections. By appropriate choice of planting dates from early September through the end of October, virtually all collections flower within a 6-wk period prior to December 20.

As a by-product of studies on racial classification, data have been collected on such agronomically useful measures as plant and ear height, lodging, and flowering date. While yield per se has not been measured, we generally have both stand counts and the number of successful pollinations from which we can calculate an index serving as a measure of barrenness or ease of maintenance. Some 1300 typical collections have been studied. Usually the data have been collected for at least 2 consecutive yr, with non-random blocking on the basis of maturity and geographic origin.

Tables 3-6 list some 400 collections which met reasonable culling levels for plant and ear height (less than 2.4 m and 1.2 m, respectively), lodging (less than two on a one (resistant) to five (susceptible) scale), and maintenance index (better than 50% successful pollinations for plots with a minimum of 30 plants). For the latest

<table>
<thead>
<tr>
<th>Race: Collection(s)</th>
<th>Race: Collection(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mexican races</strong></td>
<td></td>
</tr>
<tr>
<td>Harinoso de Ocho Occidentales: Jal 71; Nay 29, 38</td>
<td>Camelia: Chi 404</td>
</tr>
<tr>
<td>Tabloncillo: Jal 43, 63, 100, 102, 103</td>
<td>Curaguara Grande: Chi 315</td>
</tr>
<tr>
<td>Tabloncillo Perla: Nay 12, 16, 41</td>
<td>Cristalino Chico: Chi 393</td>
</tr>
<tr>
<td>Zapalote Chico: Oax 48, 50</td>
<td>Cristalino Grande: Chi 331, 340</td>
</tr>
<tr>
<td>Celaya: Gto 28, 29</td>
<td>Dentado Comercial: Chi 416, 448</td>
</tr>
<tr>
<td>Conico Norteño: Gto 21, 23, 56, 73; Qro 2</td>
<td>Cristalino Norteño: Chi 338, 349</td>
</tr>
<tr>
<td>Boliña: Oax 68</td>
<td>Dulce Golden Bantam: Chi 335, 339</td>
</tr>
<tr>
<td><strong>Guatemalan races</strong></td>
<td></td>
</tr>
<tr>
<td>Nal-Tel Amarillo Tierra Baja: Gua 111, 220</td>
<td></td>
</tr>
<tr>
<td>Nal-Tel Blanco Tierra Baja: Gua 145, 280, 765</td>
<td></td>
</tr>
<tr>
<td>Negro de Tierra Caliente: Gua 146</td>
<td></td>
</tr>
<tr>
<td><strong>Venezuelan race</strong></td>
<td></td>
</tr>
<tr>
<td>Aragüito: Ven 568, 628</td>
<td></td>
</tr>
<tr>
<td><strong>Bolivian races</strong></td>
<td></td>
</tr>
<tr>
<td>Chake-Sara: Boy 413</td>
<td></td>
</tr>
<tr>
<td>Pojoso Chico: Boy 713</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 3.** Agronomically promising typical collections of the Latin American maize races having short-day maturities equivalent to northern U.S. Corn Belt materials.
### TABLE 4. Agronomically promising typical collections of the Latin American maize races having short-day maturities equivalent to central U.S. Corn Belt materials.

<table>
<thead>
<tr>
<th>Race: Collection(s)</th>
<th>Race: Collection(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mexican races</strong></td>
<td><strong>Bolivian races</strong></td>
</tr>
<tr>
<td>Nal-Tel: Yuc 7</td>
<td>Altiplano: Bov 903</td>
</tr>
<tr>
<td>Harinoso de Ocho: Nay 24</td>
<td>Chake-Sara: Bov 952</td>
</tr>
<tr>
<td>Reventador: Nay 39</td>
<td>Aysuma: Bov 331, 443</td>
</tr>
<tr>
<td>Tepeintle: Chs 26, 225</td>
<td>Pisankalla: Bov 760</td>
</tr>
<tr>
<td>Jala: Jal 44</td>
<td>Karapampa: Bov 422, 961</td>
</tr>
<tr>
<td>Pepitilla: Gro 3; Mor 17</td>
<td>Morado: Bov 402, 584</td>
</tr>
<tr>
<td>Vandeño: Chs 25, 31</td>
<td>Perola: Bov 437, 543, 711</td>
</tr>
<tr>
<td>Celaya: Gto 36, 69, 75, 77, 84, 88</td>
<td>Pojoso Chico: Bov 741, 800</td>
</tr>
<tr>
<td>Bolita: Oax 44</td>
<td>Cholito: Bov 309, 426, 794</td>
</tr>
<tr>
<td>Dulcillo del Noroeste: Son 57</td>
<td>Cubano Dentado: Bov 585</td>
</tr>
<tr>
<td>Bofo: Dgo 110; Nay 191</td>
<td>Cateto: Bov 330</td>
</tr>
<tr>
<td>Tablilla de Ocho: Nay 185</td>
<td>Coroico Blanco: Bov 408, 792, 1052, 1060</td>
</tr>
<tr>
<td><strong>Guatemalan races</strong></td>
<td>Coroico Amarillo: Bov 403</td>
</tr>
<tr>
<td>Nal-Tel Amarillo Tierra Baja: Gua 110</td>
<td>Coroico: Bov 784</td>
</tr>
<tr>
<td>San Marcoño: Gua 447</td>
<td>Enano: Bov 995</td>
</tr>
<tr>
<td>Negro de Tierra Frii: Gua 410</td>
<td><strong>Chilean races</strong></td>
</tr>
<tr>
<td>Negro de Tierra Caliente: Gua 101, 256, 356</td>
<td>Camelia: Chi 411</td>
</tr>
<tr>
<td>Comiteco: Gua 41e</td>
<td>Curagua: Chi 311, 314</td>
</tr>
<tr>
<td>Dzit-Bacal: Gua 130, 131, 322</td>
<td>Curagua Grande: Chi 303, 305</td>
</tr>
<tr>
<td><strong>Central American race</strong></td>
<td>Cristalino Grande: Chi 302</td>
</tr>
<tr>
<td>Amarillo Salvadoreño: Composite</td>
<td>Dentado Comercial: Chi 326</td>
</tr>
<tr>
<td><strong>West Indian race</strong></td>
<td>Dulce Evergreen: Chi 332</td>
</tr>
<tr>
<td>Early Caribbean: Mar 3, 4, 6, 9</td>
<td><strong>Eastern South American races</strong></td>
</tr>
<tr>
<td><strong>Venezuelan races</strong></td>
<td>Moroti: Pr I; RGS XIX</td>
</tr>
<tr>
<td>Araguito: Ven 760</td>
<td>Moroti Precoce: Bol I</td>
</tr>
<tr>
<td>Guaribero: Ven 733</td>
<td>Caingang: SP XIII</td>
</tr>
<tr>
<td>Sabanero: Ven 514</td>
<td>Cristal Sulino: Arg VIII; Urg VIII</td>
</tr>
<tr>
<td><strong>Colombian race</strong></td>
<td>Cristal Semi-Dentado: Pag III</td>
</tr>
<tr>
<td>Costeño: Atl 314, 328, 329</td>
<td>Canario de Ocho: Arg VI</td>
</tr>
<tr>
<td><strong>Peruvian races</strong></td>
<td>Cateto Sulino: Arg II</td>
</tr>
<tr>
<td>Morocho: Apc 67</td>
<td>Cateto Sulino Escuro: Urg V-A</td>
</tr>
<tr>
<td>Huancavelicano: Hve 53</td>
<td>Cateto: Ce I</td>
</tr>
<tr>
<td>Mochero: Lbq 5</td>
<td>Cateto Assis Brasil: RGS XIV</td>
</tr>
<tr>
<td>Piricinco: Lor 9</td>
<td>Cateto Grande: Mt I</td>
</tr>
<tr>
<td>Perla: Anc 21</td>
<td>Dente Riograndense Rugoso: RGS I, IV</td>
</tr>
<tr>
<td>Morocho Cajabambino: Caj 19</td>
<td>Dente Branco Riograndense: RGS XI, XII; SC II</td>
</tr>
<tr>
<td>Sarco: Anc 85</td>
<td>Hickory King: RGS IX</td>
</tr>
<tr>
<td><strong>Chilean races</strong></td>
<td>Tusón: Bai III</td>
</tr>
<tr>
<td><strong>Eastern South American races</strong></td>
<td>Cullí: Arg 471</td>
</tr>
<tr>
<td>Moroti: Pr I; RGS XIX</td>
<td>Bola Blanca: Arg 470</td>
</tr>
<tr>
<td>Moroítí Precoce: Bol I</td>
<td>Pisincho: Arg 482</td>
</tr>
<tr>
<td>Caingang: SP XIII</td>
<td>Canario de Ocho: Arg 465</td>
</tr>
</tbody>
</table>
TABLE 5. Agronomically promising typical collections of the Latin American maize races having short-day maturities equivalent to southern U.S. Corn Belt materials.

<table>
<thead>
<tr>
<th>Race: Collection(s)</th>
<th>Race: Collection(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mexican races</strong></td>
<td><strong>Peruvian races</strong></td>
</tr>
<tr>
<td>Olotillo: Chs 56, 81</td>
<td>Enano: MD 3</td>
</tr>
<tr>
<td>Tuxpano: Oax 9</td>
<td>Pagaladroga: Lib 24, Piu 2</td>
</tr>
<tr>
<td>Vandelio: Chs 112</td>
<td>Piricinco: Lor 11; SM 1, 19, 20</td>
</tr>
<tr>
<td></td>
<td>Alazan: Lib 34</td>
</tr>
<tr>
<td></td>
<td>Huachano: Lim 3, 14</td>
</tr>
<tr>
<td><strong>Guatemalan races</strong></td>
<td></td>
</tr>
<tr>
<td>Negro de Chimaltenango: Gua 369, 590</td>
<td>Chancayano Pintado: Lim 44</td>
</tr>
<tr>
<td>Negro de Tierra Caliente: Gua 159, 358</td>
<td>Perla: Anc 20, 24; Lib 4; Lim 8, 12, 13, 48, 53, 56</td>
</tr>
<tr>
<td>Oloton: Gua 696</td>
<td>Aleman: Hco 38</td>
</tr>
<tr>
<td>Comiteco: Gua 241</td>
<td>Arizona: Ay 11, 41; Lib 2, 16</td>
</tr>
<tr>
<td>Tepecintle: Gua 65, 597, 651, 806</td>
<td>Morado Canteño: Lim 55</td>
</tr>
<tr>
<td></td>
<td>Huarmaca: Piu 65</td>
</tr>
<tr>
<td><strong>West Indian races</strong></td>
<td></td>
</tr>
<tr>
<td>Cuban Flint: Cub 63, 65</td>
<td><strong>Bolivian races</strong></td>
</tr>
<tr>
<td>Chandelito: Cub 54</td>
<td>Argentino: Bov 920</td>
</tr>
<tr>
<td>Early Caribbean: Mar 10</td>
<td>Morado: Bov 567, 786</td>
</tr>
<tr>
<td>St. Croix: 1VC 2</td>
<td>Yungueño: Bov 362, 665, 716, 747</td>
</tr>
<tr>
<td>Tusón: Cub 57, 62</td>
<td>Cholito: Bov 707</td>
</tr>
<tr>
<td><strong>Venezuelan races</strong></td>
<td></td>
</tr>
<tr>
<td>Chirimuto: Ven 703</td>
<td>Cateto: Bov 317</td>
</tr>
<tr>
<td>Canilla: Ven 693, 874, 981</td>
<td>Pororo: Bov 806</td>
</tr>
<tr>
<td>Caracas: Ven 341, 757</td>
<td></td>
</tr>
<tr>
<td>Guaribero: Ven 572, 653</td>
<td>Coroico Blanco: Bov 406, 409, 416, 582, 787, 813, 814, 820, 990, 1001, 1050, 1057, 1059, 1061, 1062, 1075, 1076, 1117</td>
</tr>
<tr>
<td>Huevito: Ven 445, 959</td>
<td>Coroico Amarillo: Bov 405, 438, 586, 592, 637, 785, 1000</td>
</tr>
<tr>
<td>Cuba Yellow Flint: Ven 650, 664</td>
<td>Coroico: Bov 396, 987, 1040, 1047, 1064</td>
</tr>
<tr>
<td>Chandelito: Ven 460</td>
<td>Enano: Bov 1044</td>
</tr>
<tr>
<td>Costeño: Ven 453, 859</td>
<td></td>
</tr>
<tr>
<td>Tuxpano: Ven 414, 598, 767</td>
<td></td>
</tr>
<tr>
<td>Común: Ven 897</td>
<td><strong>Chilean races</strong></td>
</tr>
<tr>
<td></td>
<td>Chutucuno Grande: Chi 361</td>
</tr>
<tr>
<td></td>
<td>Harinoso Tarapaceno: Chi 418</td>
</tr>
<tr>
<td><strong>Colombian races</strong></td>
<td></td>
</tr>
<tr>
<td>Pira: Cun 327</td>
<td><strong>Eastern South American races</strong></td>
</tr>
<tr>
<td>Caracas: Cor 334, 338, 342; Mag 399, 408</td>
<td>Moroti: Bol II; MT II, V; Pag V, VI</td>
</tr>
<tr>
<td>Cabuya: San 317</td>
<td>Morotí Guapi: Pag VI-A</td>
</tr>
<tr>
<td>Común: Cal 353; Val 374</td>
<td>Caingang: Pr III</td>
</tr>
<tr>
<td>Negro: Cho 339; Mag 321</td>
<td>Cristal: MG III</td>
</tr>
<tr>
<td>Puya: Mag 322, 355; San 349</td>
<td>Cateto: Des 1; SP VII</td>
</tr>
<tr>
<td>Caqueteño: Caq 305, 317</td>
<td>Dente Riograndense Rugoso: RGS II</td>
</tr>
<tr>
<td></td>
<td>Dente Riograndense Liso: RGS V, VI; SC I</td>
</tr>
<tr>
<td><strong>Ecuadorian races</strong></td>
<td></td>
</tr>
<tr>
<td>Gallina: Ecu 929</td>
<td>Dente Paulista: SP III</td>
</tr>
<tr>
<td>Cubano Amarillo Duro: Ecu 326, 370, 653, 770, 904, 926, 957, 975</td>
<td>Dente Branco Riograndense: RGS X</td>
</tr>
<tr>
<td>Cubano Tusón: Ecu 542, 659, 660, 843, 856</td>
<td>Semi-Dentado Riograndense: RGS XV, XVI</td>
</tr>
<tr>
<td>Cubano Cateto: Ecu 330, 339</td>
<td>Semi-Dentado Paulista: SP IX</td>
</tr>
<tr>
<td>Tuxpano: Ecu 629</td>
<td>Cravo Riograndense: RGS VII, VIII</td>
</tr>
<tr>
<td>Yunquillano: Ecu 710, 886, 887</td>
<td></td>
</tr>
</tbody>
</table>

37
materials (Table 6), the plant height culling level was lowered to 2.1 m. No claim is made that these collections are the most agronomically desirable, but they probably include the most desirable typical collections. Also, they represent a vast array of largely untapped germplasm, grouped by appropriate maturities, and can serve as probes to sample the germplasm banks for still more desirable collections. To a very limited extent, this has already been done with the various Coroico collections. Wolf et al. (1972) identified a particular trait associated with this race and obtained all the typical Coroico, Coroico Blanco, and Coroico Amarillo collections, not just the few that are usually distributed by ICA to routine inquiries. Several of the additional collections are superior to those listed in "Races of Maize in Bolivia" (Ramirez et al., 1960).

The entries in Tables 3-6 are organized geographically from Mexico to Chile, with eastern South America (the area once covered by the now defunct Brazilian germplasm bank at Piracicaba) listed at the end. Collections with Roman numerals represent composite collections described by Paterniani and Goodman (1977). The only other composite collection listed is that of Amarillo Salvadoreñio, although composite collections are available for most races.

All of these collections are not equally desirable for a specific purpose. A midwestern dent breeder looking for yield potential would probably be sorely disappointed with Aragüito, Chococeño, Enano, Mochero, Nap-Tel, Pisankalla, Pisinchó, and Zapalote Chico, yet a popcorn breeder might be quite pleased with several of them. A virologist might be as likely to find disease resistance in that set as in another equally diverse set. Clearly, intelligent use of these tables requires, at a minimum, consulting the appropriate "Races of Maize" booklets for the brief descriptions of the races themselves. Certainly, races having a large proportion of their typical collections included in Tables 3-6 are more promising than those that do not. In addition, races having several entries each in more than one of the tables are probably better bets than those restricted to a single table. While there may be some tendency for the most productive collections to fall into Tables 5 and 6, only in the earliest materials in Table 3 is there a high concentration of relatively low yielding materials, and even there the percentage appears to be 10% or less.

It should be recognized that many of the Argentine, Brazilian, and Chilean collections indirectly represent exports from either the U.S. and/or Europe, and these collections represent a fair amount (36%) of Table 3, which lacks any entries from the West Indies, Colombia, Ecuador, or Peru. Table 4 represents a much more diverse set of materials and lacks representation from only Ecuador.

Whether genetic diversity is measured by the number of races or racial groups of current commercial impor-

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**TABLE 6.** Agronomically promising typical collections of the Latin American maize races having short-day maturities equivalent to deep southern U.S. materials.

<table>
<thead>
<tr>
<th>Race</th>
<th>Collection(s)</th>
<th>Race</th>
<th>Collection(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mexican races</strong></td>
<td>Tehua: Chs 29, 159, 161, 204</td>
<td><strong>Ecuadorian races</strong></td>
<td>Candela: Ecu 630</td>
</tr>
<tr>
<td></td>
<td>Comitee: Chs 86</td>
<td></td>
<td>Cubano Amarillo Duró: Ecu 327</td>
</tr>
<tr>
<td><strong>Guatemalan races</strong></td>
<td>Negro de Chimaltenango: Gua 31</td>
<td></td>
<td>Cubano Cateto: Ecu 877</td>
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<td></td>
<td>Olotón: Gua 383</td>
<td></td>
<td>Tuxpeño: Ecu 942</td>
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<td></td>
<td>Tuxpeño: Gua 456</td>
<td></td>
<td>Yunquillano: Ecu 855</td>
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<td><strong>West Indian races</strong></td>
<td>Haitian Yellow: Hti 11, 13, 14</td>
<td><strong>Peruvian races</strong></td>
<td>Perla: Lim 4</td>
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<td></td>
<td>Chandelle: Cub 68</td>
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<td>Cateto: Bov 1083</td>
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<td><strong>Venezuelan races</strong></td>
<td>Canilla: Ven 604</td>
<td>Pororo: Bov 583, 587</td>
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<td></td>
<td>Caríaco: Ven 408, 631</td>
<td>Coroico Blanco: Bov 1034, 1082</td>
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<td></td>
<td>Chandelle: Ven 352, 432, 489, 717</td>
<td>Coroico Amarillo: Bov 999, 1042, 1077</td>
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</tr>
<tr>
<td></td>
<td>Costeño: Ven 947</td>
<td>Coroico: Bov 417, 1046, 1051, 1063, 1065, 1119</td>
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<td></td>
<td>Puya Grande: Ven 495</td>
<td>Enano: Bov 993</td>
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<tr>
<td><strong>Bolivian races</strong></td>
<td>Pira: Boy 462; Tol 405</td>
<td><strong>Chilean race</strong></td>
<td>Chulpi: Chi 429</td>
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<tr>
<td></td>
<td>Amauceño: Ant 343</td>
<td><strong>Eastern South American races</strong></td>
<td>Avatí Pichingó: Br 2760, 2776, 2785, 2799</td>
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<tr>
<td></td>
<td>Común: Val 390</td>
<td></td>
<td>Avatí Pichingó lhí: Br 2766; Pag 169</td>
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<td></td>
<td>Costeño: Bol 375; Cor 320: Mag 350</td>
<td></td>
<td>Moroti: Pag VII</td>
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<td></td>
<td>Puya: San 346</td>
<td></td>
<td>Caingang: SP XIV</td>
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<td></td>
<td>Chococeño: Cho 306, 348, 356; Nar 392</td>
<td></td>
<td>Cristal Semi-Dentado: Pag II</td>
</tr>
</tbody>
</table>

38
tance or by the numbers of alleles represented among our breeding materials, it is clear that only a small proportion of the total variation available is currently being utilized. In addition, the status of the maize collections at the major banks is much less than ideal and not likely to improve, since germplasm maintenance is not a high priority relative to other efforts at the major international centers. Furthermore, USDA emphasis is currently on "germplasm enhancement"; for maize, however, the USDA has failed to acquire any substantial portion of the critical Latin American collections. Almost no systematic evaluation of such collections has been completed, so there is very little basis for "germplasm enhancement" to proceed. The limited evidence available to us suggests that when an array of Latin American collections is evaluated, the resulting elite collections represent a wide array of germplasm, much of which has never been utilized (Stuber, 1978; Tables 3-6). Fortunately, most such materials are still available from the Latin American banks listed in Table 1.

How long can the world continue to rely on the goodwill of the four major maize germplasm banks (CIMMYT, ICA, INIA, and PCIM) for the maintenance and supply of such materials? The day may soon come when repeated requests for specific seed stocks, such as those listed in Tables 3-6, will remain unanswered, since maintenance of the collections is by no means guaranteed. D. N. Duvick in his presentation, "Genetic Diversity in Major Farm Crops on the Farm and in Reserve," at the 13th International Botanical Congress in Sydney, Australia (August 18, 1981), stated: "I reserve my most severe condemnation for those government agencies ultimately responsible for funding of our germplasm collections. Our national stinginess in collecting, storing, renewing, and describing the collections is inexcusable, not only in regard to our national obligations, but also in regard to our responsibility to the entire world."

LITERATURE CITED


Paterniani, E. and M. M. Goodman. 1977. Races of maize in Brazil and adjacent areas. CIMMYT, Mexico City. 95 pp.


Genetic Improvements in Maize Hybrids
During the Past 50 Years

Donald N. Duvick

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The data in this paper were presented as part of a symposium at the 1981 Crop Science Society of America Annual Convention and will be included in a special publication of the presentations in that symposium.

ABSTRACT


Genetic yield gains of maize (Zea mays) in central Iowa have averaged 0.9 quintals/hectare/year over the past 50 yr as measured by trials conducted in 1978-1980 on a series of 47 hybrids released at intervals from 1934 to 1978 and an open pollinated cultivar of 1930 vintage. Genetic yield gains were accompanied by large and consistent improvements in resistance to root lodging, stalk lodging, premature plant death, feeding of second generation European corn borer, and barrenness. The new hybrids were superior to the old hybrids in low-yield as well as in high-yield environments. Plant and ear height, leaf area index, and maturity were changed little or not at all over the years.

U.S. maize (Zea mays L.) yields per unit of surface area have increased at a reasonably steady rate for the past 50 yr (Fig. 1). Scientists and politicians wonder if this trend can continue. Scientists have attempted to assign quantitative values to the interacting factors which produce the final yield: factors such as weather, fertilizers, herbicides, and hybrid genotype. This report is intended to provide data that will help scientists estimate the amount of the genetic contribution to yield gains of maize. The report is similar to but not the same as one I presented several years ago (Duvick, 1977). The present report includes more hybrids and considers more traits.

![Graph showing maize yields](image-url)

**Fig. 1.** U.S. maize grain yields (quintals/hectare) for period of 1930-1981. Regression calculated on basis of 1930-1980 data.
MATERIALS AND METHODS

The report summarizes three separate experiments. In experiment one, 47 hybrids released by Pioneer Hi-Bred International, Inc. at intervals from 1934 to 1978 were reproduced from inbred seed that had been maintained in cold storage. All hybrids were widely grown in central Iowa in their day. In addition, a central Iowa farmer’s selection of an open pollinated cultivar, Reid’s Yellow Dent, arbitrarily was assigned a 1950 release date on the assumption that it represented Iowa corn of that period. The 48 entries were grown in small-plot yield trials in central Iowa for 3 yr (two locations in 1978, three locations in 1979, and four locations in 1980), at three densities per location and one replication per density. Plots consisted of two rows, 531 cm long and 76 cm wide. Densities were 30,000, 47,000, and 64,000 plants/hectare (ha). Plots were over-planted and thinned to the required density. The low density was typical of plant populations in the 1930’s, the medium density was typical of central Iowa in the 1970’s, and the high density is higher than is generally recommended at this time. All plots were harvested with a combine specifically modified for harvesting maize yield trials. Scores or counts were taken for several traits. All scores were on a one to nine basis, with nine representing the most favorable state.

Experiment two was designed to remove any bias that may be introduced into experiment one by the fact that in the 1960’s single cross hybrids replaced double crosses. All hybrids in experiment two were single crosses of the most widely used inbred lines of each decade from the 1930’s through the 1970’s. Five unre-plot design at three densities with two replications per cross (a total of 50) were planted in split-split-split-representative of the decade. The five sets of single crosses of the most widely used inbred lines of each decade, were crossed in diallel to give 10 single crosses. All hybrids in experiment two were single cross hybrids released by Pioneer Hi-Bred International, Inc. at intervals from 1934 to 1978, three locations in 1979, and four locations in 1980), at three densities per location and one replication per density. Plots consisted of two rows, 531 cm long and 76 cm wide. Densities were 30,000, 47,000, and 64,000 plants/hectare (ha). Plots were over-planted and thinned to the required density. The low density was typical of plant populations in the 1930’s, the medium density was typical of central Iowa in the 1970’s, and the high density is higher than is generally recommended at this time. All plots were harvested with a combine specifically modified for harvesting maize yield trials. Scores or counts were taken for several traits. All scores were on a one to nine basis, with nine representing the most favorable state.

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RESULTS

Experiment one. Several traits were highly and positively correlated with year of hybrid release. These were grain yield, resistance to root lodging, resistance to stalk lodging, stay-green (high scores indicate resistance to premature death in September), and ears per 100 plants (Table 1). Resistance to ear dropping was significantly but not very highly correlated with year of release, but at the two lower plant densities only.

Several traits were not significantly correlated with year of release (Table 1). These included: a) plant and ear height; b) maturity as measured by growing degree units to 50% pollen shed and by grain moisture; and c) seedling vigor.

Leaf angle (high scores indicate upright leaf habit) is decidedly more upright in the newer hybrids (Table 2). There was a high correlation between year of release and tolerance to second generation European corn borer (Ostrinia nubilalis Hubner). Correlations were not very high for year of release with: a) tolerance to first generation corn borer, and b) resistance to northern corn leaf blight caused by Helminthosporium turcicum Pass. There was no directional change through the years in leaf area index or its components, i.e., leaf number, length, and width.

<table>
<thead>
<tr>
<th>TABLE 1. Phenotypic correlations of several hybrid traits with year of release for period 1934 to 1978 (experiment one).*</th>
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<tbody>
<tr>
<td>Trait</td>
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<tr>
<td>-------------------------</td>
</tr>
<tr>
<td>Grain yield (q/ha)</td>
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<tr>
<td>Percent not root-lodged plants</td>
</tr>
<tr>
<td>Percent not stalk-lodged plants</td>
</tr>
<tr>
<td>Stay-green score</td>
</tr>
<tr>
<td>Ears/100 plants</td>
</tr>
<tr>
<td>Percent plants with no dropped ears</td>
</tr>
<tr>
<td>Plant height (cm)</td>
</tr>
<tr>
<td>Ear height (cm)</td>
</tr>
<tr>
<td>Growing degree units to 50% anthesis</td>
</tr>
<tr>
<td>Grain moisture percent</td>
</tr>
<tr>
<td>Seedling vigor score</td>
</tr>
</tbody>
</table>

* Means of 48 entries; nine locations, one replication/density; except stay-green taken at eight locations, plant and ear height at seven locations, and growing degree units at five locations.

Significant at 0.05 (single asterisk) and 0.01 (double asterisk) levels of probability.
Rates of gain for some traits varied according to plant density (Table 3). Thus, yield gains (linear) were 0.6 q/ha/year at the lowest plant density but 1.2 q/ha/year at the highest density. Resistance to root lodging showed the greatest gain at the high density, whereas resistance to ear dropping showed the least gain at the high density.

Experiment one can be compared with the similar experiment I conducted in 1972-73. Eleven hybrids were included in both experiments. For both there was a positive regression of yield on year of introduction but the overall yield level was lower in 1978-80 and the rate of gain was higher (Fig. 2). Thus, growing conditions influence the size of estimated rate of gain due to genetics.

Yield gains for experiment one (1978-1980 data) averaged across densities and environments were best described by a curvilinear ascending regression line (Fig. 3). The yield of the open pollinated cultivar was well below yields for hybrids and would better fit about a 1910 vintage cultivar. The single cross hybrids seemed to continue the trend established by the double crosses. One can speculate as to whether the gains after 1960 could have been achieved as shown if single crosses had not been used. The broken line in Fig. 3 extrapolates yields of the double crosses from about 1963 and indicates that yield gains probably would have continued if double crosses had been used up to the present. However, for double crosses the rate of gain might have been linear rather than curvilinear and therefore 1980 yields might have been lower on average by about 10 q/ha (10-12%).

When regressions of yield on year of release were plotted for each plant density, using best-fit regressions for each density, the nature of the interaction between density and rate of gain is readily apparent (Fig. 4). Rate of gain was least at the low density and in fact was apparently leveling off. It was linear at the medium density and rising at an ascending rate at the high density. This graph would predict that the greatest yield could be achieved from the newest hybrids by planting them at the high density, higher than now recommended for central Iowa. Fig. 4 also shows that although the old hybrids make their highest yields at
Fig. 3. Mean grain yields (quintals/hectare) of 48 maize entries regressed on year of entry release for experiment one. Square with enclosed dot indicates open pollinated; solid dots indicate double cross hybrids; circles with enclosed dot indicate single cross or modified single cross hybrids. Quadratic regression (Q) based on all 48 entries for period 1930-1980; linear regression (L) based on the 29 non-single-cross entries for period 1930-1963. Dashed line projects linear regression to 1980. Tests were made at three corn plant densities and for 3 yr.

Fig. 4. Regressions of maize grain yield (quintals/hectare) at three plant densities on year of release for the 48 maize entries of experiment one involving 3 yr and nine environments for tests.

Fig. 5. Regressions of percent not root-lodged maize plants at three plant densities on year of release for 48 maize entries of experiment one involving 3 yr and nine environments for tests.

Fig. 6. Regressions of percent not stalk-lodged maize plants at three plant densities on year of release for the 48 maize entries of experiment one involving 3 yr and nine environments for tests.
low densities, they cannot yield as much as the new ones even at low densities. Calculations of genetic yield gain through the years credit the earliest hybrids with their yield at the low density and the newest hybrids with their yield at the medium density. Average genetic gain over 50 yr then calculates to 0.9 q/ha/year.

Best-fit regressions for root lodging (Fig. 5) also show that for this trait the advantage of the new hybrids increases as plant densities increase and that old hybrids are inferior in root strength, even at the low density typical of the 1930's, compared to the newer hybrids.

Rates of gain for resistance to stalk lodging (Fig. 6) did not interact with plant densities as strongly as did those for yield and for root lodging. However, best-fit regressions indicate progress at an increasing rate if measured at medium and high densities but only straight line progress if measured at low densities.

Rates of improvement in stay-green scores (Fig. 7) showed no interaction with densities and indicate progress through the years, although relatively low $r^2$ values show that there was considerable variation among hybrids within a given era.

Tolerance to infestation with second generation corn borer has increased on the average through the years as measured in several ways (Fig. 8). Scores following natural infestation in 1978 and artificial infestation in 1979 and 1980 agreed in indicating highly significant positive gains through the years. An additional examination of inches of tunnels in split stalks (D. Guthrie, personal communication) also showed significant reductions in tunneling through the years.

The newer hybrids are revealed to be moving towards prolificacy when tested at the low density (Fig. 9), whereas at medium and high densities this trait is hidden and instead the new hybrids are shown as becoming progressively less prone to barrenness.

Good roots, sound stalks, number of ears per plant, high stay-green score, and good tolerance to second generation European corn borer had very high correlations with grain yield (Table 4). They also were very highly correlated with year of release (Table 1). In contrast, plant and ear height, growing degree units to 50% shed, and grain moisture percentage were not highly correlated with grain yield (Table 4) nor with year of release (Table 1). It would seem likely that improvements in root strength, stalk strength, stay-green, and resistance to barrenness have been important determinants in increasing yield potential and yield stability of the newer hybrids. This hypothesis is reinforced by the fact that at low planting rates, when stresses on roots, stalks, plant health, and ear producing ability are least, advantage of the new hybrids was least.

In order to answer, "Can the new hybrids yield as well as the old ones in low yield environments?", data for the
48 entries were put into four subsets grouped according to year of hybrid release. Yields of subsets at each location were regressed on yields of all 48 entries at each location (Fig. 10). Average yields per location ranged from 45 to 80 q/ha. Regressions indicated that the newer the subset the higher the yields at all yield levels; i.e., at no time did the older hybrids out-yield the newer ones even in low-yield environments. Advantage of the newer hybrids was greatest at high-yield levels, in agreement with other experiments and observations in maize as well as in other crops (Austin et al., 1980; Russell, 1974).

The major yield-limiting factors at the low-yield locations of experiment one were heat and drought. Fertilizer applications at all locations were adequate to high. An additional set of experiments has examined the effect of low vs. high rates of nitrogen fertilizer on comparative yields of old and new hybrids (Fig. 11). Four hybrids, representing four eras from 1940 through 1970, were grown at three nitrogen rates at five locations and three replications/location in 1 yr. The interaction, hybrids x nitrogen rates, was not significant; the two newest hybrids out-yielded the two oldest ones at low as well as at high levels of nitrogen.

A similar study, involving two fertility levels and about 20 hybrids and open pollinated cultivars, has been performed by R. M. Castleberry (personal communication).
His data show that DeKalb hybrids also have consistently improved in yield over the past 50 yr, that the gains are expressed at low as well as at high levels of soil fertility, and that the advantage of the new hybrids is greatest at high yield locations.

Experiment two, in which single crosses representing five decades of corn breeding were compared, gave results similar in most respects to those of experiment one. Yields improved fairly consistently; relative advantage of the newer genotypes was greatest at the high plant density (Fig. 12). Root strength improved in each decade and the greatest relative advantage of the new genotypes again was at the highest plant density (Fig. 13). Stalk strength improved rapidly at first but gains were less after 1950 (Fig. 14). Also, rates of gain in stalk strength did not interact with densities. The decrease in rate of stalk strength improvement after 1959 contrasts with the consistent improvement in stalk strength of the commercial hybrids in experiment one. Number of ears per plant increased at a steady rate (Fig. 15) and, as with experiment one, the newer genotypes were prolific at the low density and resisted barrenness at the medium and high densities.

Experiment three, in which inbreds alone were tested in tandem with their single crosses in experiment two, showed that the inbreds improved through the years in most of the same traits that were improved in the single-cross sets and in the commercial hybrids (Table 5). Grain yield, resistance to root lodging, resistance to stalk lodging, and ears per plant all showed highly

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Fig. 12. Comparison of mean grain yields (quintals/hectare) of five maize single cross diallels of experiment two based on decade of use. Tests involved 3 yr with two locations per year at three plant densities and two replications per location.

Fig. 13. Comparison of mean percent not root-lodged maize plants of five single cross diallels of experiment two based on decade of use. Tests involved 3 yr with two locations per year at three plant densities and two replications per location.

Fig. 14. Comparison of mean percent not stalk-lodged maize plants of five single cross diallels of experiment two based on decade of use. Tests involved 3 yr with two locations per year at three plant densities and two replications per location.

Fig. 15. Comparison of mean number of ears per 100 maize plants of five single cross diallels of experiment two based on decade of use. Tests involved 3 yr with two locations per year at three plant densities and two replications per location.
TABLE 5. Phenotypic correlations of several maize inbred traits with decade of use for the period 1930's through 1970's (experiment three).a |

<table>
<thead>
<tr>
<th>Trait</th>
<th>Correlation coefficient (r)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>30,000</td>
</tr>
<tr>
<td>Grain yield (q/ha)</td>
<td>.68**</td>
</tr>
<tr>
<td>Percent not root-lodged plants</td>
<td>.48*</td>
</tr>
<tr>
<td>Percent not stalk-lodged plants</td>
<td>.59**</td>
</tr>
<tr>
<td>Stay-green score</td>
<td>.28</td>
</tr>
<tr>
<td>Ears/100 plants</td>
<td>.69**</td>
</tr>
<tr>
<td>Northern corn leaf blight score</td>
<td>—</td>
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<tr>
<td>Percent plants with no dropped ears</td>
<td>.13</td>
</tr>
<tr>
<td>Plant height (cm)</td>
<td>.02</td>
</tr>
<tr>
<td>Ear height (cm)</td>
<td>-.25</td>
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<tr>
<td>Growing degree units to 50% anthesis</td>
<td>-.46*</td>
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<tr>
<td>Grain moisture percent</td>
<td>-.23</td>
</tr>
<tr>
<td>Seedling vigor score</td>
<td>.32</td>
</tr>
</tbody>
</table>

a Means of 25 inbreds (five/decade); 3 yr, two locations/year, two replications/location; except three replications only for northern corn leaf blight scores.

b Significant at 0.05 (single asterisk) and 0.01 (double asterisk) levels of probability.

significant and positive correlations with decade of use of the inbreds. Stay-green scores and resistance to ear dropping showed only low, non-significant, positive correlations with decade of use. Plant height and maturity measurements showed no or small correlation with decade of use, as with the commercial hybrids.

It is instructive to compare changes in inbreds with changes in their single crosses. In all measured traits, inbreds and their single crosses changed through the years in the same direction but usually at different rates. Resistance to root lodging increased at a faster rate in single crosses than in inbreds, but the single crosses started from a much lower base (Fig. 16). Likewise, resistance to stalk lodging increased at a faster rate in single crosses than in inbreds, but single crosses again started from a much lower base (Fig. 17). Ears per plant gave the opposite result, i.e., inbreds were at first much more barren than single crosses and then improved in resistance to barrenness at a faster rate (Fig. 18).

Plant height and maturity are two traits in which
heterosis normally is expressed very clearly. Plant height changed little through the years (Fig. 19), either in inbreds or single crosses, and the heterotic advantage of the single crosses changed very little as well, although there seemed to be a slight trend toward less heterosis for height. Maturity, measured as growing degree units to anthesis, shifted towards earlier flowering for inbreds and single crosses (Fig. 20). The relationship between the two stayed relatively constant, although as with height there was a trend towards less heterotic advantage of the single crosses.

Inbreds and single crosses both increased in yield through the years, but single crosses gained at a higher rate than inbreds (Fig. 21). Linear regressions indicate that single crosses gained at a rate of 0.8 q/ha/year, whereas their inbred parents gained at a rate of only 0.5 q/ha/year (Table 6). If heterosis is calculated as yield of the single cross in percent of mid-parent means, there is no indication of increase in amount of heterosis; in fact the correlation of heterosis percentage with decades is negative. On the other hand, if heterosis is calculated as units of yield advantage of single cross over mid-parent
means, there is a fairly consistent increase in heterosis through the decades, averaging 0.4 q/ha/year.

**DISCUSSION**

Results of this study agree closely with those of earlier reports by Russell (1974) and Duvick (1977) in showing that genetic yield capacities of hybrids for central Iowa have increased steadily over the past 50 yr and that these increases in yielding ability have been accompanied by marked improvements in resistance to root lodging, stalk lodging, premature death, and barrenness. The present study additionally shows that the newer hybrids are greatly improved in tolerance to heat, drought, low soil fertility, and second generation European corn borer. The present study shows that increases in yielding ability have not been accompanied by large changes in plant height, leaf area index, or maturity, although there has been a change toward hybrids with a more upright leaf habit. Average improvements in resistance to northern corn leaf blight and in tolerance to first generation European corn borer are measurable but not large. Inbreds also have improved markedly through the years in most of the traits that were improved in the hybrids.

Finally, tests with representative inbreds and single crosses and the plotted regression of yields of the 44 yr sequence of commercial hybrids suggest that improvements in commercial hybrids through the years have been relatively independent of the switch to single cross hybrids in the 1960’s. The gains in yielding ability and other desirable traits of the commercial hybrids seem to be due in large part to improvements in general combining ability of the inbreds used to make these hybrids. However, the rate of improvement in yield does seem to have increased since the switch from double cross to single cross hybrids in the mid-1960’s.

**LITERATURE CITED**


Identities of New Spiroplasmas Reported in Maize in the United States

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We gratefully acknowledge the excellent technical assistance of Marilyn Hale and Maggie Chang.

This research was performed under cooperative agreement No. 58-32U4-2-365 between the U. S. Department of Agriculture and the University of Maryland.

Please address requests for copies to Dr. R. E. Davis, Plant Virology Laboratory, Plant Protection Institute, U. S. Department of Agriculture, Beltsville, Md. 20705.

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ABSTRACT


A new outbreak of a corn stunting disease has been reported in California. In the present study completed in July 1982, we identified spiroplasmas, isolated by others from maize (Zea mays) plants with this disease, as strains of honey bee spiroplasma (serogroup I, subgroup B). However, we were unable to isolate honey bee spiroplasma strains from such diseased plants from California; instead, we isolated strains of the corn stunt spiroplasma (CSS) (serogroup I, subgroup C). It is not known what accounts for the difference in results between the two laboratories. Resolution of the discrepancy is important for understanding the spread of pathogenic spiroplasmas, for applying optical microscopy as an aid to corn stunt disease diagnosis, and for studying possible genetic exchange between spiroplasmas in nature.

Until a relatively few years ago, reference was commonly made to several "strains" of corn stunt disease including the Ohio corn stunt, the Louisiana corn stunt, the Mississippi corn stunt, the Rio Grande corn stunt, and the Mesa Central corn stunt. More recently, the complex of conditions, once all termed corn stunt, has been found to comprise several distinct diseases. These several corn stunting disorders are now known or presumed to be caused by several quite different pathogens, including viruses, a mycoplasmalike organism, and a spiroplasma (Ayers et al., 1978; Bascoppe, 1977; Bradfute et al., 1980; Bradfute and Robertson, 1977; Chen and Davis, 1979; Chen and Liao, 1975; Davis et al., 1972; Gordon, 1977; Gordon and Nault, 1977; Nault and Bradfute, 1979). The term "corn stunt" is now reserved for reference to what has been, and often still is, commonly known as the Rio Grande corn stunt (Davis et al., 1981). It is in association with this disease that spiroplasmas were first discovered (Davis and Worley, 1973; Davis et al., 1972), and the spiroplasma responsible for this disease is known as the corn stunt spiroplasma (CSS) (Chen and Liao, 1975; Davis et al., 1981; Davis and Worley, 1973; Williamson and Whitcomb, 1975).

Several strains of the CSS are referred to in current literature. These may differ in aspects such as geographic origin or hosts from which different strains were first isolated. However, all spiroplasmas that are termed strains of the CSS are similar to one another in serological and biochemical properties, and all derive directly or indirectly from corn (Zea mays L.) plants exhibiting symptoms of Rio Grande corn stunt or from insect vectors carrying the Rio Grande corn stunt pathogen. Those strains of CSS studied in detail thus far also share a very high degree of DNA homology with one another (Lee and Davis, 1978).

Because of their serological similarities with one another and their distinctness from other spiroplasmas, all strains of the CSS are considered members of a single serological subgroup (Davis et al., 1979; Williamson et al., 1979). This subgroup is one of several distinct subgroups that have been placed within a single major spiroplasma serogroup (serogroup I). Other subgroups within that serogroup include one containing strains of Spiroplasma citri and one containing a cluster of mutually very closely related strains isolated from bees and from flower surfaces (Daniels et al., 1980; Davis, 1978; Davis and Lee, 1982; Davis et al., 1976; Davis et al., 1979; Mouches et al., 1979; Williamson et al., 1979). In 1980, Davis and Lee (1982) proposed before the International Organization of Mycoplasmology that CSS strains and honey bee-flower spiroplasma strains be recognized and
Primary isolations of spiroplasmas from plants. Young stem tissue was surface sterilized in 70% ethanol (2 min) followed by 20% Clorox (5 min) and subsequent rinsing in three changes of sterile distilled water. A piece of tissue, about 0.5 cm², was then chopped aseptically in 9 ml of sterile liquid medium LD8A (Chen and Davis, 1979; Lee and Davis, 1984), and the resulting brei was passed through a sterile 0.45 µm pore diam Acrodisc filter. Solid LD8A agar (0.65%) medium in 6 cm Petri plates was seeded with 0.1 ml of a 1:10 dilution of filtrate. Five-ml portions of sterile liquid medium LD8A were each seeded with 0.5 ml aliquots of filtrate. Cultures were incubated at 31 or 37 C. Subcultures of primary cultures were made after 3 days. Primary cultures and first stage subcultures were observed by dark-field microscopy for growth of spiroplasmas.

Spiroplasmas. Strain 105, kindly supplied by J. W. Kloepper, was isolated by Kloepper et al. (1982b) from diseased corn collected in a field in Kings County, California. Strain PU8-17 was previously isolated in our laboratory from a maize plant having symptoms of Rio Grande corn stunt disease (Davis, 1979); the plants had been collected in 1978 from Peru (Nault et al., 1979) and kindly supplied by L. R. Nault (The Ohio State University, Ohio Agricultural Research and Development Center, Wooster). Strain 1747 [American Type Culture Collection (ATCC 29051)], isolated by Chen and Liao (1975) from a greenhouse-grown maize plant with symptoms of Rio Grande corn stunt, was obtained from the ATCC, Rockville, MD. Strains G1 and BW were isolated in our laboratory from flowers of *Bidens pilosa* L. and of *Tilia americana* L. (basswood), respectively (Davis, 1978b; Davis 1979).

Polycrylamide gel electrophoresis (PAGE) of cellular proteins. Organisms triply cloned by a procedure described elsewhere (Davis, 1978a) were grown in broth medium LD8 (Chen and Davis, 1979; Lee and Davis, 1978) or LD8A at 31 C. Spiroplasma strains G1, BW, and 105 were grown in LD8 for 24 hr; strains 1747 and PU8-17 were grown for 4 days and strain SC51-1 for 5 days in LD8A. Previous work has shown that spiroplasma strains BW and G1 cultured in medium LD8 or in LD8A yield identical or nearly identical PAGE patterns of cellular proteins (Davis, unpublished). The

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**MATERIALS AND METHODS**

Source plants for spiroplasma isolations. Source plants in two separate batches were supplied by J. W. Kloepper (formerly Department of Plant Pathology, University of California, Berkeley). The first batch consisted of five maize plants; four of the plants exhibited symptoms of disease and one was healthy in appearance. Plants in this first batch had been collected in maize fields in California (Kloepper et al., 1982). These reports indicate that some of these spiroplasmas may differ in some important respects from CSS. For example, Kloepper et al. (1982a, b) have reported the isolation, from stunted maize with reddened leaves in California, of a spiroplasma remarkably different from CSS. These authors also reported the isolation of the same spiroplasma from individuals of the leafhopper *Dalbulus maidis* (DeLong & Wolcott), a known vector of the CSS.

In cooperation with Kloepper and colleagues, we have attempted to verify the identity of this spiroplasma and to isolate and identify spiroplasmas from samples of maize sent to us from Kloepper's laboratory. The results we reported at the Second International Maize Virus Disease Colloquium and Workshop (2-6 August 1982) represent a part of our studies on the ecology of the corn stunt spiroplasma.

**TABLE 1. Isolation of spiroplasmas from maize plants from California.**

<table>
<thead>
<tr>
<th>Plant batch and shipment</th>
<th>Symptoms in plants</th>
<th>Number of plants</th>
<th>Number of plants with spiroplasmas in expressed juice</th>
<th>Number of plants yielding spiroplasma cultures at 31 C</th>
<th>Number of plants yielding spiroplasma cultures at 37 C</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Rio Grande</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>Symptomless</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>N.D.</td>
</tr>
<tr>
<td>B</td>
<td>Rio Grande</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

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*Plants were kindly supplied by J. W. Kloepper (Plant Pathology Department, University of California, Berkeley) in two separate batches. See text for details.


*N.D. signifies test for spiroplasma culture was not done.
organisms were harvested by centrifugation, washed, and solubilized according to Mouches et al. (1979). Electrophoresis was performed after the method of Laemmli (1970) in 10% acrylamide gels in a Bio-Rad electrophoresis apparatus. The gels were stained with Coomassie blue.

RESULTS

All diseased plant specimens received from California exhibited broad intermittent chlorotic stripes in leaves, symptoms typical of the Rio Grande corn stunt disease (Davis et al., 1981; Gordon and Nault, 1977; Maramorosch, 1955; Nault and Bradfute, 1979).

Isolation of spiroplasmas from plants. Spiroplasmas were observed by dark field optical microscopy in juice freshly expressed from all nine plants with disease symptoms, but none could be found in juice expressed from the one symptomless plant. All plants exhibiting symptoms yielded spiroplasma cultures at 31°C, but no spiroplasma was isolated from the symptomless plant (Table 1). No spiroplasma isolations were obtained when cultures were incubated at 37°C. One isolate has been triply cloned and designated CSCaI-1. Strain CSCaI-1 and all other spiroplasmas isolated from the California maize in our Beltsville laboratory grew much more slowly than did strain 105 isolated from maize at the Berkeley laboratory.

In common with results of Kloepper et al. (personal communication), we found that strain 105 grew rapidly in medium LD8A as well as in L/D8 at 31 and 37°C. Strains BW and G1 also grew well at 31 and 37°C in media LD8 and LD8A. Growth of strain 105 at 37°C contrasts with poor or no growth at 37°C of CSS and S. citri strains isolated from plants or insects (Chen and Davis, 1979; Chen and Liao, 1975; Davis, 1979; Williamson and Whitcomb, 1975).

PAGE analysis of cellular proteins. Electrophoretic patterns of cellular proteins for spiroplasma strains BW and G1 (both members of the honey bee spiroplasma serological subgroup [Davis, 1978b; Davis, 1979; Davis et al., 1979]), for CSS strains I747 and PU8-17, for strain 105 isolated by Kloepper et al. (1982b) from maize in California, and for strain CSCaI-1 isolated from California maize in our work at Beltsville are illustrated in Fig. 1. All of the patterns bear some similarities to one another in positions of some bands. However, the patterns fall into two easily distinguishable groups. Patterns from strains BW, G1, and 105 very closely resemble one another but are distinct from the mutually very similar patterns exhibited by strains PU8-17, I747, and CSCaI-1. These findings suggest that strain 105 is a strain of the honey bee spiroplasma and that strain CSCaI-1 is a strain of CSS. This conclusion is consistent with results from cultural and serological tests (Davis and Lee, unpublished).

DISCUSSION

In agreement with conclusions by Kloepper et al. (1982b), our findings indicate that spiroplasma strain 105, which they isolated from field-grown maize in California, is unlike S. citri and CSS. Both our PAGE analyses of cellular proteins (this paper) and our serological data (Davis and Lee, unpublished) suggest that strain 105 differs fundamentally from S. citri and CSS strains. In addition, strain 105 and other strains isolated by Kloepper et al. (1982b) from California maize have been found by Kloepper et al. (personal communication) and by ourselves to grow well not only at 31°C but also at 37°C. However, no naturally occurring strains of S. citri or of the CSS have been reported to be capable of good growth at 37°C. We also found strain 105 grew much more rapidly than CSS strains in medium LD8 and LD8A (Davis and Lee, unpublished).

Our data verify the claim by Kloepper et al. (1982b) that the spiroplasma isolated in their work from field-grown maize in California during 1981 is neither S. citri nor CSS. These latter are the only two spiroplasmas previously known to reside internally in and to induce disease in plants. The reported isolation of a different spiroplasma from 96% of field-collected maize plants (Kloepper, et al., 1982b), therefore, is of paramount importance to plant pathology. For example, the occurrence of a spiroplasma other than CSS in maize could have significant implications for use of direct observation of spiroplasmas as an aid to diagnosis of corn stunt disease (Davis, 1978; Davis, 1974a,b; Davis, 1977; Davis et al., 1981). As noted earlier (Davis et al., 1981), the broad usefulness of this aid to diagnosis
would be weakened if a helical spiroplasma distinct from CSS were to infect maize in nature.

The actual identity of the spiroplasma from field-collected maize in California is highly intriguing. Our data from serological tests (Davis and Lee, unpublished) and PAGE analyses of cellular proteins (see Fig. 1), as well as other studies (Davis, unpublished), suggest that strain AS576 is a strain of the honey bee spiroplasma serological subgroup IB. Although Kloepper et al. (1982b) reported that strain 105 and others from California maize differ from honey bee spiroplasma strain AS576, this difference may be minor. Strain 105 still appears to be a honey bee spiroplasma strain. Why we failed to isolate such a strain from California maize, from which Kloepper et al. (personal communication) have recovered such strains, remains unanswered. Strain 105 and other strains isolated from California maize grow rapidly at 31 and 37°C (this paper; Kloepper et al., unpublished), and such strains should be isolated from plants with relative ease.

Instead of spiroplasmas similar to strain 105, the spiroplasmas isolated in our laboratory from California maize appear to be strains of the CSS. Indeed, from several specific plants, we isolated CSS strains (Davis and Lee, unpublished), whereas Kloepper et al. (personal communication) have isolated strains resembling honey bee spiroplasma from the same individual plants. Why one laboratory isolated only honey bee spiroplasma and the other laboratory isolated only CSS from the same plants is unexplained, but it is possible that differences in isolation procedures may account for the discrepancy. The major procedural difference is the utilization by Kloepper et al. (1982b) of a step involving centrifugation to concentrate spiroplasmas in, and to remove soluble inhibitors from, extracts from diseased plants. Our isolation procedure involved no centrifugation step.

If CSS strains and honey bee spiroplasma strains in nature actually share an ecological niche, e.g., phloem of maize, important implications might be drawn concerning opportunities for genetic exchange between these spiroplasmas. Our earlier observation (Davis and Lee, 1982) that these spiroplasmas are mutually independent in natural habitat was based on knowledge available at that time. The prospect that a natural habitat may be shared by these spiroplasmas deserves further research, especially in view of the startling report by Kloepper et al. (1982a,b) that a spiroplasma now identified as belonging to the honey bee spiroplasma serological subgroup is found in maize in California.

LITERATURE CITED


The Transmission of Corn Stunt Spiroplasma by Natural and Experimental Vectors

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ABSTRACT


*Dalbulus maidis*, the most important natural vector of corn stunt spiroplasma (CSS), is compared with *Euscelidius variegatus*, an experimental vector. *Cicadulina mbila* and *Macrosteles sexnotatus* were also included in the study. Infection of the leafhoppers was achieved by allowing insects to feed on infected plants, by feeding insects on suspensions of spiroplasma contained between parafilm membranes, or by injecting insects with cultured organisms. Following access to plants, *D. maidis* was an exceptionally efficient vector, *E. variegatus* and *M. sexnotatus* were very poor vectors, and *C. mbila* was very efficient at acquisition but less efficient at transmission. The effect of CSS on the vectors, multiplication in the vectors, the pathway through the salivary glands, inoculum doses, and incubation periods in plant and vector are also described and discussed. The importance of host plant and feeding behavior in vector efficiency was emphasized. An extended experimental plant host range for CSS was reported, including radish (*Raphanus sativus*), mustard (*Sinapis alba*), and spinach (*Spinacia oleracea*).

Corn stunt (CS) was first described as a disease of maize (*Zea mays L.*.) in 1945 (Altstatt, 1945) and the most important vector, *Dalbulus maidis* (DeLong and Wolcott), was discovered soon after (Kunkel, 1946). Over the next 36 yr other vectors and graminaceous hosts were implicated, and the distribution of CS throughout the growing areas of the USA and Mexico was reported. In 1975 CS was shown to be caused by a spiroplasma (CSS) (Chen and Liao, 1975; Williamson and Whitcomb, 1975). However, it is now accepted that although some of those earlier reports were indeed of a disease caused by a spiroplasma, others diagnosed as "corn stunt" were in fact caused by agents such as virus or mycoplasma or even complexes of any of the three pathogens (Nault and Bradfute, 1979). Reevaluation of the data suggests that: a) the plant hosts of CSS are *Z. mays*; *Z. mays mexicana* (Schrad.) Itis; *Z. diploperennis* Itis, Döebly and Guzman; *Z. perennis* (Hitchc.) Reeves and Mangel; *Z. mays X Tripsacum flavidum* Porter et Vasey L.; and *Z. luxurians* (Durieu and Ascherson) Bird; and b) CSS is transmitted in the field by *D. elimatus* (Ball), *D. maidis*, and experimentally by *Graminella nigrifrons* (Forbes), *Stirillus bicolor* (van Duzee), *Exitianus exitiosus* (Uhler), and *Euscelidius variegatus* (Kirschbaum) (Davis et al., 1981; Nault and Knocle, 1981).

Following injection of cultured organisms, *E. variegatus* has been shown in laboratory tests to transmit CSS to maize, *Vicia faba L.*, *Catharanthus roseus* (L.) G. Don, and *Lolium perenne* L. (Calavan and Oldfield, 1979; Markham et al., 1977).

Recent surveys have failed to establish any natural vectors other than *Dalbulus* species or any plant species other than maize and teosinte and so leave unanswered many of the questions on the ecology of CS. This paper describes some laboratory research on the transmission of CSS, using both natural and experimental vectors.

THE PATHOGEN AND LEAFHOPPERS

Corn stunt. This is primarily a disease of maize caused by a spiroplasma and reported from El Salvador, Mexico, Venezuela, Peru, the USA, and the Caribbean (Davis et al., 1981). Strain J2 (ATCC 27954) of the Rio Grande corn stunt (kindly provided by R. F. Whitcomb) and strain B656 from Jamaica (kindly provided by S. J. Eden-Green) were used for these experiments. These two strains produce similar symptoms in plants, similar plasmid profiles on agarose gels, and similar protein patterns on polyacrylamide gels. The Jamaican and Rio Grande strains are serologically identical by deformation and metabolic inhibition tests and significantly different from *Spiroplasma citri* (SPA). However, B656 appears to have a longer cell doubling time during exponential growth *in vitro* (in SMC medium J2 = 15 hr, B656 = 22 hr; and in A6 medium J2 = 19 hr, B656 = 22 hr).
Dalbulus maidis. *D. maidis*, the American corn leafhopper, is found abundantly on maize from the southern USA to Argentina (Nault, 1980). Its main food and breeding host is maize, although it is capable of feeding for short periods on a few other plants (Pitre, 1967). *Dalbulus* species also transmit a virus (maize rayado fino) and a mycoplasma (maize bushy stunt) (Nault et al., 1979). There are six vector species of *Dalbulus* transmitting several pathogens including viruses and spiroplasmas (L. R. Nault, personal communication).

*Cicadulina mbila* (Naude). Known as the African corn leafhopper, *C. mbila* is found widely on maize in Africa and has been reported from Russia, India, and the Cape Verde Islands (Ruppel, 1965). The genus *Cicadulina* is found in both the Old and New World and may be distributed throughout the Caribbean and southward in the Andes to Bolivia. Species of *Cicadulina* are found primarily on grasses, but *C. mbila* is frequently found on maize and sugarcane (*Saccharum officinarum* L.). Although there are few species of *Cicadulina*, their importance is in the pathogens they spread in graminaceous crops, e.g., maize streak virus. Populations are never as great as those of the related genera *Macrosteles* or *Dalbulus*. The genus has been reviewed by Ruppel (1965). There are six vector species transmitting five plant pathogens (Nielson, 1979).

*Macrosteles sexnotatus* (Fallen). The European six spotted leafhopper is commonly found on grasses but will breed on maize and some dicotyledons. *M. sexnotatus* is found widely throughout Europe and Asia and is a vector for several yellows-type mycoplasma pathogens. Probably the best known vector in the genus is *M. fascifrons* (Stal), a vector of the aster yellows pathogen in America. There are six vector species transmitting eight plant pathogens (Nielson, 1979).

*Euscelidius variegatus*. The variegate leafhopper is commonly found on grasses, particularly ryegrass (*Lolium perenne*). It will also breed on maize and a number of dicotyledons. This species occurs in Europe, Asia, North Africa, and North America, and transmits mycoplasmalike pathogens and spiroplasmas (Markham and Townsend, 1979; Markham et al., 1977). One vector species transmits three plant pathogens.

All four species were cultured on maize cv. Golden Bantam. *E. variegatus* and *M. sexnotatus* were also cultured on Italian ryegrass (*Lolium multiflorum* Lam. cv. Westerwoods).

**ACQUISITION AND TRANSMISSION OF CORN STUNT SPIROPLASMA**

Natural feeding. *D. maidis* is unusual among vectors of mycoplasmalike organisms in its very high efficiency of acquisition and transmission of CSS. With an acquisition access period (AAP) of as little as 15 min, 5% of the insects can later transmit CSS. An AAP of 7 days will ensure that eventually all become vectors. Transmission is also very efficient; when tested 15 days after a 7-day AAP, 22% will transmit with a 1-hr transmission access period (TAP) and 100% transmit with a TAP of 72 hr (males) or as little as 12 hr (females). Using females, by day 22 (following a 7-day AAP) most or all (93-100%) will transmit during a 24-hr TAP. The proportion of insects transmitting each day decreases as the insects age (Alivizatos, 1981). Females transmit 3-4 days before males.

*C. mbila* is also an efficient vector. Following a 7-day AAP, or if the insects are bred on CSS-infected maize, 60% of the population will transmit the pathogen to corn seedlings (Fig. 1). However, if haemolymph samples are tested for the presence of spiroplasmas by dark-field microscopy, all of the insects (out of 50 tested) contain CSS after a 7-day AAP.

*E. variegatus* and *M. sexnotatus* are very inefficient vectors, transmitting at 8.8% and 1-2%, respectively. But again, if haemolymph samples are assayed, then acquisition is shown to be better, e.g., 60-85% for *E. variegatus* but only after long AAP's.

One can conclude that the ability of the CSS to penetrate the first barrier, the gut, of a number of vectors is high.

**Membrane feeding (in vitro).** *D. maidis* is equally efficient at acquiring cultured CSS *in vitro* through membranes. With feeds as little as 2 min, some insects will later transmit CSS, and all the insects given a 7-day *in vitro* AAP will become vectors (Alivizatos, 1981). Transmission *in vitro* is approximately 10% less than to plants (Fig. 2). However, this may only reflect the limitations of the system used in these experiments.

*E. variegatus* will also transmit CSS *in vitro*, with 29% of the population of infective insects transmitting by day 14 (Fig. 2). It is interesting to note that this percentage is considerably higher than that recorded when these leafhoppers inoculated CSS to seedlings.
Injection. The use of microinjection of CSS cultures was a third method of infecting insects (Markham and Townsend, 1979). It ensured that all insects became infected at the same time. In vitro and in vivo transmissions following injection produced some unexpected results. Both *D. maidis* and *E. variegatus* began transmitting CSS 3-5 days after injection; in vitro transmission increased steadily until about day 13 when *D. maidis* reached a maximum of 60% and *E. variegatus* about 30% (Fig. 2). However, in vivo transmission for *D. maidis* began at 2 days and increased rapidly to about 75%, while *E. variegatus* transmission began between days 10-14 and only reached 7.5% (Fig. 2). In one experiment transmission occurred to broad beans (*V. faba*) at 7.5%, but no transmission occurred to maize in over 400 plants tested, probably because the insects were moved from ryegrass to maize.

The ability of CSS to pass out in the saliva after such a short period was also found for *S. citri* transmission in vitro by *E. variegatus*. This suggests that spiroplasmas may be passing directly from the haemolymph into the salivary ducts or directly to the ducts via the acini of the glands. It is also interesting that the in vitro and in vivo transmission are similar for *D. maidis* and so dissimilar for *E. variegatus* (Fig. 2).

**BEHAVIOR OF CSS WITHIN THE VECTOR**

It is possible to follow the multiplication of CSS in the vectors following injection (Fig. 5).

The titer of organisms injected is usually about 10⁶ colony forming units (cfu) per insect. Multiplication is very rapid for about 5 days when a maximum titer is reached and maintained at just over 10⁶ cfu per insect. Higher titers may be induced by increasing the inoculum dose or raising the temperature; the time to reach maximum titer (which is usually ½ to 1 log unit higher) is reduced.

Following injection it is possible to show that within about 2 hr spiroplasmas are firmly adsorbed onto the salivary glands and cannot be washed off. The multiplication rate is then rapid, reaching a titer of between 10⁶-10⁷ cfu per salivary gland. In *D. maidis* the peak in numbers within the salivary gland occurs before maximum transmission is reached, and in *E. variegatus* maximum numbers and maximum transmission coincide.

Examination of infected salivary glands by electron microscopy reveals CSS at the periphery of the lobes and always between the cell membrane and an outer "membrane". It therefore appears that CSS is intercellular and probably reaches the salivary ducts by passing between cells rather than through them. This could also account for the early transmissions obtained, especially in the in vitro tests. Staining infected salivary glands with the DNA stain, Hoechst-33258, and then examining them by fluorescence microscopy (Alivizatos, 1981; Chen, 1977) reveals patches of CSS on the surface of the lobes and concentrations of organisms between the lobes.

In all four species the CSS can be seen in the haemolymph as spirals. However, when seen in the salivary glands of *D. maidis* and *E. variegatus*, CSS assumes the typical mycoplasmalike morphology.

**Pathogenicity.** Assessment of any deleterious effect that CSS has on vectors is complex. Superficially it would appear that, following natural feeding, *C. mbila* is unaffected by the presence of CSS, as the longest
surviving insects were always infected. *D. maidis* suffers losses of about 10% or less, and *M. sexnotatus* and *E. variegatus* show little difference in survival following feeds on infected plants. The difference may be in the lower proportion acquiring the spiroplasma in the latter two species. For *D. maidis* the pathogenic effect seems to be the same whether acquisition is by natural or membrane feeding or following injection. For injected vs. fed *E. variegatus*, the difference in survival due to infection is about 12-15%.

However, the host plants may play a role and when infected insects were placed on unfamiliar plants (e.g., from ryegrass to maize) the pathogenic effect was doubled in *E. variegatus*. *M. sexnotatus* differed from the other species in that there was a significant effect due to the injection process (about 40%) which was further exacerbated by infection with CSS (by 30%). Also, survival depended on the temperature, being better at 26 C than at 29 C. This was probably due to the slower multiplication of organisms at the lower temperature.

The greatest difference in mortality between CSS-infected and uninfected insects seems to occur over a period of about 6-10 days at about the time the organisms are reaching the maximum titer, which may suggest that susceptible insects are killed while those that survive infection are able to limit any further increase in titer of spiroplasmas or are analogous to a chemostat.

In both *D. maidis* and *E. variegatus*, the CSS doubling time during exponential growth in the insects is about 18 hr at 29 C and the titer reached in the insect is between 10^8-10^10 cfu; while in culture the doubling time is 22 hr and the titer reached is 10^3-10^9 cfu per ml. If the cfu in the insect is calculated as per ml, then these are equivalent titers. It has been suggested that the haemolymph is the prime site for multiplication in the insect. In both *E. variegatus* and *D. maidis*, the difference between maximum titers in the whole insect and the maximum titer in the salivary gland is about 1 log unit, but initial multiplication appears to be more rapid in the salivary glands. Corrected for body weight, the titers are slightly higher in *D. maidis* (6.78 x 10^8 cfu mg^-1) than *E. variegatus* (2.81 x 10^8 cfu mg^-1); however the initial dose in the experiment was less in *E. variegatus* (0.77 x 10^6 cfu mg^-1) than *D. maidis* (1.58 x 10^6 cfu mg^-1). When the titer in the salivary glands is corrected for weight, then the titer in the salivary glands of *D. maidis* is one log unit higher than in the whole insect, whereas in *E. variegatus* the numbers per mg of tissue are approximately the same in the salivary gland and the whole insect.

**INOCULUM POTENTIAL OF CSS**

Incubation periods (IP) in both plants and insects appear to be a good indication of inoculum doses. IP in both *E. variegatus* and *D. maidis* varied inversely with AAP; the longer the AAP the shorter the IP. For *D. maidis* the IP ranged from 8-32 days, corresponding to an AAP range of 7 days to 20 min. For *C. mbila* the IP was between 19-26 days following a 12-day AAP. Following injection, the shortest IP was 10 days for *E. variegatus* and 2 days for *D. maidis*.

IP in plants also varied with TAP. In maize the IP was 37 days when 1-hr TAP was used, decreasing with increasing TAP, and was 16 days after a 6-day TAP by *D. maidis* at 29 ± 1 C.

Feeding *E. variegatus* in *vitro* and recovering the feeding solution for enumeration of cfu shows that initially very few organisms (3 cfu) were released, but by days 14-18 approximately 70-80 cfu were released in a 16-hr TAP. However, even in the small sample used (ten replicates at each of five sample times), on two occasions an insect released about 600 cfu (at days 14 and 18).

**FEEDING BEHAVIOR**

Both *D. maidis* and *C. mbila* feed well on maize and are reluctant to move unless disturbed. *D. maidis* females appear to feed more than males and are less active, which may account for the shorter IP. All females transmit with a 12-hr TAP compared to 62% of the males at 12 hr; males required a 72-hr TAP before all transmitted. However, for a short TAP of 1 hr, males were more efficient (38% for males vs. 8% for females) at 15 days after a 7-day AAP. *E. variegatus* and *M. sexnotatus* are more restless, which may be characteristic of a "generalist" feeder or may indicate a low preference for maize.

If one accepts that transmission occurs through infected saliva, then consideration of the sequence of events in feeding may be significant. There is more than one type of saliva produced during feeding. One type gels and produces the salivary feeding track. Analysis of the feeding tracks has been reported to show in what tissue the insect fed, its ability to find that tissue (i.e., low frequency of branching indicates high preference for a host plant), and the amount it fed (by the quantity of saliva produced) (Carle and Moutous, 1965; Moreau and Boulay, 1967). Efficient vectors feed frequently in the tissue susceptible to the pathogen and for extended periods (Day and Bennett, 1954; Day et al., 1952). It has also been reported that the most efficient vectors cause the least damage and that leafhoppers transmit most efficiently to those plants on which they most prefer to feed (Lehmann and Claus, 1970).

*D. maidis* and *E. variegatus* both feed in the phloem, the only tissue in which CSS is found, but *D. maidis* produces small feeding tracks and little saliva, while *E. variegatus* produces large feeding tracks and large quantities of saliva but apparently no cell damage. The same is true for saliva production in *vitro*. Furthermore, *D. maidis* produces copious quantities of "honeydew" (undigested phloem sap) and large numbers of insects will kill a plant solely by feeding. Both facts suggest that large volumes of phloem sap and hence spiroplasmas are ingested. Acquisition experiments with *E. variegatus* feeding in *vitro* on *S. citri* cultures show that the greater the volume ingested the more certain it is that the insect will become a vector. *D. maidis* will produce an average of 4 μl (range 1.2-6.2 μl)
of "honeydew" in vitro in a 24-hr period and the number of spiroplasmas in the honeydew is the same as that in the feeding solution. At the end of a 7-day AAP, there are about $4 \times 10^8$ organisms within the insect.

*C. mbila* also produces copious quantities of "honeydew" and is also capable of producing severe vein swelling due to feeding.

**TRANSMISSION TO PLANTS**

All four species acquired CSS from maize and transmitted it to maize seedlings. *D. maidis, M. sexnotatus,* and *E. variegatus* transmitted to maize following injection of CSS. *E. variegatus* also transmitted CSS to spinach (*Spinacia oleracea* L. cv. Round seeded type), mustard (*Sinapis alba* L. cv. Tendergreen), and radish (*Raphanus sativus* L. cv. French Breakfast Crimson). *E. variegatus* could also transmit CSS from mustard to mustard.

**DISCUSSION**

The relationship of a propagative plant pathogen with its vector follows a basic pathway through the insect vector (Harris, 1979). Although the hypothesis was pioneered by workers such as Kunkel and Storey in the 1930’s, it is only now that a detailed analysis of the pathway is emerging. CSS and its vectors offer a considerable potential for assisting in this analysis.

The unusual efficiency of *D. maidis* makes that species perfect for bioassay. *C. mbila* has proven genetic variability for the transmission of a plant virus (Storey, 1932) and may prove equally useful in the study of spiroplasmas. Could some genetically variable characteristic of the salivary gland account for the fact that only 60% of the population become vectors, despite all being infected? Other vectors, such as *E. variegatus* and *Macrosteles* species can extend the range of variability for analysis.

In the past the gut and the salivary glands have been suggested as major barriers in the transmission pathway, but the plant must also play a key role in the transmission of CSS, as does the feeding behavior of the vector species. Perhaps the plant can resist the challenge of very low numbers of spiroplasmas, which would explain the difference between very early transmissions in *vivo* and in *vitro*, and it may require the release of large numbers, such as those released occasionally (600 cfu) by *E. variegatus* for infection to occur. This could also explain the significant difference in *vivo* and *vitro* transmissions by *E. variegatus*. It is also important to consider why closely related plants may vary in susceptibility, e.g., perennial and Italian ryegrass, despite good feeding by the potential vector, *E. variegatus*. The feeding behavior, the type of salivary sheath, the preference for certain plants, and the previous host plant may all play a role. We still have to explain the anomaly that when a poor vector, such as *E. variegatus*, is injected with spiroplasma the salivary glands of most, if not all, the insects are infected and yet the transmission to plants is still very poor. Is too much salivary material produced?

More general points also arise. Techniques such as enzyme linked immunosorbent assay or culturing methods are often used to test for vectors, but these should only be used to indicate possible vectors, since pathogens such as CSS may be able to penetrate the gut of many leafhoppers which may be unable to transmit the pathogen. The transmission efficiency of the African corn leafhopper shows that the potential for the spread of CSS is present in Africa, should the disease ever be imported. The increase in the dicotyledonous plant range must warrant further investigation in the field for alternate hosts.

Perhaps the most challenging aspect is that in such a complex relationship as a vector-pathogen-plant system, there are so many variables that each combination of pathogen and vector needs careful analysis, and that generalizations can at best only be guidelines.
LITERATURE CITED


Maize Rayado Fino Disease: The Virus-Host-Vector Interaction in Neotropical Environments

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Abstract


Maize rayado fino virus (MRFV) and its leafhopper vector Dalbulus maidis have closely followed the successful adaptation of their maize (Zea mays) host to widely different tropical ecosystems and maize rayado fino disease is presently found throughout most of the neotropical maize growing areas of the American continent. The complex interaction of the virus, the maize host, and the insect vector is considered under the environmental conditions of a tropical pre-montane moist forest area in Alajuela, Costa Rica. The emerging picture of the development of epidemics of maize rayado fino is analyzed. MRFV appears to represent a typical "cultivated plant-adapted virus". The epidemiology of MRFV is also examined considering its relation to current ecological principles based on the strategies and abilities of plants and animals to reproduce and disperse in relation to the type of habitat they exploit.

One of the characteristics of the maize plant (Zea mays L. ssp. mays), exhibited to a degree not found in other cultivated plants, is the diversity of forms which allow it to thrive or at least survive in a wide variety of environmental conditions (Mangelsdorf, 1974). Maize rayado fino virus (MRFV) (Gámez, 1980) and its leafhopper vector, Dalbulus maidis (DeLong & Wolcott), have closely followed this successful adaptation of their maize host, so that maize rayado fino disease is widely distributed and frequently found in maize fields in the most diverse ecological conditions of the neотropics (Gámez, 1982; Gámez et al., 1979). The American tropics comprise those regions lying approximately from the Tropic of Cancer to the Tropic of Capricorn, and include ecosystem types ranging from deserts and savannas through deciduous forests and montane forests to lowland tropical rainy forests (Jordan, 1981).

From an ecological point of view, the development of a leafhopper-borne virus disease of this nature can best be viewed as a complex interaction of the virus, the plant host, the insect vector, the environment and time (Cowling and Horsfall, 1978; Frazer, 1977; Harrison, 1981; Thresh, 1980; Zitter, 1977). This paper examines the available information on these major components of the epidemiology of maize rayado fino within a broader ecological context in an attempt to understand how each of these variables contributes to the complexity of the disease.

The epidemiology of maize rayado fino is also considered in relation to current ecological principles. These principles have been recently applied to the epidemiology of plant virus diseases, which have many features in common with the colonization and subsequent exploitation of natural habitats by plants and animals (Thresh, 1980). The kind of survival system of some plant viruses seems likely to have advantages over others in particular ecological niches, and different kinds of plant communities favor different groups of
viruses (Harrison, 1981). This paper also examines how specialized plant communities such as maize crops favor the survival of MRFV and its leafhopper vector.

**CHARACTERISTICS OF THE VIRUS-VECTOR-HOST SYSTEM**

The main properties and characteristics of MRFV, the virus-vector, and the virus-host relationships have been recently summarized (Gámez, 1980; 1982; León and Gámez, 1981).

**Virus.** MRFV is a small isometric ssRNA virus, which shares several characteristics with oat blue dwarf virus (Banttari and Zeyen, 1973). Both viruses differ from all other known small spherical viruses in the combination of their biological, biophysical, and biochemical properties (León and Gámez, 1981).

**Virus-vector relationship.** The transmission of MRFV by *Dalbulus* spp. is typically persistent and intermittent (Gámez, 1973). Multiplication of MRFV in *D. maidis* has been demonstrated (Gámez et al., 1981; Rivera, 1981; Rivera et al., 1981).

The proportion of transmitters in a colony is normally low and varies from 10-34%. Transmission ability is under genetic control and vectors do not appear to have a selective advantage for survival in the laboratory or field (Gámez, 1982; Nault et al., 1980; Paniagua and Gámez, 1976). The number of viruliferous insects detected by the enzyme-linked immunosorbent assay (ELISA) is higher than the number of transmitters determined by infectivity assay, suggesting that many insects acquire but do not transmit MRFV (Rivera, 1981; Rivera et al., 1981).

Electron microscopy observations revealed the presence of the virus in the internal organs of the insect vector, but no cytopathic effects were evident as a result of the viral infection (Kawaijima and Gámez, 1982). The absence of deleterious effects had already been noted in previous studies, when no difference in longevity (González and Gámez, 1974) and oviposition (Gámez, unpublished) were observed.

Other species of *Dalbulus* from Mexico and other leafhoppers common in North America transmit MRFV under experimental conditions (Nault et al., 1980; L. R. Nault, personal communication), but all are less efficient vectors than *D. maidis*. The geographical distribution of these other species of *Dalbulus* is apparently restricted to southern Mexico (Nault and DeLong, 1980; Nault et al., 1989). *D. maidis*, better adapted to maize than other *Dalbulus* species, is found widespread throughout the American tropics and is presently considered the main natural vector of MRFV (Gámez, 1982; Gámez et al., 1979) and of the mycoplasmas and spiroplasmas associated with the maize stunt complex in Mexico and Central America. These three pathogens may be simultaneously transmitted by *D. maidis*.

There is a close association between *D. maidis* and maize; the insect has a narrow host range which includes only maize, the annual teosintes (*Z. mays* ssp. *parviglumis* and *mexicana*) (Barnes, 1954) and the perennial teosintes (*Z. diploperennis* (Iltis, Doebley and Guzmán) and *Z. perennis* (Hitchc.) Reeves and Mangelsdorf) and *Tripsacum lanceolatum* Rupr. (Nault and DeLong, 1980; Nault et al., 1983). Apart from maize, the other host species are all restricted to southern and central Mexico in their distribution. Other grasses may serve as feeding hosts but survival of the leafhopper on wild and cultivated species of Gramineae is poor; maize is presently considered the only known natural host in areas where both virus and vector are endemic (Gámez, 1982).

**Virus-host relationship.** The host range of the virus is similarly narrow; a taxonomic analysis of available information (see review by Gámez, 1982) indicates that the virus is restricted to the Andropogonoids and mainly to the genus *Zea*. Among the teosintes, the closest maize relatives (Doebley and Iltis, 1980; Nault et al., 1980), all annuals, and one of the two known perennials are susceptible to the virus. Similarly, all maize genotypes tested, including numerous races and several hundred cultivars and inbreds, were found susceptible to the virus. *Tripsacum austral* Cutler and Anderson and *Rottboellia exaltata* L., distantly related to maize, are the only species not in the genus *Zea* that are susceptible to the virus (Nault et al., 1980).

Field observations of rayado fino from numerous localities in tropical areas of the continent and at diverse planting seasons revealed variable incidences from 0-40% in most locations to nearly 100% in some others like Zautotlán in El Salvador (Gámez et al., 1979). Reports from Mexico, Central America, and Colombia (see review by Gámez, 1982) show that the susceptibility of maize cultivars to MRFV may be variable. Depending on the cultivar, average losses of 40-50% of weight of ears are common on locally adapted materials, while introduced or newly developed genotypes may sustain losses of nearly 100%. The different susceptibilities of maize genotypes have been considered to indicate that MRFV plays an important role in their adaptation in the American tropics, a fact congruent with the hypothesis that the virus was probably an important selective force in the evolution of maize from its wild teosinte ancestors (Gámez, 1982; Nault, 1982; Nault and DeLong, 1980).

The evident close association between MRFV and its plant host and insect vector appears to be a feature not common to other propagative, leafhopper- or planthopper-borne viruses. Evidence of co-evolution of *Dalbulus* leafhoppers with maize and its ancestors has been recently presented (Nault, 1982; Nault and DeLong, 1980; Nault et al. 1983). The capacity of the small genome of MRFV to replicate in both types of organisms suggests a close adaptation of this viral genome to the *Zea-Dalbulus* system. MRFV may have constituted a selective pressure in their evolution, as well as a third coevolving element in this biological system (Gámez and Leon, 1983).

**DISTRIBUTION OF MAIZE RAYADO FINO**

The distribution of maize rayado fino is wide, both geographically and ecologically (Gámez, 1982). The disease has been recorded by several investigators from the southern USA through Mexico, Central America,
Colombia, Venezuela, Peru, Brazil, and Paraguay in South America, which indicates a neotropical distribution. The range of ecological conditions in which MRFV is found in the tropics is also very wide, from sea level to more than 3500 m. This includes nearly 20 different life zones in the basal, premontane, lower montane, and montane altitudinal belts, which differ in altitude, annual mean temperature, amount and seasonal distribution of rainfall, light intensity, soil conditions, and physiography (Holdridge, 1978). *D. maidis*, MRFV, and maize overlap in their distribution. It is evident that if maize, as it is presently believed, originated in the southern Mexican highlands and was dispersed throughout the American continent by pre-Columbian man, *D. maidis* and MRFV have also successfully followed the distribution of their host plant, paralleling its extraordinary capacity of adaptation to diverse neotropical ecological conditions (Gámez, 1982).

THE DEVELOPMENT OF MAIZE RAYADO FINO DISEASE IN MAIZE POPULATIONS UNDER TROPICAL PREMONTANE FOREST ENVIRONMENTS

As stated above, the complex interaction of virus, host, vector, and environment that determines the development of the maize rayado fino disease is successfully attained under widely different ecological conditions. In an attempt to explain how each of the components of the system contributes to the development of the disease, a long term analysis of epidemics of maize rayado fino has been undertaken in Alajuela, Costa Rica, an area where the disease is endemic. Relevant aspects of this research, conducted by several authors (Gámez, V. Quiroga, and R. Pereira, unpublished; Mora, 1978; Saavedra, 1982), are briefly summarized.

Some general characteristics of the area. The area corresponds to a tropical premontane moist forest (Holdridge, 1978). Two clearly defined seasons exist: a dry season from December to April and a rainy season from May through November. Annual precipitation is approximately 2100 mm distributed in two distinct periods during the rainy season, with a short 1-2 wk dry spell between periods; some sporadic rains fall during this dry season. Mean temperature varies between 20 and 25 C, with maxima of 24.5 to 31 C and minima of 15 to 19 C. Relative humidity is between 72-95% and radiation is 158-509 calories/cm².

Although there are two main planting dates in May and September, small maize fields are planted throughout the rainy season and more sporadically under irrigation during the dry season. Overlapping crops found through most of the year are grown under typical small-farm multiple-cropping systems. Plots vary in size from about 0.01 hectare (ha) to 1.0 ha. During the rainy season, fields are separated by not more than a few hundred meters but during the dry season they may be kilometers apart.

The genetic constitution of the maize population is heterogeneous, as local and introduced cultivars and mixtures of materials are planted in the area.

Seasonal incidence and progress of the disease in time. The seasonal variations in overall incidence of MRFV at the Estación Experimental Fabio Baudrit in Alajuela are presented in Fig. 1. The data were compiled from surveys during the dry season and early and late rainy season in representative fields, approximately 60 days after planting. Consecutive surveys were done from January 1981 to February 1982 (Saavedra, 1982; Gámez and F. Saavedra, unpublished). The available information suggests that the incidence of MRFV is low during the dry season, increases steadily during the
rainy season, and drops abruptly at the onset of the dry season. Disease progress curves for consecutive planting seasons in 1981-1982, constructed from data compiled from the same surveys described before, showed typical sigmoid trends in time but within their general overall pattern there are differences in the onset, rate, and total amount of spread (Fig. 2). Similar rates have been reported for other virus diseases (Thresh, 1980).

The vector population. Vector population studies were done in the same representative fields where incidence and progress of the disease were monitored (Saavedra, 1982). The seasonal variations in total populations, estimated by three different methods, appear in Fig. 1. Insect numbers increased steadily with fluctuations from the onset of the rainy season and decreased abruptly at the beginning of the dry season.

The percentage of viruliferous insects in the total population was 10-16% during the early plantings of the rainy season and increased to 28-36% in the late rainy season (Saavedra, 1982), paralleling the seasonal pattern of the total disease incidence and vector population (Fig. 1).

Maize fields in all seasons were first infested by adult leafhoppers in the first 4-5 wk, but later nymphs and young adults became prevalent. Existing information (Saavedra, 1982) suggests that leafhoppers migrate from other fields to the new and more vulnerable crop. A new generation of *Dalbulus* develops and moves out of the field about the 7th-8th wk, when plants approach tasseling. At this stage the physiologically mature plants become inadequate hosts for the insects, which also lose their normal and favorable habitat provided by the leaf whorl. Their movement out of the field in search for new maize hosts could be easily assisted by prevailing surface winds.

Patterns of spread. A typical pattern of natural spread of MRFV in small fields is shown in Fig. 3. Nonrandom aggregation of infected plants in small plots had been previously observed (Gámez, 1982) and was confirmed in the 1981-1982 surveys (Saavedra, 1982). The number of pairs of adjacent infected plants (Van der Plank, 1960) detected in these fields was always higher than that expected by chance. It is difficult to interpret virus disease gradients in small plots, which among other factors are subjected to pronounced edge, positional, or exposure effects (Thresh, 1976).
With these considerations the available data on the patterns of spread of MRFV suggest that immigrant viruliferous leafhoppers tend to concentrate in certain areas of the field, possibly dispersing the virus in limited foci.

The environmental conditions. The effect of environmental conditions on the occurrence and spread of virus disease is by necessity closely tied to the ecology of the insect vector and its population dynamics (Zitter, 1977). In regards to MRFV and *D. maidis*, the general characteristics of the Alajuela area where both are endemic were previously described. Total precipitation and rainfall distribution, temperature, relative humidity, radiation, and wind velocities and prevalent directions were within the normal range of variations in the 1981-1982 period of study (Saavedra, 1982). The main climatic variations appear to be those associated with the change of the rainy season to the dry season and vice versa (Fig. 1), for within a given season the main meteorological parameters are relatively stable. Nevertheless, it should be noted that the climatic conditions examined are those general to the area; the microclimate of the field or the individual plants was not examined and its relevance to the epidemiology of MRFV is unknown.

Correlations among virus incidence, insect populations, and climatic conditions. Correlation coefficients developed by regression analysis were used as indicators of correlations among factors affecting the development of maize rayado fino (Saavedra, 1982). Much variation appears to exist in the coefficients and, as stated by Kranz (1978), only the correlations with large coefficient values were considered. Precipitation is clearly correlated with insect population and virus incidence; e.g., higher insect populations determine higher virus incidence within the rainy season (Fig. 1). Seasons, as related to precipitation, affect insect populations and incidence.

The emerging picture of the development of epidemics of maize rayado fino disease. In considering the available ecological and epidemiological information on maize rayado fino and its limitations, it seems possible to draw a tentative picture of the characteristics of the development of the disease under the environmental conditions of Alajuela.

The maize fields planted in the early rainy season in May are readily infested by immigrant *D. maidis* adults coming from distant fields, possibly from the more humid areas of the country. Oviposition occurs shortly after arrival as the new generation begins to emerge at approximately the 7th wk after planting. The immigrant leafhoppers appear responsible for the spread of virus within the crop as appearance of the new generation occurs when virus incidence is reaching its maximum (Fig. 2). Insects are known to transmit MRFV only after long incubation periods of 14-19 days and retain their ability to transmit for most of their lives (Gámez, 1973). The new generation is viruliferous by the time they lose their natural habitat, when plants mature and tassel. Young insects move to neighboring fields as maize crops overlap during the rainy season.

This fact and favorable climatic conditions prevailing during the season allow for a continuous build-up of *Dalbulus* populations and virus inoculum leading to a wider spread of MRFV, as suggested by Figs. 1 and 2, and the increasing number of viruliferous leafhoppers recorded by serological tests. With the onset of the dry season, fields rapidly dry out. Coincident with this, northerly and easterly winds prevail for the first part of the dry season (Coen, 1983). It is known that leafhoppers can be easily borne aloft by convection currents and if weather conditions are favorable, they can be carried for long distances depending on their flying capacity (Kisimoto, 1973; Rosenberg and Magor, 1981). *D. maidis*, devoid of its protective habitat of the leaf whorl, could be easily carried long distances by surface winds. In addition to local winds determined by topographical and other environmental conditions, there are eight prevalent directions of surface winds in Costa Rica differing in daily or seasonal frequency. Wind velocities are up to 180 km/day with approximate temperatures of 15-20°C and relative humidities of 80% (Coen, 1983). It is hypothesized that these winds may carry and disperse viruliferous leafhoppers to any part of the Costa Rican territory within a day, and as maize may be found at any time of the year due to the diversity of ecological conditions of the country, new disease cycles could possibly develop. If this assumption is correct, *D. maidis* and MRFV have developed the required mobility and ability for rapidly invading and exploiting the relatively short-lived host and ephemeral habitat provided by the maize plant, the only known host for both parasites. This would also explain the natural sources and the overseasing of virus and vector especially during the dry season, as no alternate hosts have been determined or are known to exist in the area (Gámez, 1982).

**ECOLOGICAL ASPECTS OF THE SURVIVAL OF *D. MAIDIS* AND MRFV**

MRFV as a CULPAD virus. There are some general conclusions that may be drawn from the analysis of subjects presented in this paper. One relates to the evident adaptation of MRFV to maize crops. As stated by Harrison (1981), “crops comprise some of the most specialized types of plant communities. Unlike natural communities they consist mostly of dense populations of genetically very similar individuals, all at a uniform stage of growth and often occupying large areas of land.” In this sense maize crops are rather specialized, particularly in tropical environments in which one of the outstanding characteristic of natural communities is their species richness and genetic diversity (Jordan, 1981). Certain groups of plant viruses seem to be more prevalent in cultivated crops than others, and are referred to as cultivated plant-adapted viruses or CULPAD viruses (Harrison, 1981). All of these viruses have narrow natural host ranges. MRFV and its insect vector are presently known to be prevalent only in maize crops and have rather narrow natural host ranges, limited to some species of Andropogonids of restricted distribution (Gámez, 1982). MRFV may be
considered a tropical CULPAd virus, seemingly adapted to survive only in maize (Gamez and Leon, 1983). Maize, in turn, is one of the most domesticated of the food plants, unable to survive without human intervention (Mangelsdorf, 1974).

**Dalbulus maidis** and MRFV as "r-selected" species.

In an ecological approach to virus epidemiology, Thresh (1980) has stressed the similarities between the spread of pests and diseases into and within crops with the colonization and subsequent exploitation of natural habitats by plants and animals. The author mentions the considerable attention given by ecologists to the reproductive strategies and dispersive abilities of plants and animals in relation to the type and stability of the habitats they exploit. Within this context, current ecological concepts distinguish between opportunist, colonizing species as "r-selected" and equilibrium "K-selected" species. Longevity, large size, limited mobility, and low, delayed fecundity characterize K strategists, while opportunistic, mobile species with well adapted abilities for rapidly invading and exploiting ephemeral sites are typical of r type (Thresh, 1980).

The information presented in this paper suggests that *D. maidis* has the main features typical of r-selected species. If we can speak at all in terms of r-K selected viruses (Harrison, 1981), the method of perennation of MRFV (Gamez and Leon, 1983) of alternating cycles in its plant and insect hosts suggests that the virus is not an extreme r-strategist, but it rather occupies a more intermediate position in an r-K continuum, as visualized by Pianka (1970). Further ecological studies on the maize rayado fino system would evidently contribute to the final elucidation of the attributes of virus and vector as r or K species.

**Possible explanations of the wide geographical range of *D. maidis***.

This leafhopper-virus system appears to be a good example of virus spread by vectors migrating from maturing crops or otherwise deteriorating environments, typical of some virus diseases (Thresh, 1980). Spread is to nearby crops at an earlier and more vulnerable stage of development or to entirely new areas at greater distances. The spread of *D. maidis* to nearby crops seems to be evident in Costa Rica. It is possible that long range migrations of *D. maidis* through Mexico, Central, and South America occur, similar to those observed for other leafhoppers in Asia (Rosenberg and Magor, 1981). Although this phenomenon was not observed in Mexico (Barnes, 1954), field observations have indicated that this leafhopper migrates north and easterly from southwest USA or possibly Mexico each year, reaching areas where the insect is unable to survive in winter (Pitre et al., 1967). Similar situations may exist in tropical areas of the continent and could explain the appearance of the virus and vector in such diverse ecological life zones as the high Peruvian Andes (Nault et al., 1979), the Brazilian Cerrado, the Yucatan Peninsula, or the Mexican Highlands (Gamez, 1982; Gamez et al., 1979) where maize is grown only during one season and ecological conditions appear unfavorable for the survival of the vector between planting seasons.

Extensive knowledge on the biometrics of leafhopper vectors has contributed to the elucidation of the complex epidemiology of maize streak disease in Africa (Rose, 1978). The need for a better understanding of the biology and ecology of *Dalbulus* in the American tropics is evident. Although migratory movements of this species may occur as discussed above, other factors may contribute in the determination of its wide geographical range. It is known that certain species of leafhoppers with wide geographical and ecological distributions are complexes of genetic mutants or genetically different populations. Furthermore, isolated populations of these species are biologically, ecologically, or physiologically specific and cannot be separated morphologically (DeLong, 1971). Although the available information on the biology of *D. maidis* was obtained originally with populations from Mexico, USA, Costa Rica, and Colombia, this information is congruent and suggests a similar biological behavior of the different geographical collections. However, genetic variability of this species could also explain its extraordinary adaption to different ecological conditions, paralleling that exhibited by its maize host (Gamez, 1982).

**LITERATURE CITED**


Maize Stripe Virus

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ABSTRACT


Maize stripe disease, caused by the maize stripe virus (MStpV), occurs in many tropical areas of the world. The virus is persistently transmitted and transovarially passed by its only known vector, the delphacid plant-hopper, *Peregrinus maidis*. Infectivity was associated with a filamentous nucleoprotein (NP) 5 nm in diam. This was shown by infectivity of partially purified NP preparations, neutralization of infectivity by NP-antiserum, and correlation between the presence of NP and transmissibility by vectors. The nucleoprotein sedimented slowly (50-70S) and heterodispersely (from 4 to 6 zones) in sucrose density gradients and it banded isopycnically in CsCl at 1.280 g/ml. Purified NP contained 5.5% RNA and a single protein species of molecular weight 32,700 daltons. The nucleoprotein replicated in *P. maidis* and it was found in many planthopper organs. A noncapsid protein (NCP), serologically unrelated to the protein of the NP, was produced in large amounts (up to 2 mg/g tissue) in maize stripe-infected plants. The function of NCP is not known. MStpV is serologically related to the rice stripe virus. These viruses represent a new virus group.

The maize stripe disease was first described in detail by Storey (1956) in maize (*Zea mays L.*) in East Africa. A similar disease that may have been maize stripe had been noted 7 yr earlier in Mauritius (Shepherd, 1929). Storey described two leaf symptom types: one with narrow yellow stripes and the other with broad yellow stripes. Later, Kulkarni (1973) showed that these symptom types were associated with two distinct diseases and designated the one with narrow stripes as maize line [since shown to be maize mosaic (Bock et al., 1976)] and retained the name maize stripe for the disease with wider stripes. The corn delphacid, *Peregrinus maidis* (Ashmead), transmitted both pathogens in a persistent manner. Neither was transmitted mechanically. Prior to Kulkarni's work, and to a limited extent since, confusion between maize stripe and maize mosaic has occurred. However, the diseases are readily distinguishable because the causal agent of maize mosaic is a rhabdovirus (Herold, 1972). Other diseases with symptoms similar to the East African maize stripe and whose agents were readily transmitted by *P. maidis* were reported from Australia in 1943 by Blackford (Simmonds, 1966), from Venezuela in 1974 (Trujillo et al., 1974), and from the United States (Florida) in 1974 (Tsai, 1975).

Kulkarni (1973) found "empty" and complete isometric particles, 35 and 40 nm in diam, respectively, associated with partially purified preparations from maize stripe-diseased plants. He concluded that these particles were the causal agent. Trujillo et al. (1974) reported isometric particles, 55-60 nm in diam, associated with the hoja blanca disease, their original designation for the *P. maidis*-transmitted disease in Venezuela. However, repeated examinations of extracts or tissue thin sections from diseased plants from Florida or test plants serially inoculated with the Florida pathogen failed to reveal any isometric particles (Bradfute and Robertson, 1977). Also, no isometric particles were detected from maize stripe-diseased plants from Mauritius (Autrey, 1983) or Australia (Greber, 1981), and Lastra and Carballo (1983) have been unable to repeat the isolation of isometric particles from diseased plants in Venezuela. They now postulate that the 55-60 nm particles may have been latent *P. maidis* viruses not pathogenic to maize (R. J. Lastra, personal communication). The isometric particles found by Kulkarni (1973) were probably not the causal virus either because: a) partially purified or purified preparations of the isometric particles were not infective; b) Kulkarni's antiserum, widely used to diagnose maize stripe and supposedly prepared against the isometric particles, was in fact prepared against material in "light-scattering" zones from sucrose density gradients that were not shown to contain isometric particles; c) during the period that Kulkarni reported isometric particles associated with maize stripe, he also reported isometric particles associated with maize line which, as later shown by Bock et al. (1976), is actually caused by the rhabdovirus, MMV. Further, Bock et al. demonstrated that a 35 nm diam isometric particle could be isolated from randomly selected maize plants without symptoms. Apparently the 35 and 40 nm particles described by Kulkarni were contaminants in his cultures and were unrelated to MStpV.
Kulkarni's antiserum reacted with sap from plants infected with the Florida pathogen and the hoja blanca virus from Venezuela (Gingery et al., 1979b), indicating that some common factor other than isometric particles existed among these diseases. We suspected that the common factor was probably an unusual nucleoprotein (NP) consistently associated with maize stripe-diseased, but not healthy, maize from Florida (Gingery et al., 1979a, 1981) (Fig. 1). Extracts from such plants as well as purified NP reacted with Kulkarni's antiserum. Although it cannot be proven, the light-scattering zones used by Kulkarni for antiserum production probably contained, among other things, a nucleoprotein similar, if not identical, to that found in diseased Florida maize. In fact, recent evidence suggests that Kulkarni's antiserum reacts with at least three known maize viruses and two unidentified isometric particles in immune-specific electron microscopy (Jones, 1983).

Other countries in which virus isolates serologically related to the East African MStpV have been identified include Peru (Nault et al., 1979), Nigeria and Sao Tome, an island off the western coast of Africa (H. W. Rossel, personal communication), Australia (Greber, 1981), Mauritius (Autrey, 1983), Guadeloupe (Migliori and Lastra, 1980), and Botswana (P. Jones, personal communication). A disease with similar symptoms whose causal agent is transmitted by P. maidis has been described from the Philippines (Exconde, 1977), but it has not been serologically tested. Losses caused by maize stripe have generally been minor, although serious outbreaks have occurred in Florida (Niblett et al., 1981), Sao Tome (Rossel, 1982), and Venezuela (Lastra and Carballo, 1983).

The rice stripe virus from Japan also appears to be composed of a 3 nm nucleoprotein strand (Koganezawa et al., 1975) and has recently been shown to be serologically related to the maize stripe NP (Gingery et al., 1983; E. Shikata, personal communication).

PROPERTIES OF THE MAIZE STRIPE NUCLEOPROTEIN

The 3 nm nucleoprotein purified by the method of Gingery et al. (1981) exhibited slow (50-70S), heterodisperse (from 4 to 6 zones) sedimentation on sucrose density gradients. The buoyant density of all the zones from sucrose gradients was 1.280 g/ml in CsCl. The extinction coefficient of purified NP was 2.3 cm²/mg at 260 nm, and the A280/A260 ratio was 0.72. The NP contained 5.5% RNA and a single protein subunit of molecular weight 32,700 daltons. From Venezuelan

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Fig. 1. Electron micrograph of negatively stained maize stripe nucleoprotein from sucrose density gradient zone showing fine strands, approximately 3 nm wide. For comparison, note PVY-type flexuous rod (maize dwarf mosaic virus, strain B) and phytoferritin molecules (light rings with dense centers approximately 11 nm in diam). The MDMV-B particle was introduced as a size and resolution standard; phytoferritin was frequently found in preparations at this level of purity. Magnification bar 100 nm long. From Gingery et al. (1981). (Photo reprinted with permission of Academic Press, Inc.)
maize stripe-infected plants, Lastra and Carballo (1983) isolated a similar ribonucleoprotein that contained a single protein species of about 33,000 daltons. Greber (1981) also reported a 33,000 dalton protein associated with the Australian isolate.

The descriptions of several other particles associated with plant diseases resemble that of the maize stripe NP. For example, the rice hoja blanca virus (RHBV) was described by Shikata and Galvez (1969) as a fine, flexuous, threadlike particle. However, it was considerably thicker (8-10 nm in diam) and much less flexuous than maize stripe NP. RHBV appears morphologically similar to beet yellows virus, a member of the closterovirus group. Other structures that resemble the NP are the gene-5 product-DNA complex found in cells infected with filamentous bacterial viruses such as fd and M13 (Alberts and Frey, 1972) and the fibrillar form of aggregated winter wheat mosaic virus protein (Atabekov et al., 1968). Whether these chemically different entities have any structural similarities to the NP is not known.

The NP resembles partially degraded nucleocapsids isolated from large, enveloped, single-stranded RNA viruses, such as those in the Arenavirus, Bunyavirus, myxovirus, Paramyxovirus, Rhabdovirus, and Retrovirus groups. For example, electron micrographs of nucleocapsid isolated from vesicular stomatitis virus, a member of the Rhabdovirus group, revealed structures quite similar to the NP from maize stripe-infected plants (Simpson and Hauser, 1966). Of the animal enveloped virus groups, only Rhabdoviruses are known to occur in plants (Francki et al., 1981; Jackson et al., 1981). One other possibly enveloped plant virus is the tomato spotted wilt virus, but its characteristics are not well known (Francki and Hatta, 1981).

### INFECTIVITY OF MAIZE STRIPE NUCLEOPROTEIN

Although NP was consistently found in MStpV-infected plants, direct evidence relating it to infectivity has been difficult to obtain because of marked instability during purification. Results of experiments designed to purify the infective agent are presented in Table 1. Pelleting by centrifugation apparently was detrimental because infectivity was much higher if the extract was pelleted through a 40% sucrose cushion and higher still if not pelleted at all but rather recovered from the top of a 60% sucrose-D_2O cushion. Material from the sucrose-D_2O cushion was then isopycnically banded in either CsCl, Cs_2SO_4, or sucrose-D_2O. Significant infectivity was obtained only from the sucrose-D_2O gradient. Examination of the infective fraction from these gradients again revealed the fine-stranded material.

Experimental results using other methods also showed a correlation between infectivity and the NP. The most compelling evidence came from neutralization of infectivity studies in which the infectivity of extracts was blocked by treatment with antiserum prepared against purified NP. In five separate experiments, a total of 40/149 (27%) *P. maidis* injected with clarified extracts transmitted MStpV. Transmission after treatment of the extract with preimmune serum was 33/132 (25%), whereas treatment with NP-antiserum completely neutralized infectivity (0/147) (Gingery et al., 1981). A relationship between infectivity and NP was also shown by experiments correlating transmissibility by and the presence of NP in *P. maidis* (see next section).

### VECTOR TRANSMISSION OF MStpV

MStpV was transmitted in a persistent, intermittent pattern by *P. maidis* with a mean latent period of 15.6

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**TABLE 1. Infectivity during maize stripe virus purification.**

<table>
<thead>
<tr>
<th>Purification step*</th>
<th>Transmission by injected <em>P. maidis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial extract</td>
<td>394/1057 (37)*</td>
</tr>
<tr>
<td>Clarified extract (CHCl_3)</td>
<td>29/92 (5)</td>
</tr>
<tr>
<td>Concentrated preparation:</td>
<td></td>
</tr>
<tr>
<td>Resuspended pellet after high-speed centrifugation</td>
<td>1/154 (5) 0-4%</td>
</tr>
<tr>
<td>Resuspended pellet after high-speed centrifugation through 40% sucrose cushion</td>
<td>12/84 (3) 11-9%</td>
</tr>
<tr>
<td>Zone isolated from top of 60% sucrose-D_2O cushion after high-speed centrifugation</td>
<td>13/63 (2) 7-31%</td>
</tr>
<tr>
<td>Isopycnically banded preparation* in:</td>
<td></td>
</tr>
<tr>
<td>Sucrose-D_2O</td>
<td>9/62 (2) 7-19%</td>
</tr>
<tr>
<td>CsCl</td>
<td>0/50 (1) 0%</td>
</tr>
<tr>
<td>Cs_2SO_4</td>
<td>1/44 (1) 2%</td>
</tr>
</tbody>
</table>

* Purification was performed as described in Gingery et al. (1981).

* Rate of transmission. In each experiment about 40 *P. maidis* were injected. The fraction is the number of insects that transmitted MStpV to test plants over the number of injected insects that were placed on test plants. The number in parentheses is the number of experiments.

* The range of transmission among individual experiments.

* Material from the top of 60% sucrose D_2O cushions was used.
days (Gingery et al., 1979b; Nault et al., 1983; L. R. Nault and D. T. Gordon, personal communication). In one series of experiments, 59% of the offspring of nine viruliferous females also transovarially transmitted the virus (Gingery et al., 1981). MStpV did not shorten the life span of viruliferous individuals, but in some experiments it reduced fecundity by as much as 50% (L. R. Nault, personal communication).

By enzyme-linked immunosorbent assay (ELISA), NP was found in viruliferous P. maidis in the muscle, brain, midgut, hindgut, Malpighian tubules, salivary glands, ovaries, eggs, spermatheca, and male sperm sac, but only once in ten trials from the testes (Nault et al., 1983; L. R. Nault and D. T. Gordon, personal communication). In experiments in which individual organs were assayed for NP at various times after acquisition, NP was detected first in the midgut and later in the ovaries and salivary glands; in all three organs the amount of NP increased with time. The presence of high concentrations of NP in the salivary glands was highly correlated with the ability of the insect to be a vector of MStpV, further associating the NP with infectivity. Persistent transmission, transovarial passage, and impaired fecundity strongly suggested that the infective virus replicated in P. maidis, whereas the increase of NP with time demonstrated replication in P. maidis (D. T. Gordon and L. R. Nault, personal communication). Thus, an additional link was established between the infective agent and NP.

THE RELATIONSHIP BETWEEN MAIZE STRIPE AND RICE STRIPE VIRUSES

The rice stripe virus (RSV) from Japan is similar to MStpV in many ways. RSV, like MStpV, was first reported to have an isometric particle (Kitani and Kiso, 1969), but more recently has been described as a 3 nm diam filament that can assume several configurations. One of these was thought to be a supercoiled, circular configuration designated as a branched filamentous particle (Koganezawa et al., 1975; Koganezawa, 1977). Other configurations included an open circular form and linear fragments. The branched filamentous particles were about 400 nm long and the contour length of the open circular form and the longest linear form was about 800 nm. The branched filamentous particles were infective as determined by injection into its delphacid vector, Laodelphax striatellus (Fallen) (Koganezawa et al., 1975). Koganezawa (1977) reported that the different zones in centrifuged sucrose density gradients contained different configurations of the 3 nm filament. Micrographs of the filaments in a linear configuration looked remarkably similar to those observed for maize stripe NP. Branched filamentous structures have not been observed for the maize stripe NP even after using the RSV purification procedure, nor have differences in filament configuration been seen from the various sucrose density gradient zones. Recently, E. Shikata (personal communication) has purified branched filamentous particles from RSV-infected rice (Oryza sativa L.) and maize by the procedure used for maize stripe NP purification. The apparent ease of isolation of branched filamentous structures for RSV and the inability of doing so for MStpV suggests that MStpV branched filamentous forms are less stable than those of RSV, or that they do not occur.

Toriyama (1982) recently described an 8 nm wide rod-shaped particle of uncertain length that occurred in extremely low amounts in RSV-infected plants and was thought to be yet another configuration of the 3 nm filament. In his report, the rod-shaped configuration, not the branched filamentous configuration, was infective. Structures in maize stripe NP preparations which may be similar to the rod-shaped form have been observed occasionally (Gingery, unpublished).

Both MStpV and RSV: a) are composed of 3 nm nucleoprotein filaments; b) are propagative in and transovarially transmitted by delphacid planthoppers; c) exhibit slow, heterodisperse sedimentation in sucrose density gradients; d) have single-stranded RNA genomes; e) have a single protein species [molecular weight (MW) of 32-33,000 daltons] associated with the 3 nm filament; and f) elicit large quantities of a noncapsid protein (MW of 16-17,000 daltons) in infected leaves (see discussion below). These similarities suggest that RSV and MStpV may be members of a new group of viruses. This conclusion is supported by recent work showing a close serological relationship between MStpV and RSV. In agar-gel double-diffusion tests (Gingery et al., 1983), MStpV and RSV antisera reacted with both viruses, with no detectable spurring of precipitation zones. In neutralization of infectivity tests, MStpV and RSV antisera both eliminated MStpV infectivity (Gingery et al., 1983). E. Shikata (personal communication) observed by electron microscopy MStpV antibody decoration of RSV branched filamentous structures.

European wheat striate mosaic virus (EWSMV) is another delphacid-transmitted, transovarially passed, viruslike disease of uncertain etiology (Serjeant, 1967). No typical viruslike particles were identified in infective fractions from centrifuged sucrose density gradients, suggesting that EWSMV may be similar to MStpV and RSV.

NONCAPSID PROTEIN

A remarkable feature of the maize stripe disease is the production of very large amounts (up to 2 mg/g fresh tissue) of a 16,300 dalton protein in MStpV-infected leaves (Gingery et al., 1981). By phase-contrast microscopy, the protein was frequently seen in expressed sap as 10-50 μm needle-shaped crystals. The crystals dissolved above pH 6.0, recrystallized at pH 5.4 or below, and isopycnically banded in CsCl gradients at 1.28 g/ml. This protein is referred to as the noncapsid protein (NCP) because it was serologically unrelated to the 32,700 dalton protein associated with the NP. A serologically similar protein was detected in MStpV-infected Rottboellia exaltata L. (Gingery, unpublished). RSV-infected rice plants also contained large amounts of an NCP that produced needle-shaped crystals (Kiso and Yamamoto, 1973). Neither the function of these NCP's nor their effect on host plants is known. The presence of
the NCP in MStpV-infected plants was the basis for an assay for maize stripe developed by Falk and Tsai (1983).

**SPECULATIONS ABOUT MStpV**

It has not yet been irrefutably established that the NP is the infective agent of the maize stripe disease despite the fact that infectivity has been demonstrated for highly purified NP. It is clear, however, that the NP is serologically related to the infective MStpV. Therefore, it can be concluded either that the NP is one of two or more components that make up the infective virus, or that the NP is the only component of the infective virus whose infective configuration is uncertain. Even in the case of RSV, which apparently is more stable than MStpV, structures associated with infectivity are not necessarily the infective form in situ, especially considering that a discrepancy exists as to whether the infective RSV is a branched, filamentous (Koganezawa et al., 1975) or a rod-shaped particle (Toriyama, 1982). Both could conceivably be infective and still be degradation products of the native virus. The above reservations, of course, apply to all described viruses. The main difference between MStpV or RSV and most other viruses is that the other viruses have structures that better fit classical ideas about virus morphology and that are observed in both purified preparations and in situ. Structures associated with maize and rice stripe are quite unusual and have not been identified in infected tissue. Such studies are needed.

It can be hypothesized that MStpV and RSV are aberrant forms of viruses that at one time had a more classical morphology. The 3 nm filament might reflect a changed attraction between coat protein and genome such that the original morphology has been altered, or the filament may be a component of the ancestral virus whose other structural components have been lost (e.g., nucleocapsid of an enveloped virus). Thus, studies comparing MStpV, RSV, and other enveloped viruses need to be done, even if the other viruses seem unlikely to be related.

The origin and function of the NCP is a mystery at this time. If it is a product of the virus, it is difficult to imagine why the energy used to synthesize the massive amounts of NCP found has been expended for a protein which no longer serves a vital function. Of course, the NCP may play an important but unrecognized role in the maize stripe disease cycle. One hypothesis suggests that NCP was at one time a virion structural protein that is now no longer functional. During this alteration, the regulation of its synthesis may have been altered also and it now accumulates unrestricted. If this is true, one would expect NCP to increase in *P. maida* also. However, Falk and Tsai (1983) were unable to detect NCP in viruliferous *P. maida*.

**LITERATURE CITED**


présente sur mais en Guadeloupe et transmise par le delphacide


Origins of Leafhopper Vectors of Maize Pathogens in Mesoamerica

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ABSTRACT


Two auchenorrhyncha homopterans, the corn leafhopper (Dalbulus maidis) and the corn delphacid (Peregrinus maidis), are responsible for vectoring five maize pathogens in Mesoamerica. The leafhopper transmits the corn stunt spiroplasma (CSS), the maize bushy stunt mycoplasma, MBSM, and the maize rayado fino virus (MRFV). The delphacid vector transmits the maize mosaic virus (MStpV) and maize stripe virus (MStpV). Evidence strongly suggests that the corn leafhopper and its Dalbulus relatives have a long history of association with maize (Zea mays) and its teosinte (Zea spp.) and gamagrass (Tripsacum) ancestors in Mexico. The corn leafhopper and seven other Dalbulus leafhoppers have been collected from these grassy relatives of maize over the past 1 yr in Mexico. Three of these species were discovered for the first time, and the first definitive field hosts were discovered for three other species. Laboratory studies have provided evidence that two species, the corn leafhopper and the Mexican corn leafhopper (D. elimatus) are maize specialists and behave like r-selected species. When compared to D. gelbus, whose field hosts are several perennial Tripsa-

Fewer than 10 viral and mycoplasmal diseases of maize (Zea mays L.) are known to cause serious or potentially serious losses to maize in the Americas. Two insect species are responsible for the transmission of five pathogens that incite five of these diseases. The first of these, the corn leafhopper [Dalbulus maidis (DeLong & Wolcott)], transmits the maize rayado fino virus (MRFV), the corn stunt spiroplasma (CSS), and the maize bushy stunt mycoplasma (MBSM). The second vector, the corn delphacid [Peregrinus maidis (Ashmead)], transmits the maize mosaic virus (MMV) and the maize stripe virus (MStpV). The corn leafhopper occurs only in the Americas, from the southern U.S. to Argentina in South America (Oman, 1948; Nault and Knoke, 1981). The three maize pathogens vectored by D. maidis occur throughout the range of their vector. The corn delphacid occurs worldwide in tropical and subtropical regions, as do MMV and MStpV. The recent discovery that these five pathogens cause most of the principal maize viral and mycoplasmal diseases in Peru (Castillo and Nault, 1982; Nault et al., 1979, 1981), and perhaps in the rest of Latin America, raised questions concerning the origins of these pathogens and their vectors. Answers to these questions could reveal critical information on the biology and ecology of vectors and pathogens and, hopefully, lead to new approaches for disease control. In the following discussion I shall concentrate on the difficult task of vector origins. Informa-
tion concerning pathogen origins is scant and interpretation of the data is highly speculative.

**ORIGIN OF DALBULUS MAIDIS**

High populations of *D. maidis* occur on maize throughout its range at both low and high altitudes. Corn stunt, the most important of the three diseases associated with the vector, is highly damaging to maize from low-lying areas of the Pacific coast of Central America (R. Gamez, personal communication) to the high valleys of the Peruvian Andes (Nault et al., 1981). However, neither Central nor South America is a likely site of origin for *D. maidis*, and recent evidence suggests that the high valleys of Central Mexico are its place of origin and that its evolution is closely tied to the wild ancestors of maize that also occur in this region.

Until I began my investigation in 1979, little was known about leafhoppers in the genus *Dalbulus* with the exception of *D. maidis* and the Mexican corn leafhopper, *D. elimatus* (Ball). The latter species is known principally from Mexico, the southwestern U.S. (Barnes, 1954), and possibly Central America (R. Gamez, personal communication). *D. maidis* was originally described by DeLong in the genus *Cicadula* (DeLong and Wolcott, 1923) but was later placed in *Dalbulus* by Dorst (1937). DeLong (1950) erected the genus *Dalbulus* and separated it from *Dalbulus* by characteristics of the male aedeagus and the shape of the head (vertex). *Dalbulus* was represented by *D. elimatus* (the genotype), *D. maidis*, and four Mexican species DeLong described at that time: *D. guevarai* DeLong, *D. longulus* DeLong, *D. gelbus* DeLong, and *D. acus* DeLong. Left in *Dalbulus* by Dorst were *B. montanus* Oman (the genotype) from southern Arizona and two Mexican species described by DeLong: *B. bilineatus* DeLong and *B. tropicus* DeLong. Of the nine leafhopper species, hosts were known for only *D. maidis* and *D. elimatus*. The others were swept from unknown grasses by DeLong, Oman, and other collectors.

Barnes (1954) conducted an extensive survey of leafhoppers on maize in Mexico in the early 1950's and found that *D. maidis* was most prevalent at low elevations, whereas *D. elimatus* was found principally at high elevations. Low numbers of *D. gelbus* and *D. longulus* were also collected, but there was no evidence that they were breeding on maize. Later Ramirez et al. (1975) collected *D. guevarai* from maize plots at the Tlatizapan, Morelos Experiment Station of the International Center for the Improvement of Maize and Wheat (CIMMYT). The species was successfully reared on maize in the laboratory and used as a vector of one of the corn stunting mollicutes (either CSS or MBSM). Indeed, *D. guevarai* was later experimentally used as a vector of *Meso Central corn stunt* (= MBSM) by Granados and Whitcomb (1971).

*Tripsacum* (gamagrass) along with the teosintes (*Zea* spp.) are the closest relatives of maize (Doebly, 1983; Doebly and Itis, 1980; Galinat, 1977; Itis and Doebly, 1980; Wilkes, 1972). The gamagrass and teosinte species are more abundant in southern Mexico than elsewhere. Moreover, I noted that the Mexican *Baldulus* and *Dalbulus* described by DeLong in 1950 had a distribution that overlapped that of wild relatives of maize (Nault and DeLong, 1980). I proposed the hypothesis that these leafhoppers utilize teosinte and gamagrass as hosts and that these insects have a long history of association with these grasses, perhaps co-evolving with them. Field studies in Mexico (Nault, 1983, unpublished; Nault and DeLong, 1980; Nault et al., 1983) as well as laboratory studies (Nault, unpublished) strongly support this hypothesis.

From 1979 through 1982 *Tripsacum*, maize, and the teosintes were examined for presence of leafhoppers in seven Mexican states: Durango, Mexico, Morelos, Guerrero, Jalisco, Oaxaca, and Veracruz. Eight *Dalbulus* spp. were collected, including three new species (Table 1, Fig. 1) (Nault, unpublished; Nault and DeLong, 1980; Nault et al., 1983), but no *Dalbulus* leafhoppers were taken. *D. maidis* and *D. elimatus* were the two most commonly collected species from maize and teosinte. Also occasionally found on maize were *D. guevarai*, *D. gelbus*, and *D. longulus*. Interestingly, *D. guevarai*, *D. longulus*, and *D. elimatus* were never found together on maize, although each occurred in mixed populations with *D. maidis*, *D. guevarai*, *D. longulus*, *D. elimatus*, and *D. guzmani* DeLong and Nault form a sister species group. Isolating mechanisms may prevent *D. guevarai*, *D. longulus*, and *D. elimatus* from occurring sympatrically (Fig. 2). At only one locality was *D. maidis* consistently taken from *Tripsacum*; the site was adjacent to maize fields where *D. maidis* was abundant. Perhaps *D. maidis* was utilizing *Tripsacum* as a feeding host only, having migrated from the nearby maize that matured and dried more quickly than the perennial *Tripsacum*.

*Tripsacum* served as a principal source of *D. guevarai*, *D. longulus*, and *D. gelbus* as well as the three new species, *D. guzmani*, *D. triopsacoides* DeLong and Nault, and *D. quinquenotatus* DeLong and Nault. There does not appear to be a host specific relationship between gamagrasses and *Dalbulus* leafhoppers, i.e., a given *Tripsacum* sp. does not harbor a specific *Dalbulus* sp. (Table 1). For example, *D. quinquenotatus* was found on six of the nine *Tripsacum* species surveyed and seven *Dalbulus* spp. were taken from *T. pilosum* Schrib. and Merrill. It is possible that certain *Dalbulus* spp. may prefer and thrive best on a specific *Tripsacum* sp., but field collections to date are insufficient to indicate this. Planned laboratory experiments will test this possibility of host preference for various *Dalbulus* and *Baldulus* spp.

In other laboratory tests we have compared the biol-
TABLE 1. Collections of *Dalbulus* leafhoppers from *Tripsacum* and *Zea* spp from 1979-1981 in Mexico.

<table>
<thead>
<tr>
<th>Plant species</th>
<th><em>maidis</em></th>
<th><em>elimatus</em></th>
<th><em>longulus</em></th>
<th><em>guevarai</em></th>
<th><em>gelbus</em></th>
<th><em>quinquenotatus</em></th>
<th><em>tripsacoides</em></th>
<th><em>guzmani</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Z. m. mays</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td><em>teosinte</em></td>
<td>+</td>
<td>+</td>
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<tr>
<td><em>T. andersonii</em></td>
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<td>+</td>
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<tr>
<td><em>T. bravum</em></td>
<td>+</td>
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* Includes *Z. m. mexicana*, *Z. m. parviglumis*, *Z. diploperennis* and *Z. perennis*.

* Nymphs as well as adults collected.

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**Fig. 1.** Preliminary phylogeny of eight Mexican *Dalbulus* leafhopper species showing lateral views of aedeagus (male genitalia). The two major groups are divided by number of head spots (two or four).
ology and behavior of three Dalbulus spp.: D. maidis, considered the best adapted maize specialist and the only species reported as a maize pest (Bushing and Burton, 1974); D. elimatus, also a maize specialist; and D. gelbus, a species occasionally found on maize but best adapted to Tripsacum. I anticipated that D. maidis and D. elimatus, which exploit annuals, would respond like "r-selected" species, whereas D. gelbus which lives on a perennial would likely behave as a "K-selected" species (Pianka, 1970; Southwood, 1977). These terms are taken from the well-known logistic equation in which the rate of change in a population (dN/dt) is related to the population (N) and the intrinsic rate of reproduction (r_{max}), as modified by an additional factor (1-N/K) which is dependent on the extent to which the population approaches the carrying capacity of the habitat (K), i.e.:

\[ \frac{dN}{dt} = N \cdot r_{\text{max}} (1-N/K) \]

Rates of reproduction are greatest when N is small compared to K and other conditions favor high reproductive rates. These rates are smallest when N approaches K and where K-selected species prevail. Although insects in general are considered r-selected and vertebrates K-selected, within a group of related insects such as Dalbulus there may be a continuum of r-to K-selected species. In the stable habitat of a long-lived perennial, such as Tripsacum, a leafhopper would be expected to establish more constant population levels.

Pianka (1970) and Southwood (1977) suggested features common to r- and K-selected species. For example, when compared to a K-selected species, an r-strategist has a more rapid rate of development and a shorter life span and generation time, is more fecund and mobile, and frequents unstable habitats.

Our tests with D. maidis, D. elimatus, and D. gelbus, all performed on sweetcorn, are consistent with these features. At all temperatures from 17 to 32 C, D. maidis developed more quickly than D. elimatus which in turn developed more quickly than D. gelbus. At 32 C, only D. maidis survived from egg to adult. The number of eggs and resultant progeny produced by D. maidis was greater than for D. elimatus and both were significantly more productive than D. gelbus. The intrinsic rate of increase (r_{max}) was significantly greater for D. maidis than D. gelbus, with D. elimatus being intermediate. On the other hand, D. gelbus lived significantly longer than D. maidis with D. elimatus being intermediate.
Mobility was measured by counting leafhoppers taking flight in response to a mechanical disturbance; D. elimitus was significantly more mobile than D. gelbus with D. maidis being intermediate. Additional studies similar to the above are planned for D. guevartai, D. guzmanii, D. longulus, D. quinquenotatus, D. tripsacoides, and B. tripsaci, whose principal host is Tripsacum. These tests will be performed on Tripsacum as well as sweet corn. In preliminary trials, D. gelbus utilized T. dactyloides as a host better than did D. maidis.

Seven Dalbulus species are vectors of CSS, MBSM, and MRFV (D. guzmanii has not been tested). They are not equally efficient as vectors. One reason is that most species are highly sensitive to these plant pathogens (CSS and MBSM), i.e., they also serve as leafhopper pathogens (Madden and Nault, 1983; Nault, unpublished). With the exception of D. maidis, CSS is pathogenic to all other Dalbulus spp. as well as B. tripsaci. Although pathogenic effects are less severe, MBSM reduces the longevity of B. tripsaci and Dalbulus spp. with the exception of D. gelbus and D. elimitus. Earlier Granados and Meehan (1975) showed CSS to be highly pathogenic to D. elimitus but only slightly so to D. maidis.

Several of these findings may explain, in part, why other Dalbulus species are less successful in exploiting maize than D. maidis. The abundance of D. maidis and absence of other Dalbulus spp. on maize in the low tropics may be due to its tolerance to high temperatures that have been demonstrated as lethal to D. elimitus and D. gelbus. Also, the tolerance of D. maidis to CSS in regions where the pathogen is common would place it in a favorable position to out-compete other Dalbulus spp. D. elimitus has rarely been collected at low elevations where CSS is common but is frequently found at high elevations in Mexico where CSS rarely occurs. Unlike CSS, the distribution of MBSM in Mexico is more common at high elevations. MBSM is pathogenic to D. maidis but not to D. elimitus. The differential susceptibility of D. maidis and D. elimitus to CSS and MBSM could be an important factor in their distribution. Other Dalbulus spp. can avoid CSS and MBSM by remaining on Tripsacum which is immune to both mollicutes (Nault, 1980). Lastly, the relatively higher intrinsic rates of increase for D. maidis and D. elimitus on maize compared to that of D. gelbus and perhaps the other Dalbulus spp. can explain their success as important maize herbivores.

Much more field and laboratory work is needed before questions concerning the origins and distribution of Dalbulus spp. can be answered. Clearly, Mexico is a center of diversity for the genus. However, no surveys for leafhoppers on Tripsacum have been made in central or South America. Northern South America (Peru, Colombia, and Equador) is a second center of diversity for Tripsacum (de Wet et al., 1981) and may harbor as yet undiscovered Dalbulus spp. Among Dalbulus spp., only D. maidis is known to feed on maize in Peru (Nault et al., 1979, 1981). However, DeLong collected and co-described a leafhopper, Picchusteles inca Linnouvairi and DeLong (1976), from grasses found near the ancient Incan City of Machu Picchu. The species is remarkably similar to Dalbulus, a point noted by Linnouvari and DeLong. Although Tripsacum has not been reported from Machu Picchu, P. inca may be an indicator of the presence of gamagrass or of a close andropogonoid relative. Clearly, the type locality of P. inca should be revisited, as well as known locations of Tripsacum in Peru and elsewhere in South America.

As noted earlier, it is far more difficult to speculate on the origins of the three pathogens vectored by Dalbulus. Davis (1983) discusses the relationship of CSS to other spiroplasmas and Spiroplasma citri in particular. If Whitcomb (1981) is correct that CSS is a "maize specialist" that originated from S. citri, then it might be quite difficult to determine when and where CSS originated. S. citri is found worldwide, has a broad host range, and is transmitted by a number of deltocephaline leafhoppers. If we assume that CSS is a variant of S. citri, then this specialization took place somewhere in the neotropics. I suspect that a deltocephaline leafhopper carried S. citri from an unknown dicotyledenous host to maize or teosinte. Once in Zea, S. citri adopted an indigenous Zea dwelling leafhopper as its vector. This leafhopper was very likely D. maidis. Perhaps at first CSS was highly pathogenic to D. maidis as it currently is to other Dalbulus spp. With time, resistant mutants appeared as now exist today. My guess is that as pre-Columbian man domesticated maize and carried it with him throughout the Americas, D. maidis followed, carrying CSS with it. D. maidis and CSS may have been the first pest and disease to plague the early domesticated maize (Nault, 1983). These speculations are far from satisfactory; nevertheless, they form a basis whereby future studies can be structured.

Even less can be said about the origins of MBSM and MRFV. MBSM has not been cultured and nothing is known about its relatedness to other mycoplasmalike organisms. MBSM has a host and vector range that is narrower than that of CSS (Nault, 1980). As for MRFV, only one other plant virus, oat blue dwarf, is considered to be a distant relative (Gómez, 1983). The two viruses have distinct host ranges and leafhopper vectors. Of the three maize pathogens vectored by Dalbulus, MRFV is the only one known to infect Tripsacum where it produces mild or symptomless infections (Nault et al., 1980). MRFV is less damaging than CSS and MBSM to native land races of maize in Latin America (Gómez, 1980) and is not known to be pathogenic to D. maidis (Gómez, 1983). This, along with the susceptibility of Tripsacum to MRFV, suggests a long association of the virus with maize and its relatives.

**ORIGIN OF PEREGRINUS MAIDIS**

Recently Brewbaker (1979) presented the bold but controversial hypothesis that the MMV was responsible for the collapse of the classic Maya civilization 1000 yr ago. Based on reasons given in his lengthy treatise, Brewbaker favors the notion that a devastating disease outbreak in maize, and not foreign invasion, sustained drought, holocaust, human disease epidemic, or socio-
economic decadence was responsible for the collapse. He rather casually dismisses other insect pests and diseases as being responsible and provides his rationale for selecting MMV as the cause.

A synopsis of his argument is as follows. MMV is a devastating disease transmitted by *P. maidis*, a delphacid planthopper restricted to the humid lowland tropics. According to Brewbaker, maize and teosinte are the only definitive hosts for MMV and are the only hosts on which *P. maidis* thrives. The disease is serious only where maize is grown more or less continuously throughout the year in the wet or irrigated tropics. Resistance in maize occurs only in one known form, the Mv gene, that confers a high level of resistance but not immunity. The Mv gene occurs in all seven maize races that evolved in the Caribbean but in none of the Mexican or Central American races. He proposes that MMV originated in northern South America or the southern Caribbean Islands and was spread northward to other Caribbean Islands by the Arawak Indians around the time of Christ. The sympatric origin or selection in maize of the Mv resistant mutant in this region is assumed to have allowed its incorporation into the Caribbean maize races. Brewbaker conjectures that viruliferous *P. maidis* were blown from the Caribbean, perhaps by hurricane winds, to the Peten in northern Guatemala about the time of the eighth century. The disease became epidemic in susceptible maize races, such as Nal-Tel and Tepecintle, grown by the Peten Mayas.

I will not comment on the many non-agronomic factors that may have contributed to the Mayan collapse, nor for that matter will I review Brewbaker’s dismissal of other potentially contributing insect pests and diseases. Rather I will critique only arguments that he uses to support his hypothesis. Along the way I will suggest my own hypothesis for the origins of *P. maidis*, MMV, and MStpV as well and explain why none could have been responsible for the extinction of the Maya civilization.

I cannot dispute Brewbaker’s contention that MMV can cause “devastating” disease epidemics. Such epidemics have been reported in recent times from several tropical regions of the world. I am surprised, however, that Brewbaker ignores MStpV, the other principal virus also vectored by *P. maidis* (Gingery et al., 1981). On the American continent MStpV is known to occur in Florida, Venezuela, and Peru where both MMV and MStpV occur in the same maize fields. Surely both viruses occur together in the Caribbean and the lowlands of Mexico and Central America. No doubt maize mosaic and maize stripe virus have been confused and reported by Brewbaker and perhaps others as well as one caused solely by MMV (Nault et al., 1981). The possible presence of MStpV in this region does not argue against Brewbaker’s hypothesis, but the possible involvement of MStpV should be considered. It would be particularly instructive to test the responses of the seven Caribbean maize races that contain the Mv gene, as well as the Nal-Tel and Tepecintle races, to MStpV.

A more serious weakness in Brewbaker’s argument is his insistence that *P. maidis* and MMV are maize and teosinte specialists. In the mid-1960’s *P. maidis* was reported as a pest of sorghum [Sorghum bicolor (L.) Moench] and Pennisetum typhoides (Burm.) Stapf and C. E. Hubb. in India where it was also reported as breeding on *Setaria italica* (L.) Beauv., *Echinochloa colonum* (L.) Link, and *Paspalum scrobiculatum* L. (Chelliah and Basheer, 1966; Thontadarya and Channa-Basavanna, 1968). Moreover, these authors report that sorghum was the preferred host in laboratory tests. In recent years, *P. maidis* has become a more serious pest with the introduction of new sorghum varieties that mature at different times of the year (Agriwal et al., 1981). Delphacid feeding causes death of top leaves and prevents emergence of the sorghum ear head. Secondary damage is caused by sooty molds that grow on honeydew secreted by planthoppers. Furthermore, Brewbaker should not take lightly the reports of others that *P. maidis* can utilize hosts other than maize (Namba and Higa, 1971). I have observed *P. maidis* using *Rottboellia exaltata* L. as a host in Florida and have confirmed this in laboratory and greenhouse tests (Nault, unpublished).

I also strongly suspect that the reports of MMV in other hosts are correct (Herold, 1972). The second virus, MStpV, unequivocably has a host range that extends beyond the genus *Zea*. We have confirmed that sorghum and *R. exaltata* are hosts (Gingery et al., 1981). Greber (1981) has recently isolated MStpV from one cultivated and two wild *Sorghum* spp. in Australia and has experimentally infected barley (*Hordeum vulgare* L.), rye (*Secale cereale* L.), and triticale (*Triticosecale Wittmack*).

The above evidence would suggest that *P. maidis*, MMV, and MStpV may have a stronger link to *Sorghum* and its relatives than to *Zea*. In my 8 yr collecting from teosinte and gamagrass at mid- and high elevations in Mexico, I have never encountered *P. maidis*, MMV, or MStpV.

Africa, rather than the Americas, is the likely place of origin for the corn delphacid, MMV, and MStpV. Only one other *Peregrinus* species is known, *P. icocasta* (Fennah), and it occurs in tropical Central and West Africa. There is also the African genus, *Curtometopum*, as yet only known from females of the type species, *C. turneri Muir*, that could conceivably be allied to *Peregrinus*, but its position will remain uncertain until a male is discovered.

In a letter to me dated 19 July 1979, R. G. Fennah, British Museum of Natural History, an authority on the world’s delphacid planthoppers, stated “The facts so far available suggest that tropical Africa is the genetic headquarters of *Peregrinus*, and that (P.) *maidis* has spread into India and S.E. Asia in prehistoric times and has taken the same route into the New World as the tapi or delphacids of the genus *Ugyops* (s.l.). *Saccharosyde* is a grass or sugar cane frequenting delphacid genus represented in various warmer parts of the New World by six species, and by a single species extending through Japan, Manchuria, and China. Unlike *Peregrinus*, it has no species common to Asia and America.
Its close relative, *Neomalaxa*, includes a single species that extends from Puerto Rico to Brazil. There is a case for considering *Saccharosydne* as of American origin. If *Peregrinus maidis* were American, I should rather expect a comparable situation to exist. But it doesn’t.

I take exception to only one part of Fennah’s statement, i.e., that *P. maidis* came to the New World by the same route as the tapir. He is, of course, referring to the land bridge (Beringia) that once connected the USSR with Alaska ca. 18,000 yr ago. This land mass would have been exposed during ice age conditions, yet *P. maidis* appears to tolerate only tropical climates. There would also be the problem of adaptation to hosts other than *Sorghum*, since this genus also has a tropical or subtropical distribution.

I favor another hypothesis for the introduction of *P. maidis* to the New World as well as the Oceanic Islands. I postulate that introductions occurred in post-Columbian times as corn and sorghum seed were being transported transoceanically by early travelers. While *P. maidis* cannot feed and survive on the seed itself, I have noted that the hopper can survive for 1 or more weeks on the germinated hypocotyl of maize. The *P. maidis* habit of frequenting secluded areas of its hosts would be compatible with its being a stowaway in bags of sorghum or maize seed, a few seeds of which could germinate when exposed to moisture and serve as sustenance for the hitchhiking hoppers. The situation is also ideal for transport of MMV and MStPV in that both are long-lived in their vector and MStPV transovarially passed through the egg. The adaptation to maize by the corn planthopper and the two viruses may have occurred after their introduction to the New World or may have occurred earlier on the African continent when maize was introduced in the 1500’s.

Perhaps the strongest argument for a relatively recent dispersal of *P. maidis* worldwide is the acknowledgement from Fennah (personal communication) that there is little variation (morphological) in the spc: 0 over its range of occurrence. If dispersal occurred in prehistoric or even pre-Columbian times, particularly to oceanic isla: cts that are notorious for rapid speciation of flora and fauna, then I would have expected morphological variation in *P. maidis* to occur, but it has not. More subtle genetic variation in *P. maidis* is possible and can be measured by a study of its isoenzyme patterns. Perhaps there is more variation than is apparent from a morphologic inspection.

I agree with Fennah that *Peregrinus* has an African origin and that its evolution is more closely linked to *Sorghum* than *Zea*. If *P. maidis* spread from Africa to the Caribbean by natural means in pre-Columbian times, then Brewbaker’s theory could still be valid. However, I favor the hypothesis that the delphacid was introduced in post-Columbian times to the Caribbean where native races responded to MMV by selection and spread of the Mv gene. This leaves open the question of the Maya collapse. I remain intrigued by Brewbaker’s hypothesis, and being an entomologist and plant pathologist, I favor the notion that a pest or disease played a significant role in man’s history. Brewbaker may be right in part, but he has selected the wrong insect-transmitted pathogen. I favor CSS as the responsible pathogen (Nault, 1985).

Some might argue that the debate between Brewbaker and myself is solely academic and of little consequence to agriculture and the solution of current disease problems. I would disagree! Brewbaker has properly focused attention on the difficulties of producing corn in the low Central American tropics and has discovered a significant source of disease resistance to MMV (Brewbaker, 1981). He has also unintentionally called attention to the unresolved problem of identifying the principal maize stunting pathogens in Central America. Is MMV the most important as Brewbaker suggests, or are the other pathogens discussed in this paper important as well? The answers to such questions could provide reasons for the success or failure of current and future disease control methods (such as use of the Mv gene) in Central America.

**LITERATURE CITED**


Maize Virus Disease Problems in Venezuela

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ABSTRACT


Viruses of maize (Zea mays L.) are in order of importance: maize stripe (MStpV), maize rayado fino (MRFV), maize dwarf mosaic (MDMV), and maize mosaic (MMV) viruses. The viral situation on this crop changed radically during the last 5 yr. Previously MMV was the most prevalent pathogen, whereas today it is almost impossible to find this virus, even though the vector population has remained almost unaltered. It seems that MMV was supplanted by maize stripe virus (MStpV) which, at the time of the last survey, occurred in low incidence. MRFV was recently found in this country and in a few years has become second in importance to MStpV. Meanwhile, maize dwarf mosaic virus (MDMV) remains at about the same level as before. To increase our knowledge about the virus isolates affecting maize in Venezuela and their relationship with similar viruses from other countries, we are presently involved in a program of purification and characterization of these viruses. Some of our results are presented as follows.

Maize mosaic virus. Maize mosaic was observed for many years in Venezuela. However, the causal agent was only identified as a plant rhabdovirus in 1950 (Herold et al., 1960). Until a few years ago, MMV was the most important virus disease affecting maize in Venezuela. However, this situation has changed radically during the last 5 yr and today it is almost impossible to find this virus in the fields even though populations of its vector, Peregrinus maidis (Ashmead), have remained constant. We believe that MMV was supplanted by MStpV, since this virus is now the most prevalent and both share the same vector.

MMV is still important in other areas of the Caribbean. The virus was found in Guadaloupe and French Guyana and positively identified by electron microscopy, insect transmission, and serology (Migliori and Lastra, 1980, 1981), and also recently reported in Mexico (Rocha Peña et al., 1982). The isolates seem to be related serologically to the Venezuelan virus. MMV was also identified from samples collected in experimental fields at the Universidad del Valle in Cali, Colombia. At present, MMV is of little economic importance in Venezuela. However, it is important in neighboring regions. Furthermore, the situation could change again in the near future since both the virus and the vector are present in the country, but for reasons not understood, P. maidis at the moment is transmitting MStpV and not MMV.

The causal agent of maize mosaic was purified and some of its characteristics studied. Purification resulted in bacilliform virus particles which tend to break down at one end. The virus reached a high concentration in plants and the yield of purified particles was ca. 0.05 mg/g of tissue. P. maidis injected with purified virus was able to transmit the virus to healthy plants as indicated by development of typical symptoms. The virus particle has a density of 1.18 g/ml in sucrose density gradients and its nucleic acid was identified as a single-stranded RNA by the Pederson's diphenylamine method and by its sensitivity to nucleases. The virus was not a good immunogen; however, a serum was obtained with a titer of 1:32 in agar gel double diffusion tests (Lastra and Acosta, 1979).

Analysis of the viral protein by polyacrylamide gel electrophoresis (PAGE) identified five different constitutional proteins with the following molecular weights: 150,000 daltons corresponding to L protein; 75,000 daltons corresponding to G protein; 56,000 daltons corresponding to N protein; 45,000 daltons corresponding to M protein; and 30,000 daltons corresponding to S protein.
to Ns protein; and 33,000 daltons corresponding to M protein. These values were calculated from data of 10 different experiments using 7.5, 10, and 12% polyacrylamide gels. The G protein with a molecular weight of 75,000 daltons is actually a glycoprotein as demonstrated by a positive reaction with the Schiff reagent (Lastra, unpublished) (Fig. 1). These values are within the range for other plant rhabdoviruses affecting the Poaceae (Gramineae) (Jackson et al., 1981).

Rhabdoviruses have been reported recently from maize in several countries such as the U.S., Australia, and Spain (Jackson et al., 1981). However, a definitive proof is lacking that they are the same as MMV. Therefore, further research is needed to clarify the distribution of this virus and its importance as a maize pathogen in other countries.

Maize stripe virus. The disease was first reported in Venezuela in 1974 and its pathogen named maize hoja blanca virus (MHBV) (Trujillo et al., 1974). The causal agent was reported to have an isometric virus particle with 55-60 nm diam. However, in spite of many trials for several years, we were not able to find these particles associated with infected tissue, neither in leaf dip preparations, in situ by electron microscopy of thin sections, nor after purification of infected tissue. Since the symptoms of MHBV were quite similar to those reported in the literature for MStpV and both viruses are transmitted by P. maidis, serological studies were carried out at Wooster to determine the relationship between them. Using an antiserum prepared against an isolate of MStpV from Florida, it was found that MHBV and MStpV were related, if not the same virus (Gingery et al., 1979). We think that the virus particles mentioned as the causal agent by Trujillo et al. (1974) could be the same virus particles as found by Herold et al. (1960) in maize plants and by Herold and Munz (1967) in latent infections of P. maidis. This virus has the same size and shape as the particle mentioned for MHBV. Furthermore, virus particles were found in natural planthopper populations infecting all parts of the insect, including the salivary glands. Therefore, some of those particles could pass into the maize plant during feeding and, by chance, be detected by electron microscopy and thus give the impression of being implicated in the viral disease. Insects carrying the latent virus were kept for generations on healthy maize plants and the latter never showed symptoms, indicating that the latent virus does not infect maize plants, at least not Venezuelan lines.

The incidence of MStpV was not very high in 1976 (Lastra, 1977). Since then it has become the most important maize virus in Venezuela, displacing nearly completely MMV. The reason for this is not understood, but since they both share the same vector, either this insect preferentially transmits MStpV or plants acting as virus reservoirs are preferentially infected with MStpV rather than MMV.

Because of the increasing importance of MStpV, we are presently involved in research to characterize the Venezuelan isolate of this virus. A nucleoprotein was isolated from infected plants that was absent in non-infected controls. The purification procedure was a modification of that used by Gingery et al. (1979). Two bands were found after CsCl isopycnic centrifugation. The lighter band showed two peaks after electrophoresis in polyacrylamide gels, with molecular weights of 93,000 daltons and 53,000 daltons. However, the heavier band only showed a peak at 33,000 daltons. The absorption spectrum of the bands was characteristic of a nucleoprotein with a maximum between 261-262 nm and a minimum at 239-242 nm and a 260/280 ratio of 1.38. Nucleic acid isolated from this fraction was identified as ribonucleic acid by the Pederson's diphenylamine method and migrated as a single band after electrophoresis on agarose gels. The buoyant density of the nucleoprotein in CsCl was 1.30 g/ml (Lastra, unpublished). From the above data, we can conclude that this nucleoprotein is very similar, if not the same, as that demonstrated by Gingery et al. (1981) for the Florida isolate of MStpV.

![Fig. 1. Electrophoresis of maize mosaic virus (MMV) proteins in 10% polyacrylamide gels. The molecular weight (MW) of the protein bands corresponds to the average of 10 experiments using different gel concentrations. Markers with kilo daltons in parentheses are: phosphorylase b (94 k), albumin (67 k), ovalbumin (45 k), carbonic anhydrase (30 k), trypsin inhibitor (20.1 k), lactalbumin (14.4 k).](image)
Attempts to visualize this nucleoprotein by means of electron microscopy were unsuccessful, even after fixation of the material at different steps during purification.

**Maize rayado fino virus.** MRFV is an important maize virus in the tropical areas of the American hemisphere. It was reported from Costa Rica in 1969 and subsequently was found in several countries ranging from Uruguay to the U.S. (Gamez, 1980), but had not been found in Venezuela. However, during a 1979 survey for MMV, some plants with symptoms similar to MMV failed to yield rhabdovirus particles when examined by electron microscopy. Furthermore, serological tests using antiserum against MMV were negative. This virus was identified as MRFV by transmission with *Dalbulus maidis* (DeLong & Wolcott), the presence of isometric particles 33 nm in diam in both leaf-dip preparations and sections of infected tissue, and by a positive serological reaction against antisera prepared to MRFV from Costa Rica (supplied by R. Gamez) and rayado fino Colombiano del maiz (supplied by G. Martinez) (Lastra and Uzcategui, 1980).

The Venezuelan isolate of MRFV was characterized and compared with the Costa Rican strain (Leon and Gamez, 1981). After purification, the virus showed two distinct bands in centrifuged sucrose density gradients. The upper band was composed mainly of defective virus particles which lacked nucleic acid and with a buoyant density of 1.29 g/ml. The lower band was mostly composed of complete virus particles with a buoyant density of 1.40 g/ml. The virus particles were isometric with diam of 30-33 nm. The nucleic acid was identified as ribonucleic acid by the Pederson’s diphenylamine method and its molecular weight was determined as approximately 2 x 10^6 daltons by electrophoresis in 2.4% polyacrylamide gels. The viral protein was difficult to dissociate completely and several bands could be seen after PAGE. However, a consistent band was found with a value of 21,000 daltons, and this we considered the structural capsid protein. Several other bands with values of 44,000, 64,000, and 89,000 daltons were also found, but these are probably dimers or trimers, etc. of the main capsid protein component (Carballo, 1980).

Antiserum against the Venezuelan isolate of MRFV reacted positively against the antigen from Costa Rica.

MRFV is widespread in Venezuela and is presently the second most important virus after MStpV. The virus is prevalent in regions around 400 m above sea level. However, in Costa Rica and Colombia it seems to be more prevalent in regions over 1000 m.

**Maize dwarf mosaic virus.** This virus is frequently found infecting maize, sugarcane (*Saccharum officinarum* L.), and sorghum (*Sorghum bicolor* (L.) Moench) in Venezuela. The isolates present in Venezuela could easily be transmitted from one of these plants to the others. Sorghum is the most severely affected crop, but in maize the percentage of infection is similar to that reported in 1977 (Lastra, 1977). However, the virus is commonly found in association with both MStpV and MRFV and probably acts synergistically with both. When the plant is infected solely with MDMV, it is somewhat dwarfed but to a lesser degree than with the other viruses. We believe that as a pathogen of maize, MDMV is less damaging than any of the other three viruses.

**Corn stunt.** This disease is caused by a spiroplasma and the strain found in Venezuela is probably the Rio Grande. However, it is only occasionally found and does not seriously affect the crop in Venezuela (Lastra, 1977).

**DISCUSSION**

Viruses affecting maize in Venezuela radically changed over the last 5 yr. At the time of the last International Maize Virus Disease Colloquium and Workshop in 1976, MMV was the most prevalent virus and MRFV had not been reported. However, after extensive surveys during the last 2 yr, it was almost impossible to find any plants infected with MMV. Plants with symptoms similar to MMV were always shown to be infected with MRFV. These two diseases are rather difficult to diagnose since at least in Venezuela they both exhibit similar parallel striping and stunting, the only differences appearing to be that plants infected with MMV tend to have the top bent over, whereas plants infected with MRFV grow more erect. Otherwise, the only way to distinguish the two diseases is by electron microscopy, serology, or by transmission with vectors, since MMV is transmitted by *P. maidis* and MRFV by *D. maidis*. A practical approach for field diagnosis is to look in the whorl of the leaves for which of these two insects is present and base diagnosis on the vector present as well as symptoms.

The other big change in the viral complex of maize in Venezuela is the increase in incidence of MStpV. Five yr ago MStpV was present but in low incidence; today it is the most prevalent maize virus in this country and we believe that it almost completely displaced MMV. This could be related to both viruses being transmitted by the same vector and either there is a preference for transmission of MStpV by *P. maidis* or more wild hosts are naturally infected with MStpV than with MMV. In this latter case the vector could more readily acquire MStpV for transmission to maize and, once established in the crop, could spread more readily from one field to the next.

The maize viruses mentioned are usually present as mixed infections under field conditions, and these complexes can be quite severe if there is not some tolerance in the cultivated maize lines. During 1978, we tested 200 cultivars of sweet corn (kindly supplied by R. W. Toler from Texas A & M Univ.) which became severely diseased with these viruses. It appears that the Venezuela maize lines have some tolerance, since they survive and thrive despite this menace.
LITERATURE CITED


Present Knowledge of Virus and Mollicute Diseases of Maize in Peru

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ABSTRACT


The Andean mountain ranges determine the three major physiographic zones of Peru. They are the Coast, the Sierra, and the Selva. Maize (Zea mays) is grown all year in the irrigated valleys of the Coast. Both flint and floury cultivars are grown, principally for grain production. Surveys since 1978 for virus and mollicute diseases have revealed the presence of maize chlorotic mottle virus (MCMV), maize rayado fino virus (MRFV), maize mosaic virus (MMV), and maize dwarf mosaic virus (MDMV). All but the last virus may cause serious damage to maize within the coastal region.

In the Sierra, maize is grown seasonally from 2400 to 3500 m above sea level. Floury cultivars are grown mostly for human consumption. In addition to MCMV, MRFV, and MMV, the corn stunt spiroplasma (CSS) and the maize bushy stunt mycoplasma (MBSM) have been identified from this region. The two mollicutes cause a serious disease known as puca poncho in the department of Ayacucho.

The Selva is formed by the lowlands descending the eastern Andean slopes to the Amazon River basin. Only 8% of Peru's population live there, but the region, which encompasses 25% of Peru's territory, is being considered for greatly expanded agricultural use. The presence of MRFV, MMV, CSS, and the maize stripe virus may hamper plans to increase maize production in this region.

Peru is the third largest country in South America, with an area of more than 1,350,000 km². Its geographical location is essentially equatorial, nearly reaching the equator at the extreme northern point. Peru has been an agricultural country since long before the extensive Inca empire was formed. The organization of this empire was linked to high technical progress, achieved by the earlier civilization of farmers, and eventually led to a centrally, state-planned agriculture. This situation changed after the Spanish Conquest, when a large proportion of the Indian population was displaced as forced labor into the mines. This continued until well into the Republic Periods (Grobman et al., 1961).

During the 1960's, an average of 60% of Peru's 14 million inhabitants were engaged in agriculture. This amount diminished to 42% by 1981. Among other factors, this diminished percentage was caused by population growth in urban centers, lack of incentives for farmers, and industrialization of some areas of the coast. This resulted in a continuous flow of inhabitants from rural areas to the cities, creating serious social and economic problems.

DISEASES OCCURRING IN PHYSIOGRAPHIC REGIONS OF PERU

The Andean mountain ranges cross the country on a wide arch parallel to the coastline, from the southeast to the north, setting the background for the highly complex physiography of Peru. Three main natural regions are determined by the Andes: a) the Coast, b) the Sierra or the highlands that include the mountains and high plateaus, and c) the Selva or eastern lowlands (Fig. 1). The climate of these regions is defined by the Andean systems, the tropical location, and the cold Humboldt Current of the Pacific Ocean. A purely tropical climate typical for this location prevails only in the Selva where the Andean system or the tropical location or both exert their influence (Grobman et al., 1961).

The Coast. The Coast is the region extending in elevation from sea level to an altitude of 1000-1500 m. It comprises 12.5% of the territory of Peru and almost 50% of its population live there. Almost the entire coastal area, of which there is nearly 13 million hectares (ha), is a desert. Of this, only about one half-million ha are under cultivation. The cultivated areas are distributed along 52 river valleys. These rivers originate in lagoons at the snow level of the western range of the Andes or in small watersheds with very low precipitation and empty into the Pacific Ocean. Their water supply with few exceptions is meager and limits the area that can be successfully cultivated in each valley.

The climate in this zone is mild, characterized by a high relative humidity and the virtual absence of rain. The Humboldt Current, running parallel to the coast, lowers the temperature of the ocean and the surrounding air and produces condensation of moisture into a low canopy of clouds which may extend more than 100 km out over the ocean and 10-15 km inland for a period of several months, usually from May to November.
Fig. 1. Principal maize growing regions in Peru.
Cotton (*Gossypium hirsutum* L.), sugarcane (*Saccharum officinarum* L.), corn (*Zea mays* L.), potatoes (*Solanum tuberosum* L.), vegetables, and some tropical fruits can be grown on the coast. Two crops of corn, involving flint hybrids and floury cultivars for grain, and up to four crops for fresh consumption and forage per year are possible in the valleys given sufficient water for irrigation.

The valleys of the central coast from Lima, Chillon, Chancay, and Huaral up to the northern Barranca Valley were surveyed for maize virus diseases in 1978 (Nault et al., 1979). Maize chlorotic mottle virus (MCMV) (Castillo and Hebert, 1974), maize rayado fino virus (MRFV) (Gamez, 1977; Nault et al., 1979), maize mosaic virus (MMV) and maize dwarf mosaic virus, strain A (MDMV-A), (Nault et al., 1979) have been identified. Under favorable conditions for maize growth, virus spread depends upon vector abundance; when this occurs high disease incidence results.

All flint and floury genotypes are susceptible to MCMV; the floury cultivars Pardo, Chancayano, and Blanco Urubamba are most susceptible. In 1981 in the Chillon Valley, an entire field of the maize cultivar Pardo had to be converted into forage due to an early infection by MCMV that left little or no grain yield (V. Noriega, personal communication).

Clear symptoms of MCMV can be seen during the fall and winter plantings, whereas in the summer chlorosis is mostly restricted to the base of the leaves as a bright yellow mottle. At least two beetle species of the genus *Diabrotica* (Fig. 2) have proven to be vectors of MCMV (E. Reyes and Castillo, unpublished). Both nymphs and adults of *D. viridula* Auct. reared on corn served as vectors. A second species, *D. decempunctata* (Latrielle), was collected from the field and demonstrated to be a vector. *D. viridula* is the species most prevalent in the corn fields of the Chillon, Chancay, and Huaral up to the Barranca Valley. It is likely that infections occur early in fall plantings when *Diabrotica* populations are still high. MCMV has also been transmitted by leaf and root contact (E. Reyes and Castillo, unpublished). Another possible means of pathogen spread is from infected crop residue to corn seedlings by rootworm larvae (Uyemoto et al., 1981). Species of *Setaria, Panicum,* and *Digitaria,* common weeds in the corn fields of the coast (Castillo and Helfgott, 1981) and susceptible to MCMV, could act as reservoirs or alternate hosts for virus spread.

MMV was tentatively identified by observation of rhabdovirus particles in leaf sections from plants showing maize mosaic symptoms (Nault et al., 1979). Its spread is dependent on the only known vector *Peregra-
nus maidis Ashmead. However, attempts to transmit this virus with P. maidis from corn to corn have failed (Castillo, unpublished). Alternate weed hosts could act as MMV sources in Peruvian maize fields. Setaria spp. are common weeds, but have not been evaluated yet as virus hosts. A high population of the vector occurs during the summer and fall months when averages up to 50 adults per leaf have been found. The temperatures most favorable for P. maidis are 20 to 22.5°C (R. Marin and J. Sarmiento, personal communication). All flint and floury cultivars presently grown along the coast are susceptible. In 1980 a variety trial with a genotype containing a brachytic gene was observed having almost 100% infection.

MRFV occurs less frequently than MMV. Dalbulus maidis (DeLong and Wolcott), its vector, appears to be better adapted to lower temperatures than P. maidis. The highest population of D. maidis could be found during summer and fall. However, this population does not drop to the same level as that of P. maidis. D. maidis has been collected throughout the winter in low numbers (Marin, 1981).

MDMV-A has been observed causing mottling in corn leaves and occurs mostly during winter and cold springs. Several species of aphids could be its vectors, but the most frequently occurring aphids are Rhopalosiphum maidis (Fitch) and R. padi (L.) (Sarmiento, 1981). An unusually severe outbreak of a mottling disease was observed during the winter and spring of 1981. It was believed to be caused by MCMV, but it turned out to involve MDMV-A. The main alternate host of this virus is "Gruma china" [Sorghum halepense (L.) Pers.], which is a common weed in corn fields on the coast and which may be infected with MDMV-A (Nault et al., 1979). Continuous growth of maize and alternate hosts should provide an optimum environment for both vector multiplication and virus spread. Fortunately, the deserts located between the valleys seem to act as barriers for spread of vectors from one valley to another. A survey conducted in the department of Ica (H. O’Reilly and Castillo, unpublished) has shown less incidence of virus diseases than in Lima.

The Sierra. The Sierra includes the area from about 1500 m and above on the western Andean slopes and extending along and across the entire Andean system to an altitude of about 1000 m and above on the eastern slopes. It comprises 63% of the land area of Peru and contains 43% of its population (Anonymous, 1981). The Andean mountains run south to north along the coast, forming two knobs and three to four ranges of mountains which create the major basins and interandesian valleys. The climate of the Sierra is varied. Half of the climates of the world may be found at different latitudes and altitudes (Grobman et al., 1961). A recent agroeconomic study of the Santa Valley (Callejon de Huaylas) (Turdieu, 1976) has shown that 60% of its population is engaged in agriculture, with 88% of the land units less than 1 ha. This allows for no more than subsistence agriculture. Corn is grown in this area from 2400 to 3500 m above sea level. This range is divided into three zones: a) low, from 2400-2600 m; b) interme-

diate, from 2600-3000 m; and c) high, from 3000-3500 m. This division could be adapted as a model for the other Andean valleys; where appropriate, the low zone can be extended down to 1500 m. Important corn growing areas are found in the valleys of the Sierra and all the altitudes up to 3000 m. The greatest concentration of corn is found in Cuzco in the Urubamba Valley; other important production areas are found in Cajamarca, the Santa Valley in Ancash, and in Ayacucho and Andahuaylas.

Floury cultivars are grown mostly for fresh consumption and for grain. Ayacucho is the only department where flint hybrid production exceeds that of floury cultivars. Small farmers grow corn as a main crop associated with beans (Phaseolus vulgaris L.), quinoa (Chenopodium quinoa Willd.), peas (Pisum sativum L.), squash (Cucurbita maxima Duchesne), broadbeans (Vicia faba L.), and Andean tubers (Oxalis tuberosa, Mol., Ullucus tuberosus Logn.). This mixture of crops reduces the risk of a total loss as could happen with a single crop and also introduces variety in farmers’ diets.

D. maidis, the vector of MRFV and corn stunting mollicutes, thrives from the lowest level of the Sierras up to 2600 m. Alternate hosts for D. maidis are unknown in the Sierras where corn production is not continuous. However, environmental conditions appear to be optimum for the vector to reach high populations during the summer. The incidence of MRFV has been found to be from 23 to 33% and can cause considerable loss (Fig. 3). This virus is very important in Cajamarca (San Marcos, Cajabamba, and Condebamba valleys, the latter had a 62% incidence) and Ancash (Yungay

Fig. 3. Stipple streak symptoms of maize rayado fino virus — Peruvian isolate on leaves of maize cultivar Pardo.
and Ichujuaylas). With such high incidences, MRFV is expected to be an important production constraint (Nault et al., 1981; H. O'Reilly and Castillo, unpublished).

The corn stunt spiroplasma (CSS) (Figs. 4, 5) was first identified from Peru in 1978 by Nault et al. (1979) and its presence later confirmed by electron microscopy observations of spiroplasmas in tassel sections (Castillo et al., unpublished). It is believed that CSS and maize bushy stunt mycoplasma (MBSM) are the causal agents of the puca poncho disease that causes serious damage in the Huanta Valley in Ayacucho (Nault et al., 1981). MRFV has also been reported from Ayacucho and MBSM from Ancash (Santa Valley). MMV has been reported from the Santa Valley but is not believed to be as important as other viruses in this area. MCMV has been found from 1500 up to 3500 m above sea level where it is associated with most of the mottling disease observed in the areas of Cajamarca, Ancash, and Ayacucho. In Cajamarca D. decempunctata has been shown to transmit MCMV. There is another Diabrotica sp. in Cuzco that transmits MCMV. Fortunately, it does not feed on corn.

The Selva. The Selva is formed by the lowlands descending from the eastern Andean ranges and spur slopes into the Amazon River basin plains. It has a climatic transition. The mountain slopes and the valleys located in it have a rather mild climate; i.e., subtropical with a variable precipitation. On the other hand, the plains are characterized by a sweltering, torrid climate with a heavy rainfall that varies from 1.3 to 2.1 m per yr. It encompasses 25% of the territory of Peru and contains nearly 8% of the country's population. Some valleys are used for agriculture, but the lowlands are an object of study to increase agricultural areas for sugarcane, corn, soybean [Glycine max (L.) Merr.], and other subtropical crops. Only the valley of Tarapoto has been surveyed for maize virus diseases (Nault et al., 1979). MMV and maize stripe virus (MStpV), both of which are vectored by P. maidis, were reported from Tarapoto as well as D. maidis transmitted CSS and MRFV. The Selva appears to be an optimum environment for both of the above vectors and this could seriously threaten plans for increased corn production in the region.

CONCLUSIONS

At the time of the first Maize Virus Disease Colloquium and Workshop in 1976, little was known about maize virus diseases in Peru (Castillo, 1977). Much has been learned since then (Nault et al., 1979; Nault et al., 1981; Castillo and Nault, 1982). A total of five virus- and two mollicute-incited diseases are now known to occur in Peruvian maize. These diseases are serious constraints to maize production in Peru. The country's rough physiography limits maize growth to the Andean Valleys on the highlands, and this combined with the deserts located between the river valleys in the coastal areas could regulate both virus spread and vector dissemination. We now know that on the Coast MCMV, MMV, and MRFV can cause heavy damage; MDMV-A

Fig. 4. Corn stunt symptoms on maize in a field near Tarapoto, San Martin, Peru.

Fig. 5. Corn stunt spiroplasma symptoms on maize in a field near Ayacucho, Peru.
is also present but its effects are not yet known. High populations of *P. maidis* and *Diabrotica* spp. are found during the summer in the coastal valleys. However, their numbers decrease during the winter. These population fluctuations are related to changes in virus disease incidences. In the Sierra valleys, virus and mollicute diseases are localized, no doubt due to their geographic isolation. For example, in Cajamarca MRFV and MBSM, and MRFV are the most damaging. In the south Cuzco appears to be free, so far, of these diseases. The absence of *D. maidis* and *P. maidis* from Cuzco accounts for the lack of the five diseases. However, *Diabrotica* is present and could spread MCMV in these regions if they should be introduced. In the Selva, MMV, MStpV, and CSS could threaten new areas for maize production.

**LITERATURE CITED**


Maize Virus Diseases in Argentina

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ABSTRACT


Maize dwarf mosaic virus (MDMV) was detected in the Argentine central maize (Zea mays) growing area in the early 1970’s and was isolated from plants with typical maize dwarf mosaic disease symptoms. No appreciable yield losses were associated with the disease. Simultaneously, another disease, later called “Enfermedad de Rio Cuarto”, was recorded for the first time in 1970 near the Rio Cuarto city, Cordoba Province. It caused losses up to 90% in some fields. At present it is economically the most important virus disease in Argentina. The causal agent, maize rough dwarf virus (MRDV), was identified recently (1982) by physical, serological, and biological methods. Results of performance trials showed that certain resistant inbred lines contributed MRDV resistance to their crosses. The third maize virus identified was maize chlorotic mottle virus, which is not important at present but represents a potential threat.

In Argentina maize (Zea mays L.) is grown mainly in what is called the “Maize Central Area” located in the east central part of the country. It extends from about 31-32 to 36-37 S latitude (about 600 km from north to south). It comprises the northeastern portion of the Buenos Aires Province, the southern part of Santa Fe Province, and the eastern part of Cordoba Province (Fig. 1). The area cultivated during 1980/81 was 4,000,000 hectares (ha) which represented an increase of 20.8% over the previous season. Maize yields on farms of this central area range from 3000-4000 kg/ha in drier seasons to 6000-8000 kg/ha in more favorable ones; the mean yield is about 4800 kg/ha. Farmers seldom use fertilizers in maize production and irrigation is not used at all. The average maize yield for the whole country during 1980/81 was 3857 kg/ha which was the highest since 1910 (Table 1). Compared to the last 5 and 10 yr averages, this represented increases of 30 and 41.1%, respectively (Fig. 2). While the U.S. is the world's largest maize exporting country with 64,800,000 tons, Argentina ranks second with 5,800,000 tons.

Maize dwarf mosaic virus (MDMV) was the first maize virus disease identified in Argentina (Docampo et al., 1973). At the same time, a maize disease, later called “Enfermedad de Rio Cuarto” (Rio Cuarto disease), was reported for the first time by Lyons and Luna (1970). The disease occurred near the city of Rio Cuarto, Cordoba. The disease caused great concern to growers due to its pronounced teratologic effects and the rate of natural spread. Disease symptoms that included morphological malformed caused observers to suspect that a virus was involved.

**MAIZE DWARF MOSAIC VIRUS**

In order to estimate the relative frequency of MDMV in maize located in different Argentine maize regions (Nome et al., 1981; Nome et al., unpublished) and as a first approach to evaluate MDMV incidence, serological identification studies were conducted using field-infected leaf samples. Samples (N=189) were collected at random from 10 maize central area locations.

| TABLE 1. The average maize cultivation and yield for Argentina during the 1979-80 and 1980-81 seasons. |
| Maize | Seasonal production |
| Area cultivated (hectares; ha) | 3,310,000 | 4,000,000 | 20.8 |
| Area harvested (ha) | 2,490,000 | 3,500,000 | 40.6 |
| Yield (kg/ha) | 2,570 | 3,857 | 50.1 |
| Production (tons) | 6,400,000 | 13,500,000 | 110.9 |
Fig. 1. Principal maize growing provinces of the "Maize Central Area" of Argentina. Dots indicate the relative intensity of maize cultivation.
Antisera to strains A and B of MDMV were used. Serological results obtained by the enzyme-linked immunosorbent assay (EIA) (Table 2) demonstrated that MDMV was present in low incidence in the maize central area and also that symptomless infections occurred.

A first attempt to measure the effect of MDMV incidence on yield and ear quality of two commercial hybrids was made by S. L. Lenardon et al. (unpublished). These workers showed that 5.8% and 10.7% disease incidence produced 100 and 300 kg/ha yield losses, respectively. The effect of incidence on ear quality was represented by differences in the weight of 1000 kernels.

RIO CUARTO DISEASE

History and geographical distribution. The Rio Cuarto disease became important in 1976 when losses up to 90% were recorded in some fields. The disease was noted in the provinces of San Luis and La Pampa in 1977. In 1978 it recurred in many areas. It was also important during 1979/80 in Anguil, La Pampa, where losses of about 30-60% were recorded. During 1980/81 and 1981/82 diseased plants were also observed frequently in the central maize growing area, a clear indication that the disease had spread. Thus, it represented a real threat to Argentine maize production.

Symptomatology. The characteristic symptoms of the disease were short internodes, small leaves with enations, and general stunting. The chronically infected plants were about one-third normal size. Proliferation of ears was also observed on the infected plants and in some cases two, three, or more at each node (Fig. 3a).

Sterility of male and female flowers occurred with severe infection. Infected plants presented the most common symptoms at kernel maturity. The lower portion of the plant and internode were aborted with few or no kernels. Proliferation of small tassels and incomplete male inflorescences were observed (Fig. 3b). Sterility of male and female flowers occurred with severe infection. Infected plants presented the most common symptoms at kernel maturity. The lower portion of the plant and internode

TABLE 2. Frequency of maize dwarf mosaic virus strain A (MDMV-A) and MDMV-B for 10 locations of the Argentine Corn Belt, based on a total of 189 random leaf samples.

<table>
<thead>
<tr>
<th>Location</th>
<th>MDMV strain detected</th>
<th>Total positive reactions</th>
<th>Positive reactions per location (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>A + B</td>
</tr>
<tr>
<td>Pergamino 1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pergamino 2</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
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<tr>
<td>Rio Cuarto 1</td>
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<td>0</td>
</tr>
<tr>
<td>Rio Cuarto 2</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Number of positive samples</td>
<td>7</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Percent of positive reaction per strain</td>
<td>5.8</td>
<td>4.2</td>
<td>7.9</td>
</tr>
</tbody>
</table>

* Serological detection by enzyme-linked immunosorbent assay for MDMV-A and -B in maize samples.
length appeared quite normal, whereas internodes and leaves of the upper portion were extremely small. Leaves were usually flecked with minute necrotic spots along the veins. The stalk was flat and glassy in appearance. The male flowers were small, erect, and sterile. Ears were small with only a few kernels. The whole plant looked darker green than normal. Plants with late infection looked normal but the ears were small and produced kernels only on the basal two-thirds of the ear. The symptoms were similar to those reported for maize rough dwarf virus (MDRV) (Lovisolo, 1971).

**Epidemiology.** The erratic behavior of disease is evident through analysis of records that showed a great variation in disease incidence according to planting date, year, and cultivar. A higher incidence of severely affected plants along the borders of maize fields as compared to their centers and also in areas invaded by weeds suggested to us that this virus was transmitted by vectors and that it was present in natural hosts.

**Crop loss.** In order to establish a correlation between disease incidence (index) and yield loss (kg/ha), a trial was conducted in the area where the disease was endemic. The results showed a regression between the two evaluated parameters, disease index and yield losses (Fig. 4). At the same time, a differential genetic response to disease was expressed by different genotypes interacting with the environment.

**Identification of the causal agent.** Electron microscopy. Ultra-thin sections of roots and stems of diseased corn showed viruslike particles from samples collected at the end of the growing season (February 1980). No virus particles were observed in either the samples collected at other times of the growing season or in healthy samples at any time. Viruslike particles were present in the parenchyma phloem cells of roots and stems. Particles were isometric and measured approximately 55-60 nm in diam. Some looked empty while others had an electron dense core approximately 40-50 nm in diam surrounded by an outer electron lucent layer 10 nm thick. Particles had the appearance of a reovirus. Smaller particles with the internal core alone were found frequently mixed with the others. Another kind of protein particle, in helical or zig-zag array with double membranes, was also seen in the same infected cell. Viruslike particles embedded in a granular matrix or viroplasm were commonly found. No mycoplasma-
like organism or helical filament was observed by electron microscopy in the samples examined (Nome et al., 1981; Nome et al., unpublished). Concurrently, Bradfute et al. (1981) detected reovirus particles and inclusions similar to those associated with MRDV in samples with maize Rio Cuarto disease.

Purification. Stems and roots of mature infected plants were used as source material to purify the reovirus. The purification process described by G. Boccardo (personal communication) for MRDV was performed in view of the morphological and symptomatological similarity between MRDV and the Rio Cuarto virus.

The purification was performed at 4°C as rapidly as possible. The tissues were homogenized in a blender and extracts were exposed to the following steps. Good clarification of tissue extracts was obtained using carbon tetrachloride and the aqueous phase was recovered following low speed centrifugation. Virus was initially purified from clarified extracts by centrifugation in a sucrose (30-70%) density gradient column and recovery from the collected virus band of centrifuged columns by high speed centrifugation.

The ultraviolet absorption spectrum of the purified virus indicated a low virus concentration and maximum and minimum absorption values at 260 and 245 nm, respectively.

SeroLOGY. Purified extracts were treated by the standard method of immunosorbent electron microscopy. MRDV antiserum was used. Isometric particles of 65-70 nm in diam were observed, indicating the presence of B spiked subviral particles (SVP). In separate experiments with maize Rio Cuarto diseased samples, Bradfute et al. (1981) found that antisera to MRDV and rice black-streaked dwarf virus adhered to reolike SVP in immune electron microscopy.

By EIA and agar gel double diffusion tests, positive immune results were obtained using MRDV-B spiked SVP antiserum and purified extracts as antigen. No reaction was obtained with MRDV smooth core antiserum in agar gel diffusion tests.

Transmission. We were unable to transmit the disease agent from infected to healthy corn by mechanical transmission, but were able to transmit it to healthy corn by contact of leaf mid-veins. The plants developed...
only short internodes, small leaves, and general stunt ing but with no tissue proliferation. Thus, we were not able to induce the whole disease syndrome by this means. Field-collected Delphacoides (planthoppers) transmitted the disease agent to healthy corn in the greenhouse. Transmission studies using single species are in progress to evaluate vector efficiency.

Hosts. Symptoms of stunting, enations, and flower distortion were observed in Digitaria sanguinalis (L.) Scop. occurring in maize fields affected by the Rio Cuarto disease. Ultra-thin sections of the enations showed viruslike particles similar to the ones found in diseased maize tissue.

Disease control strategies. Results of trials conducted in the Rio Cuarto area during 3 consecutive years showed that late plantings (December) allowed plants to escape the disease. To the contrary, November plantings produced the highest infection. Research is in progress to evaluate disease incidence in herbicide treated and untreated maize fields invaded by D. sanguinalis (I.N.T.A. San Luis, personal communication). This weed has been mentioned as a natural host for MRDV (Luisoni and Conti, 1970).

Results of performance trials conducted during three consecutive seasons showed that certain inbred lines tested where this disease is endemic contribute high virus resistance to their crosses more consistently than others. Crosses between highly resistant inbred lines nearly always were more resistant than crosses of resistant x susceptible lines. This is a first step in the development of hybrids with good yield and resistance to Rio Cuarto disease.

MAIZE CHLOROTIC MOTTLE VIRUS

Field observations of isolated plants showing a varied symptomatology (e.g., stunting, leaf malformation, mosaic, mottle, ring and chlorotic spots, streaks and stripes) was evidence for the presence of other diseases apart from those mentioned above. To detect and identify the pathogens of these diseases and to determine the relative frequency of their occurrence, tests were carried out using antisera for maize chlorotic mottle virus (MCMV), MRDV, and MDMV-A and -B. The tests were performed by EIA of leaf samples collected from field-infected plants. Results (Table 3) showed the presence of MCMV and constitute the first report of this virus in Argentina. Identity of MCMV was further confirmed by other serological tests (Nome, V. Jossens, and E. Dal Bo, unpublished), mechanical transmission to test plants, physical properties, morphology of purified virus particles, and synergistic effect between MCMV and MDMV.

CONCLUSIONS

The "Enfermedad de Rio Cuarto" is economically the most important virus disease in Argentina. Apparently it has been the principal corn virus disease since the early 1970's. Although the etiology was determined recently (1982), circumstantial evidence indicates it has been present since 1960. Its distribution remained limited to Rio Cuarto and surrounding areas until 1980, when field observations indicated an outbreak in maize fields located in the central maize growing area. Even now, after several years, we do not know why the Rio Cuarto disease did not expand sooner in geographical distribution.

In contrast, MDMV distribution, characterized by isolated plants with typical symptoms of the disease, has extended throughout this central area, but has been generally limited to regions invaded by johnsongrass [Sorghum halepense (L.) Pers.] its overwintering host. Strain A of MDMV is the most prevalent.

Experimentally estimated losses caused by maize virus diseases in Argentina suggest that MDMV does not cause appreciable yield loss in corn. In contrast, MRDV losses have been as high as 90% for susceptible hybrids evaluated in the endemic area. These estimates are highly variable and further experimental work is needed to achieve reliability. It is difficult to define precisely the full potential threat of MRDV to Argentine corn production.

Recently MCMV was detected in Argentine corn fields. This virus has been involved in an economically important disease, corn lethal necrosis, in the U.S.

TABLE 3. Detection of maize viruses by enzyme-linked immunosorbent assay.

<table>
<thead>
<tr>
<th>Virus antiserum*</th>
<th>Samples with positive reaction</th>
<th>Percent of total samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDMV-A</td>
<td>105</td>
<td>42.3</td>
</tr>
<tr>
<td>MDMV-B</td>
<td>25</td>
<td>10.1</td>
</tr>
<tr>
<td>MDMV-A and -B</td>
<td>28</td>
<td>11.3</td>
</tr>
<tr>
<td>MCMV</td>
<td>8</td>
<td>3.2</td>
</tr>
<tr>
<td>MRDV</td>
<td>10</td>
<td>4.0</td>
</tr>
<tr>
<td>MDMV-A or -B + MRDV</td>
<td>9</td>
<td>3.6</td>
</tr>
<tr>
<td>Total positives</td>
<td>185</td>
<td>74.6</td>
</tr>
</tbody>
</table>

* Enzyme-linked immunosorbent assay with antisera to maize dwarf mosaic virus strain A (MDMV-A) and strain B, maize chlorotic mottle virus (MCMV), maize rough dwarf virus (MRDV), using as antigen extracts from 248 maize leaf samples with virus symptoms.

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Corn Belt. This very damaging disease is caused by joint infection of MCMV with MDMV. MCMV poses a potential serious danger to Argentine corn production.

**LITERATURE CITED**


Diseases of Maize Caused by Viruses and Mycoplasmalike Organisms in Brazil

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ABSTRACT


Virus and mycoplasmalike organism (MLO) diseases usually represent minor problems to the maize (Zea mays) crop in Brazil. Nevertheless, due to the large expansion of the area planted and the volume of grain production, they represent a potential menace and some investigations have been conducted to identify and study the pathogens. To date the following viruses and MLO have been identified as naturally infecting maize: a) common mosaic, caused by isolates of sugarcane mosaic virus; b) Brazilian corn streak virus, identical to the maize rayado fino virus described in Central America; c) chlorotic vein banding virus, a rhabdovirus which is probably a member of the maize mosaic virus complex; d) cucumber mosaic virus, inducing chlorotic streaks; and e) both chlorotic (Rio Grande type) and red forms (Mesa Central type) of corn stunt.

Maize (Zea mays L.) among Brazilian crops occupies the largest cultivated area, with nearly 12 million hectares (ha). In 1981 production was about 21 million tons of grain (Table 1). It is grown everywhere in the country, but the southern region is where most is produced, led by Parana state where growers produce roughly one-fourth of the Brazilian maize harvest. Although the total production is enough to meet the country's demand (mainly for animal feed), the average yield (1.8 t/ha) is very low. Such low productivity stems from several factors ranging from governmental policies regarding price fixation, climatic factors, use of the best soils for other crops, and lack of proper technology, to inadequate varieties. However, the social importance of the maize crop lies in providing jobs and keeping people in the rural zones, which contribute to reducing the migratory flux toward the cities and to assuring employment in the industries connected with maize processing. Because of the crop's obvious economic importance, the Brazilian government through its agencies, such as Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), is developing serious efforts to increase both productivity and cultivated area (Alves, 1981; Lauschner, 1981; Yeganizatz, 1979).

Except for occasional outbreaks that are mostly local, diseases including those caused by viruses and mycoplasmalike organisms (MLO have not been a serious factor in yield reduction. However, since some of these virus and MLO diseases are present in most maize growing areas, they represent a potential threat and deserve attention.

In the following we will briefly present a general view of these virus and MLO problems in Brazil.

VIRUS DISEASES

Common mosaic. This was the first virus disease of maize identified in Brazil. Symptoms of the disease appear as several patterns of mosaic on infected plants. The disease is caused by isolates of the sugarcane mosaic virus (SCMV), a potyvirus. Field transmission of SCMV is by aphids. Under experimental conditions, this virus is easily transmitted mechanically. Different maize varieties and lines vary in susceptibility; therefore, it may not be difficult to incorporate resistance factors if required. Common mosaic is not frequent in commercial plantings in the state of Sao Paulo, but is more frequent in the state of Rio Grande do Sul.

A potyvirus isolate that infected johnsongrass (Sorghum halepense (L.) Pers.] and induced mosaic on maize was obtained from a sorghum [Sorghum bicolor (L.) Moench] sample sent to the authors from the state of Minas Gerais in 1973 by Robert Schaffert, but further
identification was not carried out. In 1980, the maize dwarf mosaic virus (MDMV) was identified in samples collected near Campinas, SP (O. E. Bradfute and D. T. Gordon, personal communication). Although MDMV may be present in some areas in Brazil, it has not been as important in causing losses as in the USA.

**Brazilian corn streak.** The disease, known in Brazil today by the name “rica” (Portuguese equivalent to streak), was first recorded in the country around 1947 by S. C. Arruda, plant pathologist at the Instituto Biológico, São Paulo. Arruda did not name the disease, but described its symptoms and established the transmission by an unidentified leafhopper, the description of which coincides with that of *Dalbulus maidis* (DeLong & Wolcott). The authors have been familiar with the Brazilian corn streak disease since 1950, although it was not reported until 1971 (Costa et al., 1971).

Brazilian corn streak is characterized by whitish necrotic dots or lines along the leaf veinlets of infected plants. Occurrence is common in maize fields, but usually in low proportions. When maize is planted late or between seasons, high levels of infection occur that may involve 50% of the plants. Infected plants do not show noticeable changes in growth and the leaf symptoms tend to disappear in older plants (Costa et al., 1971). The Brazilian corn streak virus (BCSV) is transmitted by the leafhopper *D. maidis* in a persistent manner, with an incubation period of 2-3 wk. Under experimental conditions, the symptoms develop about 20 days after inoculation (Costa et al., 1971). Brazilian corn streak has been recorded in the following regions: Sao Paulo (Costa et al., 1971), Federal District, Minas Gerais, Goias (Gamez et al., 1979), and Rio de Janeiro (Kitajima, unpublished). The virus has an isometric particle ca. 30 nm in diam which has been purified (Kitajima et al., 1976b). Cooperative work with R. Gamez, Costa Rica, unequivocally demonstrated that BCSV is identical to the maize rayado fino virus (MRVF), described in Central America (Kitajima et al., 1975). Presumed virions were found in parenchymatous and vascular cells, usually in the vacuole of infected plants (Kitajima et al., 1976b). In viruliferous leafhoppers, virions were found in several organs including salivary glands, commonly within membrane-bounded cavities in the cytoplasm of cells (Gamez et al., 1981).

**Chlorotic vein banding.** This disease is encountered much less frequently than the Brazilian corn streak. It is probably identical to maize mosaic as induced by rhabdoviruses in Venezuela, Hawaii, and other parts of the world, notwithstanding slight differences in virus particle measurements. Symptoms are quite conspicuous, with large chlorotic stripes along the veins of infected leaves; it might be confused with nutritional disorders, but one main difference is that in the latter the chlorotic stripes appear between the veins. It was first reported in Sao Paulo state (Costa et al., 1971), but corn chlorotic vein banding was also found in the Federal District and Manaus, AM (Kitajima and van der Pahlen 1977), as well as Recife, PE, and Rio de Janeiro (Kitajima, unpublished). The causal agent is a rhabdovirus, 250-300 nm x 70 nm; it is transmitted by the delphacid *Peregrinus maidis* (Ashmead) in a persistent manner (Costa et al., 1971). Corn chlorotic vein banding virus (CCVBV) was partially purified (Kitajima et al., 1976a) and is related serologically with the Venezuela maize mosaic virus (M. T. Lin, personal communication); in thin sections of the infected leaf tissues, CCVBV occurs within a membrane-bounded complex of cavities. Occasionally, naked inner components of the particles appear sandwiched between adjacent chloroplasts and/or tonoplasts. In the viruliferous vector, the particles were devoid of the outer membrane and were found in several organs (Kitajima and Costa, 1982).

**Cucumber mosaic virus.** Maize plants exhibiting mosaic symptoms, more conspicuous than those induced by SCMV, were found at Campinas, SP. The infected leaves presented irregularly distributed, long chlorotic streaks, either in the veins or in the interveinal parenchyma tissue. Infected plants showed a pronounced reduction in size. Recovery tests identified the pathogen as an isolate of the cucumbe mosaic virus (CMV) group. Experiments using *Rhopalosiphum maidis* (Fitch) failed to transmit CMV from cucumber (*Cucumis sativus* L.) to maize. Possibly other aphids may transmit CMV to maize in nature. The disease is very rare and presently does not justify control measures (Costa and Kitajima, 1972).

**MYCOPLASMALIKE DISEASES**

**Corn stunt—chlorotic form.** Brazilian corn stunt in the chlorotic form probably corresponds to the Rio Grande type of corn stunt described in the USA. Infected plants show large whitish foliar areas. There is some reduction in the size of the plants, but not as large as described in Mexico and the USA. There is a shortening of the internodes only in the upper parts of the plant. Branching of the stem is not common (Costa et al., 1971). MLO’s were found in the sieve tubes of infected plants and in some organs of the viruliferous vector, *D. maidis* (Kitajima and Costa, 1970), but their spiroplasmal nature has yet to be demonstrated.

The leafhopper *D. maidis* transmits the disease agent persistently, with an incubation period of about 3 wk. Experimentally infected plants took about 1 mo to exhibit initial symptoms (Costa et al., 1971).

It is quite difficult to find corn stunt—chlorotic form in a maize field grown in the normal spring/summer season. However, when maize is planted in the winter, for sweet corn or breeding purposes, incidence of corn stunt may be high and probably connected with the movement of vector populations (Costa et al., 1971).

**Corn stunt—red form.** It occurs more frequently than the former and is similar to the Mesa Central type of the corn stunt found in Mexico. Infected plants show leaves with yellowing at the edges, followed by reddening. Under conditions in Sao Paulo, there is not as much reduction in plant size as described for corn in Mexico. Branching of the stem at the basal part of the plant is rare. MLO’s were found in the sieve tubes (Kitajima and Costa, 1970). The pathogen is vectored by *D. maidis* (Costa et al., 1971).
LITERATURE CITED


Maize Viruses and Virus Diseases in Italy and Other Mediterranean Countries

Maurizio Conti


ABSTRACT


Four different viruses have been found to infect maize (Zea mays) in Italy: sugarcane mosaic (SCMV), maize rough dwarf (MRDV), maize white line mosaic (MWLMV), and barley yellow dwarf (BYDV). SCMV occurs throughout the country, causes mosaic but not dwarfing in maize, and is prevalently transmitted by the aphid Rhopalosiphum padi. The virus isolates are of the johnsongrass (Sorghum halepense)-infecting type. MRDV causes on maize the following symptoms: dark green color, severe dwarfing, leaf vein enations, and longitudinal splitting of roots. The virus is typically prevalent in the Po Valley and its natural vector is the planthopper Laodelphax striatellus. Its incidence is generally negligible but, in the last few years, new serious epiphytotics have occurred in the northwest part of Italy. The reasons for these are still under investigation, although early sowing of maize seems to be the most important. MWLMV has been found in a very small area of northeastern Italy where it infected in low percentage only one of 60 maize cultivars grown for research purposes. It was no longer detected after its initial appearance in 1978. The virus is serologically related or identical to North American isolates of MWLMV and to the "Nanisme et anneaux foliaires du maïs" described in France. BYDV infections in maize have been reported in Italy since 1980. Infected plants show slight stunting and reddening of basal leaves in autumn but are symptomless in summer. Severe damage to the crops has been observed when BYDV occurs in mixed infection with SCMV or when infected plants also suffer from insect infestation and cold weather.

SUGARCANE MOSAIC VIRUS

History and pathogen characteristics. The first evidence for the presence of SCMV in Italy came from the description of the "arroccamento striato del sorgo" (= sorghum red stripe disease) by Goidanich (1938). He excluded fungal and bacterial pathogens as incitants and suggested that the etiological agent might be a virus. This was later demonstrated contemporaneously but separately by Grancini (1957) and Lovisolo (1957), who both concluded that the viruses causing the sorghum red stripe and maize mosaic diseases were the same and closely resembled SCMV. Grancini (1957), in particular, comparing different virus isolates from Digitaria sanguinalis (L.) Scop., Z. mays, and S. vul-
showed that they were all transmissible from and to the various plant species, causing in each identical symptoms. The experimental host-range of the virus was more widely investigated by Lovisolo (1957) who found that susceptible species of Gramineae occurred in the tribes Andropogoneae [Saccharum officinarum L., Sorghum halepense (L.) Pers., S. sudanense Stapf., S. vulgare var. saccharatum and var. technicum], Maydeae (Z. mays), Paniceae [Setaria viridis (L.) Beauv.] and Festuceae (Arundo donax L.), but observed no infection in several species of the tribes Hordeae, Agrostideae, and Aveneae.

The identification of the virus was completed by Dijkstra and Grancini (1960) who detected its particles by electron microscopy and confirmed serologically that it was closely related to SCMV. The virus was subsequently reported in other Mediterranean countries such as Yugoslavia, France, and Israel and in several parts of central and eastern Europe (cf. Grancini and Mariani, 1974). The experimental host-range, properties in vitro, and particle morphology of two Yugoslavian isolates of SCMV from maize and S. halepense were studied by Stefanac (1967). Her results were very similar to those previously reported by Grancini (1957), Lovisolo (1957), and Dijkstra and Grancini (1960). Moreover, she demonstrated that the two virus isolates were serologically closely related or identical.

More recently, Tosic et al. (1977) compared two SCMV isolates from northern and central Italy, one SCMV isolate from Yugoslavia (SCMV Yu), the SCMV strains A, B, D, E, H, I, and Jg from the USA, and the maize dwarf mosaic virus (MDMV) strains A and B also from the USA. On the basis of plant reactions, electron microscopy, and serology, they concluded that both the Italian SCMV isolates from maize resembled more closely MDMV-A, SCMV-Yu, and SCMV-Jg than the other virus isolates tested. They also confirmed that the SCMV isolates which infect maize in Italy and Yugoslavia are of the type infecting S. halepense, which then can be regarded as the main overwintering host of the virus in these countries and probably also in other parts of Europe, as suggested by other authors (Dijkstra and Grancini, 1960; Grancini, 1957; Lovisolo, 1957, 1958).

**Symptoms and epidemiology.** SCMV is very common in maize and sorghum in northern and central Italy, although precise data on its incidence in the various parts of the country are not available. The symptoms in maize consist of moderate to mild mosaic, which is generally more evident in the top leaves (Fig. 1). Symptoms are clearly distinguishable in spring, when plants are at the sixth-eighth leaf stage, fade in summer, and reappear in the new vegetation produced in autumn. Low temperatures in spring may induce some necrosis and anthocyanin pigmentation in infected plants of particular maize lines (Grancini, 1957). The growth and yield of infected plants appear only slightly reduced but have never been properly compared with those of healthy controls. Severe dwarfing has never been observed in maize as a consequence of infection by SCMV alone.

Two types of SCMV-induced diseases have been described in field-infected sorghum. The former consists of a light mosaic, resembling that in maize and having the same seasonal symptoms. The latter is initially

![Figs. 1-2. Symptoms of sugarcane mosaic virus (SCMV) in maize (Zea mays) and sorghum (Sorghum vulgare). 1) Mosaic symptoms in maize. 2) Reddish-brown necrotic stripes in sorghum. (From Grancini and Mariani, 1974.)](image)
characterized by the same type of mosaic but this is then followed by necrotic stripping which becomes reddish-brown (Fig. 2), thus accounting for the original disease name (Goidanich, 1938). In the former case the disease is economically negligible while in the latter the necrotic symptoms gradually intensify and may be complicated by invading saprophytic fungi and bacteria. Plants remain seriously stunted and eventually die.

The epidemiology of SCMV in Italy and other Mediterranean countries has been little studied, and most of the data are 10 or more years old. Beside maize and cultivated sorghum species, SCMV is known to infect *D. sanguinalis*, *Setaria* spp., and *S. halepense* (Gran- cini, 1957; Lovisolo, 1957; Stefanac, 1967). *S. halepense* is unanimously considered as the principal if not the only primary source of inoculum for maize and sorghum, and a consistent correlation has been found between the presence and density of infected *S. halepense* in the field and high virus incidences in maize (Stefanac, 1967). Other studies have confirmed that the SCMV isolates from maize are all of the *S. halepense*-infecting type (Lovisolo, 1958; Tosic et al., 1977). The importance of the other known susceptible grasses as SCMV sources has been generally disregarded because they are all annual species which do not account for virus overwintering. It cannot be excluded, however, that they might be important as virus donors for the aphid vectors which transmit SCMV to the secondary, summer-sown maize crops.

Different SCMV isolates from Italy and Yugoslavia have been experimentally transmitted by the aphids *Rhopalosiphum maidis* (Fitch), *R. padi* (L.), *Myzus persicae* (Sulzer), and *Schizaphis graminum* (Rondani) (Conti, unpublished; Lovisolo, 1958; Sutic et al., 1970; Tosic et al., 1977). The retention of infectivity by fasting aphids has been shown to be up to 6 hr for *S. graminum* and up to 4 hr for *M. persicae* and *R. maidis* (Sutic et al., 1970; Tosic et al., 1977). The maximum retention time published for MDMV strains and aphid vectors tested in the USA is 4 hr, although most reports give maxima of 30 min to 2 hr (cf. Gordon et al., 1981).

Another means of transmission of SCMV is apparently by maize seeds (Kerlan et al., 1974), although it occurs rarely and in some cases could not be demonstrated (Tosic et al., 1977). MDMV-A and MDMV-B have also been reported to be seed-transmissible in maize (cf. Gordon et al., 1981); in the USA, SCMV has been found to be also transmitted through the soil (Bond and Pirone, 1970).

Lovisolo (1965) has outlined as follows the possible epidemiological cycle of SCMV in Italy. The virus overwinters in *S. halepense*, which remains a host throughout the year, and is transferred from it to the spring-sown maize and sorghum by the aphid vectors. Transmission to the summer-sown maize and sorghum can occur in July-August and the virus can be returned from the infected crops to *S. halepense* in late autumn. The most important natural vector of SCMV in the Mediterranean area is believed to be *R. padi*, based on the heavy infestations of maize by this aphid species. However, transmission experiments of SCMV from maize to maize, using winged *R. padi* as vectors according to a technique reproducing the natural conditions of transmission, gave infection rates not exceeding 6%. Preliminary results on aphid catches in a suction trap (Rothamsted Insect Survey-Suction Trap) in the Piedmont, northwest Italy, indicate that when spring-sown maize is emerging, the flight activity of *R. padi* is rather low while it is high in autumn (Conti, unpublished). These results suggest that other aphids, beside *R. padi*, may be important for field transmission of SCMV in Italy.

### MAIZE ROUGH DWARF VIRUS

History and geographical distribution. MRDV was first recorded in Italy in 1949, when severe outbreaks in North American maize hybrids caused serious problems to the growers and stimulated the early investigations on the disease (Biraghi, 1949; Fenaroli, 1949). In that period, North American maize cultivars had just been introduced in Italy and were gradually replacing old local varieties of the flint type. The latter were less productive but, as became evident later, had developed some resistance or tolerance to the virus. This suggests that MRDV has long been present in the Mediterranean area and, in fact, the virus was soon found in Israel, France, Spain, and Yugoslavia, as well as in central Europe (cf. Lovisolo, 1971). In central Europe, however, MRDV epiphytotic have never been recorded.

Although MRDV can be considered a typical Mediterranean pathogen, it is now known that other viruses closely related serologically to it do occur in other parts of the world. Two of these, rice black-streaked dwarf virus (RBSDV) and cereal tillering disease virus (CTDV), have been fully characterized and are regarded as MRDV strains. RBSDV severely damages rice (*Oryza sativa* L.) and maize in Japan while CTDV has been found in barley (*Hordeum vulgare* L.) and oats (*Avena sativa* L.) in a small area in Sweden (cf. Milne and Lovisolo, 1977). Recently, MRDV has been reported in China (Kung et al., 1981). It occurs in the northwest part of that country and in some restricted areas near the Yangtse River. The virus has been identified on the basis of symptoms in maize, electron microscopy, and identity of its vector, but not serologically. Preliminary host-range studies seem to indicate that it might be a new strain of MRDV (T.-H. Kung, personal communication).

Another maize-infecting virus resembling MRDV in many aspects has been reported in Argentina, where it causes the "Mal de Rio Cuarto" (= Cuarto River disease) (Nome et al., 1980). Although studies on this virus are still in progress, its close serological affinity to MRDV has already been proven (S. F. Nome and R. G. Milne, personal communication). These results clearly indicate that the world distribution of MRDV and closely related viruses is much wider than was believed a few years ago (cf. Milne and Lovisolo, 1977).

After the early records of MRDV in Italy, the etiology of the disease still remained obscure for several years. Even after Harpaz (1961) demonstrated that it was transmitted by the planthopper *Laodelphax striatellus*
(Fallen), the infective nature of maize rough dwarf was questioned by Q. L. Holdeman and W. O. McCartney (cf. Lovisolo, 1971). There were two contrasting opinions among plant pathologists and entomologists: one, that the disease might be induced by a virus, and the other that it could be due to insect salivary toxins. In 1966, however, Gerola et al. (1966) succeeded in detecting the virus particles in infected plant tissues and finally Koch’s postulates were fully satisfied by reproducing the disease in maize which had been inoculated with planthoppers made infective by abdominal injection of purified virus suspensions (Milne et al., 1973).

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Figs. 3-6. Maize rough dwarf virus (MRDV). 3) Virus particles showing the double protein shell. One particle (arrow) is lacking the outer shell. 4) Field-infected maize (Zea mays) with dwarfing, about 3 mo after infection. 5) Typical leaf-vein enations in naturally infected maize. 6) Roots from an infected maize plant showing longitudinal splittings caused by neoplastic proliferation of the phloem. (Photographs 3 and 6 courtesy of R. G. Milne.)
Virus characteristics and relationships with other European Fijiviruses. MRDV belongs to the Family Reoviridae which includes plant, insect, avian, and mammalian viruses. Plant reoviruses have 65-70 nm spherical or slightly angular, double-shelled particles with icosahedral symmetry, and contain a double-stranded RNA genome. The latter consists of 12 segments in plant reovirus subgroup 1 (Phytoreovirus) and 10 segments in subgroup 2 (Fijivirus). Phytoreoviruses are transmitted by leafhoppers (Fam. Cicadellidae) and bear no spikes on the outer protein capsid. Fijiviruses, which include MRDV, are transmitted by planthoppers (Fam. Delphacidae), have outer spikes, and easily lose parts of the external shell to give a spiked inner shell (Fig. 3).

The other European Fijiviruses are the Swedish CTDV, which has the same natural vector as MRDV, and oat sterile dwarf virus (OSDV). The latter virus is serologically unrelated to MRDV, occurs in northern and central Europe, and is transmitted by the delphacid hopper Javesella pellicuda F. which is an experimental vector of MRDV (cf. Milne and Lovisolo, 1977). Two other viruses, agents of the Arrhenatherium blue dwarf and Lolium enation diseases described in central Europe, are serologically indistinguishable from OSDV. Maize is apparently not susceptible to OSDV while it can be infected experimentally with CTDV, reacting with symptoms identical to those caused by MRDV.

MRDV is not transmissible by sap inoculation, using the current rubbing techniques, but I. Harpez was able to transmit it in very low percentages by needle punctures (cf. Lovisolo, 1971). The in vitro properties of the virus have been determined by injecting crude sap from maize leaf enations into MRDV-free plant-hoppers, which were then tested on indicator maize plants. Such extracts were still infective when heated for 10 min at 50°C but not at 55°C, diluted with buffered saline up to $10^{-2}$ but not to $10^{-3}$, and stored for 1 but not 2 days at room temperature, or 3 but not 5 days at 5°C. MRDV could be recovered from maize enations kept frozen at $-25$ C for 153 but not for 180 days (Conti, unpublished).

Symptoms and epidemiology. The name "nanismo ruvido" (= rough dwarf) was attributed by Biraghi (1949) to the disease caused by MRDV in maize because the infected plants become dwarfed and develop numerous vein swellings on the undersurface of leaves, rendering them rough to the touch. Such vein swellings, or enations, derive from neoplastic proliferation of phloem cells and are the most characteristic symptom of the disease. They are suitable for field diagnosis to distinguish MRDV infections from other dwarfing diseases of maize induced by other pathogens, soil deficiencies, or infestations by insects, e.g., aphids and thrips. Virus particles can easily be detected in the crude sap extracted from maize enations and negatively stained with uranyl acetate (cf. Milne et al., 1973). Using MRDV antiserum, the immune-electron microscopy decoration test can be applied to these preparations for rapid confirmation of the presence of virus.

MRDV symptoms in maize have been fully described by several authors (Biraghi, 1949; Conti, 1976; Fenaroli, 1949; Lovisolo, 1971; Milne and Lovisolo, 1977). Early-infected plants become clearly distinguishable in the field about 20-25 days after emergence. They are darker green than normal and their leaves are unusually erect and stiff. A slight dwarfing may already be evident, and some minute swellings of the leaf veins can be seen. As the symptoms develop, stem elongation is inhibited so that the reduction in growth becomes more and more evident (Fig. 4). Meanwhile, vein swellings increase in size, assuming the appearance of whitish enations protruding from the undersurface of leaves (Fig. 5). Plants infected early remain 20-30 cm high and may die prematurely, while plants infected later develop symptoms of variable intensity. Dwarfing, in particular, may be more or less severe, and the characteristic enations may appear only on the top leaves, and on ears and leaf sheaths. Another symptom induced by MRDV and common to all the infected maize plants at the end of the vegetative period is a premature reddening of leaves, starting at their edges. Plants which are infected within 20-30 days after emergence and survive until autumn produce no ears, while plants infected later can yield some ears, although these are reduced in size, bear only a few kernels, and show obvious malformation. The roots of infected plants develop longitudinal splits due to the abnormal proliferation of the phloem (Fig. 6).

The delphacid planthopper L. striatellus is the only known natural vector of MRDV (Harpaz, 1961), but the following other species transmit it experimentally: Delphacodes propinquus Fieber, Javesella pellicuda, and Sogatella vibix (Haupt) (cf. Milne and Lovisolo, 1977). MRDV propagates in the vector and can be transmitted transovarially to the offspring of infected females.

L. striatellus has two generations per year in the Mediterranean area and one generation in northern Europe. It overwinters as young nymphs in diapause. During the winter, these can be found in the weedy borders of drainage channels, in permanent meadows, and on weeds growing in uncultivated fields or along their edges. Its preferred winter hosts are Agropyron repens (L.) Beauv., Arrhenatherum elatius (L.) Presl, Cynodon dactylon (L.) Pers., and Poa annua. L. Of these, only C. dactylon has been reported as a possible host of MRDV in Israel (cf. Milne and Lovisolo, 1977), where it may serve to maintain the virus from one vegetative cycle to another. Repeated attempts to detect MRDV in this grassy weed in Italy were negative (Conti and E. Luisoni, unpublished).

During winter, MRDV survives mainly in its planthopper vector which becomes infected before entering diapause (Conti, 1972). The insects emerge from diapause in spring and turn into adults which migrate to the surrounding crops in April and May (in northern Italy). It is during this period that maize is exposed to MRDV inoculation by planthoppers which have completed the incubation period and are infective. The longer the young plants remain exposed to such hoppers, the higher the incidence of MRDV infection.
The absence of weeds in maize fields, which are all routinely treated with herbicides at sowing, prevents the insects from settling in the crops in spring. This is because *L. striatellus* does not breed on maize but feeds only occasionally on it. The planthopper breeds predominantly on several grasses of the Panicoideae and on small grain cereals which are preferred when at their early stages of growth. In June, maize crops are further invaded by adult *L. striatellus* derived from eggs laid that same spring. These become permanently established on the new weeds now growing in the crops. *Digitaria sanguinalis* and *Echinochloa crusgalli* (L.) Beauv. are the preferred food and breeding hosts of the planthopper and both are susceptible to MRDV (Conti, 1972; Luisoni and Conti, 1970). They are infected by immigrating planthoppers and become the principal source of MRDV for nymphs of the second generation. These acquire the virus, enter into diapause, and overwinter, thus perpetuating MRDV from one vegetative cycle to the next.

MRDV-infected *D. sanguinalis* plants can be easily recognized in the maize fields in autumn by their bushy appearance and the reddening and malformation of the leaves. Infected *E. crusgalli* are dwarfed, dark green, and show some occasional thickening of leaf veins.

**Present status in Italy.** The epidemiology of MRDV in Italy has been studied in the Piedmont, in the northwest part of the country (Conti, 1972, 1974, 1976; Luisoni and Conti, 1970). The area where the virus occurs may be subdivided into two smaller areas which differ in agricultural character. First, there is a typical rice-growing area northeast of Turin, including large farms whose acreage of 100 hectares (ha) or so is mostly devoted to rice, maize, and permanent meadows. Rice is generally grown for 2 consecutive years followed by maize for 2 yr. The second area, extending from the first to the southwestern part of the Piedmont, includes smaller farms with a great variety of crops but not including rice.

During field surveys carried out from 1970 to 1975, the highest MRDV incidence observed in maize was about 15%. Such high levels, however, were exceptional, and the usual incidence of infected plants was 0.7 to 6.4% (Conti, 1977). In the last 2 yr (1981, 1982) this situation has drastically changed, as serious MRDV outbreaks have been reported from various parts of the Piedmont. Incidences are as high as 30 to 60% and the great majority of infected plants are severely stunted, indicating predominantly early infections. New epidemiological investigations are underway to identify reasons for these outbreaks.

Field observations and information collected from the growers and the main maize seed companies operating in Italy show that in the last few years there has been a tendency to sow the maize about 1 mo earlier, i.e., from the end of March to the first week of April. This practice has apparently been stimulated by the following facts: a) availability of new, cold-tolerant maize cultivars; b) demonstration that yield is considerably increased by prolonging the vegetative period (each additional day of vegetation has been shown to increase yield by about 50 kg of dried grain per ha); and c) desire to devote more time to other crops during the season. The latter is of particular importance where maize is grown in rotation with rice, as the latter has to be sown immediately after maize and requires a great deal of careful work.

Several reports of severe MRDV outbreaks came to the Istituto di Fitovirologia Applicata, CNR, Turin, during the spring of 1982. In all the cases examined, maize had been sown very early (from 20 March to 8 April) and the majority of reports were from the rice-growing area. At present it is possible to conclude that early sowing is the main factor determining the increased incidence of MRDV in the Piedmont. However, severely infected maize has sometimes been observed close to other largely healthy crops of equally susceptible cultivars, sown more or less at the same time. This has not yet been explained satisfactorily.

It is clear that early sowing can raise MRDV incidence for at least two reasons: first, plants remain exposed to inoculation much longer than plants sown later, e.g., at the end of April; and second, early sown maize grows more slowly because the weather is cooler, thereby remaining very susceptible to infection for a longer time. In conclusion, it seems likely that severe MRDV outbreaks may occur when maize is sown early in fields either heavily infected by overwintering *L. striatellus* or particularly exposed, or attractive, to infective planthoppers migrating from other fields.

**MAIZE WHITE LINE MOSAIC VIRUS**

Detection in Italy. MWLMV was found in 1978 in Friuli, northeastern Italy (Conti et al., 1980). In this region, maize is extensively grown and occupies about 20% of the cultivated area which includes the eastern and larger part of the Po Valley. The disease outbreak was limited to a very small area and would probably have passed unobserved if it had not occurred in an experimental field of the Centro di Sperimentazione Agraria per il Friuli-Venezia Giulia, Udine, a local station for agricultural research. The experimental field was about 80 km north of Udine, on the pre-Alpine hills outside the maize-growing area, and had been sown at the end of May in a trial including 60 different maize lines and cultivars. Only one of these, the North American dent hybrid 'Wolf', developed symptoms described below which were noted in June by M. Snidaro and P. G. Cocceano (personal communication) (Centro di Sperimentazione Agraria, Udine, and Osservatorio per le Malattie delle Piante, Gorizia). Some infected plants were sent to the Istituto di Fitovirologia Applicata, Turin, and tests revealed that they were infected with MWLMV, a virus not previously reported from Italy.

**Virus characteristics and field observations.** The infected maize plants were slightly stunted and showed a vivid leaf mosaic or mottle, with short, whitish stripes over the veins and adjacent interveinal tissues (Fig. 7). Such symptoms appeared much brighter than and, on the whole, clearly different from those induced by SCMV which commonly infects maize in northern
Attempts to transmit the disease agent to glasshouse-grown maize by sap inoculation were negative as were other attempts using as vectors the aphid *R. maidis* and the following leaf- or planthoppers: *Macrosteles sexnotatus* Fallen, *Psammoettix striatus* (L.), *P confinis* Dahlb., *Forcipata obtusa* Vid., and *L. striatellus*. Soil samples were also collected at the base of field-infected plants, and healthy maize from seed was grown in them for 3 mo. No virus transmission was observed, although several MWLVM isolates have now been demonstrated to be transmissible through the soil (cf. Gordon *et al.*, 1981).

Isometric virus particles, about 30 nm in diam, were found by electron microscopy in leaf dip preparations from infected maize. Identical particles were seen in purified virus suspensions obtained from field maize as described by Conti *et al.* (1980). The particles resembled tombusviruses in morphology (Fig. 8).

The MWLVM-infested area was surveyed in September 1978 to collect more data on virus incidence and distribution. The virus was found only in the cultivar 'Wolf' in two fields, one being where it had originally been detected. In this field, plants had been sown as a 10-row strip around the plantation. Plants with symptoms were most numerous in the three-four rows bordering permanent meadows and were less numerous in the inner rows or in the edge rows not adjacent to meadows. The total virus incidence was about 6%. The other field of corn infected with MWLVM contained only very few plants with symptoms and these were near the middle of the field.

Grassy weeds growing inside or near the infected maize crops and showing apparent virus disease symptoms were collected and checked for MWLVM by electron microscopy with negative results. As MWLVM could not be transmitted artificially and was no longer detected in that area in the following years, all the work on its identification was done on infected plant material collected in the field during 1978.

Two possible explanations for the sudden but isolated outbreak of MWLVM in Italy might be that it had been transmitted through the seed of the maize ‘Wolf’, or transmitted to this field from some unknown natural host. Neither of these hypotheses could be confirmed by investigations carried out in the years from 1978 to 1980 (Conti *et al.*, 1980, unpublished).

**Serology.** Two antisera prepared to the Italian isolate of MWLVM (MWLVM-I) had titres of 1:1024 against the virus and 1:2 against healthy maize sap in agar gel diffusion tests. Serological tests were performed with MWLVM-I, as the antigen and with three antisera to different strains of tomato bushy stunt virus (TBSV), one antiserum to maize chlorotic dwarf virus (MCDV), and another to an Ohio isolate of MWLVM (MWLVM-OH). The two latter antisera were kindly supplied by D. T. Gordon (Wooster, OH). In agar gel diffusion tests, MWLVM-I did not react with the three TBSV antisera to strains BS-3, petunia asteroid mosaic, and pelargonium leaf curl, nor with that to MCDV. Instead, it reacted in microprecipitin assays with the antiserum to MWLVM-OH up to its homologous titre (1:128/1:256). Cross-reaction tests carried out in the USA confirmed the non-affinity between MWLVM-I and MCDV, and the close serological relationship between MWLVM-I and U.S. isolates of MWLVM (cf. de Zoeten *et al.*, 1980; D. T. Gordon, *personal communication*).

More recently, MWLVM-I has been compared serologically with a virus described in France under the
BARLEY YELLOW DWARF VIRUS

Early records and epiphytotics in rice. The story of BYDV in Italy is a good example of the importance that new growing techniques and changes in agricultural practices may have in modifying the ecology of plant viruses, sometimes favoring their escalation from endemic to epiphytotic status.

BYDV was first reported in Italy in grasses and small grain cereals by Slykhuis (1958) near Rome and by Grancini (1963) in northern Italy. BYDV started to become of economic importance in the years around 1967, when the early epiphytistics of "giallume" (= yellowing) were observed in rice in Northern Italy (Corbetta, 1967). The etiology and epidemiology of the disease were investigated at the Plant Pathology Institute, Faculty of Agriculture, University of Milan, where it was found that the disease was caused by a strain of BYDV and that its most important natural vector was the aphid, *R. padi*. The principal source of virus to rice was shown to be the perennial weed, *Leersia oryzoides* (L.) Swartz. BYDV could also be found in a few other Gramineae growing near infected rice fields, namely *Avena sativa*, *Echinocloa crusgalli*, *Holcus lanatus* L., *Lolium perenne* L., and *Panicum dichotomiflorum* Michx. *L. oryzoides* is the most important as a primary virus source because it is the most common grass weed of the rice fields and multiplies very easily by stolons, thereby preserving BYDV from one vegetative cycle to another (Amici et al., 1978; Baldacci et al., 1970; Osler et al., 1980).

In Italy, BYDV epiphytotics in rice or other cereals had never been observed prior to 1967. Their appearance in rice was determined by the new agricultural practices adopted for the complete mechanization of this crop, which was made necessary by the shortage and increasing cost of manual labor. Sowing rice directly in the field rather than transplanting it from the more protected nurseries, as was done previously, left plants exposed to BYDV aphid vectors for a longer period of time when plants are very susceptible. The practice of monoculture over large areas was favorable to the permanent establishment of an increasing number of virus and vector sources within the rice-growing area. It also made necessary the massive use of herbicides which resulted in certain weed species disappearing gradually and the spread of other less herbicide sensitive species. The plant species more favored by this situation were those which propagate both by seed and vegetatively, as with *L. oryzoides*. Another factor which increased considerably the spread of this weed was the increased use of mechanical cultivators which cut the underground stolons into fragments and helped to spread them through the fields. The stolons of BYDV-infected *L. oryzoides* were dispersed every year in this way and gave rise to new foci of infection.

The problem of rice "giallume", which was quite serious for about 10 yr after its first appearance, has now been largely overcome with the use of new resistant cultivars of rice derived mainly from the old cultivar Vialone Nero.

**Epiphytotics in other small grain cereals and maize.** BYDV started to affect wheat, barley, and oats greatly in 1975-76 in northern and central Italy (Conti, 1978; Giunchedi and Credi, 1973). In 1977, about 120 ha sown to barley in the autumn of the previous year in the province of Udine, northeastern Italy, had to be plowed under and sown again in the spring due to 100% BYDV infection (P. G. Cocceano, personal communication).

In the same years, the first cases of infection were observed in maize. This species has sometimes been reported to be a symptomless host of BYDV, but laboratory studies have shown that the growth and yield of infected plants may be considerably reduced (Panayotou, 1977). Experimentally infected maize plants develop two main types of symptoms: a) irregular, elongated chlorotic spots with a water-soaked appearance, mostly on the margins of the first two leaves; and b) fine chlorotic lesions along the small veins of the leaves, extending either along the whole leaf or towards its base only and, more rarely, white or yellowish stripes on the leaves (Panayotou, 1977). Maize plants infected in the field by BYDV may be symptomless in summer but then, near maturity, show reduction of the growth and reddening of the edges in the basal leaves. Such symptoms may appear earlier and become more severe in plants infected by both BYDV and SCMV (Belli et al., 1980) or in plants suffering heavy aphid
investments on the BYDV epiphytotic in barley and wheat in northern Italy were started at the Istituto di Fitovirologia Applicata, CNR, Turin, in 1980. Preliminary results have given some idea why BYDV has reached epiphytotic levels. The area devoted to the cropping of barley in the Piedmont, northwest Italy, has increased about 10 times in the last 6 or 7 yr, and this has certainly favored increased incidence of BYDV, as barley is its best natural host and is easily colonized by its main vector, *R. padi*. The availability of a higher number of susceptible plants has progressively increased the virus pool and the proportion of aphids becoming viruliferous. Also, since about 1970, there has been a tendency to sow the barley earlier, so that most crops are now sown from mid-September to the first week of October. This causes the plants to emerge and remain exposed in their early stages of growth in the open when the flight activities of *R. padi* and other aphid vectors are still relatively intense. The aphids can colonize barley and produce an additional generation of insects. These may overwinter on it and spread virus within the crops during late autumn and early spring.

Under the climatic conditions of northern Italy, *R. padi*, the most efficient vector of BYDV, overwinters mainly on *Prunus padus* L. as eggs. In mild winters it can also survive on the roots of some grasses and cereals as immature forms which develop into the early migrating individuals the next spring. Investigations on the biology of *R. padi* in the Piedmont showed that it also can lay its winter eggs on the oriental cherry, *P. serrulata* Lindl. Thus, in spring this species may harbor dense colonies of the aphid (Conti, unpublished). In the last 20 yr or so, the oriental cherry has become one of the most widespread ornamental plants in the Piedmont, and this may have favored the multiplication of *R. padi* and its survival during cold winters. The role of this cherry as an alternative winter host of the aphid may have been of great importance, as the natural primary host of the aphid, *P. padus*, is no longer common in the Piedmont due to the drastic reduction of natural woodland where it grows.

**LITERATURE CITED**


Maize Virus Diseases in France

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ABSTRACT


For the moment, the only economically important maize (Zea mays) virus disease in France is maize dwarf mosaic. In surveys, maize dwarf mosaic virus (MDMV) strain A was associated with mosaic symptoms and was mainly present in areas of southern France where johnsongrass (Sorghum halepense), the overwintering host, occurred. In 1973 an outbreak due to MDMV strain B was present in an area of seed corn production. For several years viruslike symptoms have been identified as caused by a strain of barley yellow dwarf virus (BYDV) and the reactions of some cultivars were checked. In 1974 a new virus disease was found in east-central France and named “Nanisme et anneaux foliaires du Mais.” Finally, in our surveys a virus disease similar to maize mosaic was found in 1977 and another distinct viruslike disease, associated with rhabdovirus particles, was observed in 1981 and 1982.

MAIZE DWARF MOSAIC VIRUS

In the fall of 1968, a viruslike disease was observed in various Sorghum bicolor (L.) Moench hybrid nurseries near Montpellier. A study was undertaken on this disease in 1969. The causal agent was mechanically transmitted to several different genera in the Gramineae. Properties of the virus isolate were determined and an antisera was prepared. From the results of our studies on symptoms, host range, physical properties, and electron microscopy, it was concluded that this newly described virus from France (Signoret, 1970), which infected johnsongrass [S. halepense (L.) Pers.], was identical to the maize dwarf mosaic virus (MDMV)-johnsongrass or A strain (MDMV-A) (Signoret, 1971). Since 1970 we have conducted several surveys in corn nurseries and fields mainly in southern France. We have found mosaic symptoms and dwarfing of corn (Fig. 1A) which was most prevalent in zones where mosaic-diseased johnsongrass occurred. Laboratory studies of the different isolates led us to the identification of MDMV-A previously described from sorghum in other European countries (Conti, 1983; Tosic, 1983). The virus was also present on other foxtails (Setaria spp.) and on crabgrass (Digitaria sanguinalis (L.) Scop.). However, some differences between sorghum and corn isolates have been observed based mainly on infectivity and reactions on sorghum test plants and on foxtails.

In 1973 the spread of the virus was most important on sorghum and corn. Some sorghum fields in the southern part of the Rhone Valley exhibited yield decreases of 95%. The virus was prevalent mainly in seed corn production fields in the south. Nevertheless, the virus occurred for the first time in the north of France in the corn nurseries of the Institut Nationale de la Recherche Agronomique (INRA) at Versailles. This new isolate was determined to be MDMV-A with some slight differences from previous isolates (Kerlan et al., 1974; Signoret, 1974). Again in August 1973, a great number of dwarfed plants with mosaic symptoms were observed in several fields for hybrid seed production. After several trials on test plants and in comparison with the previous isolate (MDMV-A) and MDMV strain B from the USA, we have identified MDMV-B as a new strain occurring in France (Signoret, 1974). Our results were confirmed by D. T. Gordon (Wooster, OH) and J. H. Eill (Ames, IA) in host range and serological tests. Initially identified in France in 1973, it was found again only in 1974, and then only in a few plants. In 1981 MDMV-A was identified in Alsace, south of Strasbourg.

Studies on seed transmission of MDMV-A have shown a rate of one in 10,000 or less. These results are similar to those reported by other authors (Gordon et al., 1983).
Fig. 1. Symptoms of maize viruses in France: A) Maize dwarf mosaic virus on corn: Mosaic symptoms and dwarfing. B) Pattern of dashes and dots on leaf of corn inbred F7 infected by barley yellow dwarf virus. C) Plant infected by the causal agent of "Nanisme et anneaux foliaires du Mais". D) Maize plant infected by a rhabdovirus.
In collaboration with my entomologist colleague, F. Leclant, studies on aphid transmission have been done (Leclant, 1974). *Myzus persicae* (Sulzer) and *Sitobion avenae* (F.) were less efficient than *Schizaphis graminum* Rondani as vectors. However, *S. avenae* was more frequent and abundant in corn fields. We also estimated the potential incidence of maize dwarf mosaic in the field, using weekly distributed trap plants consisting of 50 pots each containing *Oh43* x *WF9* and *P3179* seedlings from mid-May to mid-September 1975. Beginning at the end of May, the incidence of maize dwarf mosaic remained above 41% in the *Oh43* x *WF9* trap plants, and after July 9 it fluctuated between 0 and 20%. With the more resistant corn hybrid, *P3179*, 40% incidence was reached only on June 11. Later the disease intensity fluctuated between 20% and 64% until the end of August, decreasing then slowly to 0%. With *P3179*, the incidence curve was comparable to that obtained by Louie et al. (1974), but the maximum virus incidence was obtained earlier.

**BARLEY YELLOW DWARF VIRUS**

Some years ago in the fall season, we saw viruslike symptoms in some seed corn production fields in southwestern France. Plants were generally stunted with yellow spots and stripes mainly on the youngest leaves. Yield was severely reduced. Unfortunately, it was not possible then to identify the causal agent. Subsequently, similar symptoms have been found on several inbreds and hybrid maize cultivars. We tried to identify the causal agent of this disease. In tests with the planthopper *Lepidopteran striatellus* Fallen and several aphids (*Rhopalosiphum padi* (L.), *S. avenae*, and *S. graminum*), *R. padi* transmitted the causal agent to *Avena byzantina*, K. Koch, cv. Coast Black) and to the French maize inbred F7 with a high efficiency; *S. avenae* and *S. graminum* were poor vectors and *L. striatellus* was not a vector. Symptoms produced on Coast Black oats were identical to those produced by barley yellow dwarf virus (BYDV). On F7 the symptoms were similar to those seen in naturally infected fields, i.e., patterns of yellow dashes and dots which rapidly elongated along the small veins of the youngest leaves (Fig. 1B). The time of symptoms’ appearance on corn was from 6 to 15 days after inoculation, depending on temperature. At 15°C, no symptoms were produced, but after transfer of plants to a greenhouse, typical symptoms developed. In the field and greenhouse, the symptoms tended to disappear with plant aging.

Purification of the virus with methods used for BYDV followed by electron microscopic observations allowed us to find isometric particles 28 nm in diam. Serological tests using enzyme-linked immunosorbent assay (ELISA) gave a positive reaction against antisera prepared with BYDV isolates RPV and PAV (Signoret and Alliot, 1980). From these results, we believe that the symptoms seen in naturally infected plants were caused by a strain of BYDV. However, the symptoms were different from those generally described for BYDV infection (Leclant, personal communication; Stoner, 1977), but were similar to those reported by

Panayotou (1977). Since aphid vectors of BYDV (mainly *R. padi*) are present on maize in the fall and some of these plants are infected with BYDV, corn fields could play an important role as a reservoir of BYDV for autumn-sown cereals, as suggested by different authors (Moreau and Lapierre, 1977; Fargette and Lister, 1982). Fortunately, it has been demonstrated that aphids leaving corn are less infective compared to those coming from volunteer cereals (Bayon et al., 1982).

**NANISME ET ANNEAUX DU MAIS (MAIZE DWARF RINGSPOT)**

In 1974 Lapierre and Signoret (1975) observed in east-central France a viruslike disorder on corn hybrids. The disease was characterized by delayed growth and development, with infected plants appearing dwarfed. On leaves, a mosaic symptom, different from that caused by MDMV, was present with numerous yellow dots, ringspots, and dashes (Fig. 1C) plus discoloration of the leaf sheath. Infected plants were sterile or produced small ears with few kernels.

Electron microscopic observations of purified preparations revealed two kinds of spherical particles, 17 nm and 26 nm in diam. The former mainly precipitated using 10% polyethylene glycol (PEG 6000) and the latter by 15% PEG plus 0.6 M NaCl (Lapierre et al., 1976). Treatments with ribonuclease at different ionic strengths showed a sensitivity of the particles to the enzyme, indicating presence of a single-stranded RNA molecule. Properties of the particles were sedimentation coefficients ($S_{20,w}$) of ca. 121 S and 48 S for the 26 nm and 17 nm diam particles, respectively, and molecular weights of the RNA of $1.5 \times 10^{8}$ and $0.4 \times 10^{8}$ daltons for the larger and smaller particles, respectively. These properties are similar to those of the particles of tobacco necrosis virus and its satellite virus (Uyemoto, 1981). There was no serological relationship between the two types of particles. In 1980 we found a serological reaction between the 26 nm particle and the antisera prepared by de Zoeten (de Zoeten et al., 1980) against maize white line mosaic virus (MWLMV). Both mechanical and insect transmission (several aphid species and the planthopper, *Javesella marginata* Fall) were unsuccessful. We also tried soil transmission without success, but additional experiments are in progress. In conclusion, based on these results and those presented by Conti (1983), it seems possible that maize dwarf ringspot virus is identical or closely related to MWLMV. One remaining problem is that the French isolate had two sizes of particles, whereas reports on MWLMV (Gordon et al., 1981) give only one particle of the approximate size of the larger particle.

**MAIZE RHABDOVIRUS**

In July 1977 we visited two seed increase fields for maize inbred lines in southern France. There was a high incidence of dwarfed plants showing a pronounced mosaic characterized by an alternation of large bands, yellow and pale green, on the leaves (Fig. 1D). On many plants most leaves were almost com-
completely yellow. The yellow parts became rapidly necrotic. The tops of some plants were bent over. We have tried mechanical transmission to different Gramineae species without success. Also, transmission trials with the planthopper *L. striatellus* were not successful. However, a leaf-dip preparation stained with ammonium molybdate allowed us to observe numerous bacilliform particles not found in healthy plants. These particles, measuring 220 x 60 nm, resembled those of the rhabdovirus group. In several purification trials we found only bacilliform particles which unfortunately were partly disintegrated. It is unlikely that this rhabdovirus is the maize mosaic virus (MMV) which occurs in many tropical and subtropical countries (Francki et al., 1981). Greber (1982) has reported another maize-infecting rhabdovirus in Australia that he named maize sterile stunt virus (MSSV). The virus is located in the cell cytoplasm as is the virus we found in France. These two viruses also closely resemble each other in particle size. In conclusion, the maize rhabdovirus found in France seems to be similar, if not identical, to MSSV rather than to MMV. That this virus is not MMV is supported by the fact that *Peregrinus maidis* Ashmead, the vector of MMV, has not been found in France. MSSV is transmitted by the delphacid hopper, *Sogatella longifurcifera* (Easaki & Ishihara), and several hoppers of this group, which are possible vectors of the virus, occur in France. However, since 1977 symptoms of rhabdovirus infection have not been observed.

**OTHER STUDIES**

Last year we found severely stunted plants with typical viruslike symptoms in some seed corn production fields. Yellow streaks of different sizes appeared on the leaves. Some streaks were spindle shaped and others yellow dashes appearing mainly on the secondary veins. These bands or dashes rapidly coalesced into long stripes, while the leaves turned yellow beginning from the tips. The early infected plants died in August, but those showing symptoms later were less dwarfed and produced some ears. In electron microscope studies of the causal agent, we found numerous bacilliform particles in preparations from diseased leaf samples. Transmission trials are in progress with hoppers collected in and around fields with diseased plants. Conti (1983) reported similar symptoms on some maize plants in the Bergamo area of Italy at the end of July 1982. He also observed bacilliform particles from these diseased plants. Plans are to compare the two viruses.

**LITERATURE CITED**


Investigations of Maize Mosaic in Yugoslavia

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ABSTRACT


Maize mosaic in Yugoslavia was first recorded in 1960 and is presently found throughout the country in high incidence. The incitant, maize mosaic virus (MMV-YU), is a potyvirus and is closely related to maize dwarf mosaic virus strain A (MDMV-A) and sugarcane mosaic virus, johnsongrass strain (SCMV-Jg). MMV-YU is distinct from MMV, which is a rhabdovirus found in tropical and subtropical areas of the world. Important hosts of MMV-YU are maize (Zea mays), sorghum (Sorghum bicolor), and johnsongrass (S. halepense). MMV-YU, MDMV-A, and SCMV-Jg had identical host ranges in comparative tests. Principal symptoms induced by MMV-YU were mosaic on leaves that developed following inoculation, reduction in plant height (18.5%), reduced seed germination (19%), and increased sterility following inoculation, reduction in plant height (18.5%), and increased sterility (26%). Oil content of grain was reduced (31%). MMV-YU infected sugarcane mosaic virus (SCMV) and maize dwarf mosaic virus (MDMV) strains and their relationship to MMV-YU. Partial cross protection was demonstrated between MDMV-A and MDMV-B and between MDMV-A and strain I of SCMV. MMV-YU infection increased susceptibility of maize to infection by Gibberella zeae, Helminthosporium turcicum, and Ustilago maydis. Johnsongrass served as overwintering host for MMV-YU in Yugoslavia. Estimates of crop loss were based on a formula which considered the number of sterile plants resulting from infection plus the reduced productivity of infected fertile plants. Yield of fertile plants was decreased by up to 42%. MMV-YU resistant corn genotypes were identified and crosses to produce resistant hybrids were made. Based on these studies the following three groups of related maize-infecting viruses were proposed: Group 1—MDMV-A, SCMV-Jg, MMV-YU, European maize mosaic virus, karlikovaja mosaika kukuruzi (Soviet Union), mosaik zakrljalog kukuruza (Yugoslavia), and sorghum red stripe virus; Group 2—MDMV-B and strain E of SCMV; Group 3—SCMV-A, -B, -D, -H, and -I.

Virus mosaic on maize (Zea mays L.) in Yugoslavia was first described in 1960 (Panjan, 1960). Since that time mosaic on maize became established in many parts of the country on a high percentage of maize plants. It appears that virus mosaic was not present in Yugoslavia before 1960. Since 1960, this disease and its causal agent, termed the Yugoslav maize mosaic virus (MMV-YU), have been intensively investigated in Yugoslavia. The acronym MMV-YU is used to indicate the difference between this virus and the rhabdovirus MMV referred to by other authors in these proceedings (Autrey, 1983; Greber, 1983; Lastra and Carballo, 1983). Results of investigations in Yugoslavia will be reviewed in this paper. I will also make some comments concerning sugarcane mosaic virus (SCMV) and maize dwarf mosaic virus (MDMV) strains and their relationship to MMV-YU.


Symptoms vary depending on the host plant. Mosaic on maize seedlings appears first on the base of youngest leaves as chlorotic dots and streaks. Later, mosaic symptoms appear on the whole blade of the youngest leaf as well as on all new leaves. Mosaic symptoms do not develop on leaves which expand before infection occurs. There is no regularity in the type of mosaic even on one infected plant. Virus symptoms on infected sorghum and johnsongrass are more uniform and frequently chlorotic streaks are present (Tosic, 1965). Symptoms on P. miliaceum, infected under greenhouse conditions during May-June, include chlorotic streaks and dots with chlorotic stripes along veins; these stripes often turn necrotic (Tosic, 1965). Symptoms on M. frondosa...
and *M. racemosa* include mottling and chlorotic lines and dots on leaves (Tosic, 1974b).

**Virus identification. Morphology.** MMV-YU in Yugoslavia has a filamentous particle with average length of 750 nm (Stefanac, 1967; Tosic, 1965). The earlier report of 620 nm for the length may have been in error (Panjan, 1960). Its particle morphology clearly distinguishes MMV-YU from MMV which has a rhabdovirus particle (Autrey, 1983; Greber, 1983; Lastra and Carballo, 1983).

**Properties-in-sap.** It was established that MMV-YU had the following properties-in-sap: thermal inactivation point (TIP), 52 C (Tosic, 1965), 56 C (Panjan, 1969), and 60 C (Stefanac, 1967); dilution end point (DEP), 12.5 x 10^-3 (Tosic, 1965) and 10^-3 (Panjan, 1969; Stefanac, 1967); and longevity in vitro (LIV), 24 hr (Tosic, 1965) and 13-16 hr (Panjan, 1969).

**Transmission.** Isolates of MMV-YU in Yugoslavia have been transmitted mechanically by rubbing leaves with juice of diseased plants (Panjan, 1960; Stefanac, 1967; Sutic et al., 1969; Tosic, 1962) and both experimentally and naturally by the aphid species: *Rhopalosiphum maidis* (Fitch), *Schizaphis graminum* (Rondani), and *Myzus persicae* (Sulzer) (Sutic et al., 1969; Tosic and Simova, 1967; Tosic and Sutic, 1968). Tests with aphids show that this virus is nonpersistent in these vectors since aphids can acquire MMV-YU from diseased plants within 1 min (*S. graminum* and *M. persicae*) or 2 min (*R. maidis*). There is no latent period in the vector. Inoculation feeding is 1 min for *S. graminum* and *M. persicae* and 2 min for *R. maidis*.

Seeds obtained from maize plants infected with MMV-YU in the field transmitted virus to seedlings at the rate of 0.008% (Tosic and Sutic, 1977). Since about 60,000 seeds of corn are planted per hectare (ha), this low percentage of seed transmission of MMV-YU provides an initial inoculum in fields of about five infected plants per ha.

**Serology.** Antisera prepared against MMV-YU reacted positively with virus isolates from maize as well as those from sorghum and johnsongrass (Stefanac, 1967; Tosic, 1965). Some results are contradictory (Panjan, 1969).

MMV-YU reacted positively with antisera prepared to MDMV strains A and B and to SCMV strain D. After cross absorption with heterologous antigens, the Yugoslavian isolates reacted positively with homologous antisera and antisera prepared to MDMV-A (Tosic, 1974b).

**Relationship of Yugoslavian maize mosaic virus to maize dwarf mosaic and sugarcane mosaic viruses.** All of the above mentioned data have shown that the virus causing maize mosaic in Yugoslavia is similar to MDMV and SCMV described in the USA and elsewhere. Since many strains of MDMV and SCMV have been identified, it was necessary to check in more detail the relationship between viruses causing maize mosaic in Yugoslavia and MDMV and SCMV strains isolated in the USA. The following properties of viruses and strains were compared.

**Infectivity on differential hosts.** In tests with the differential hosts proposed for MDMV and SCMV, strains (Tosic and Ford, 1972), MMV-YU, like MDMV-A, infected *Muhlenbergia frondosa*, *M. racemosa*, *Paspalum dilatatum*, *Sorghum alburnum*, *S. halepense*, and *Sporobolus poiretii*, but not *Bromus rubens* L. and *Heteranthelium piliferum* Hochst. (Tosic, 1974b).

**Pathogenicity on sorghum cultivars.** Tested under the same experimental conditions, MMV-YU, MDMV-A, and the johnsongrass strain of SCMV (SCMV-Jg) showed similar pathogenicity to different sorghum cultivars, but differed from that shown by MDMV-B and SCMV strains A, B, D, E, H, and I (Tosic and Malak, 1973).

**Epidemiology of maize mosaic in Yugoslavia.** *S. halepense* serves as a perennial host for MMV-YU survival in the field in Yugoslavia (Sutic and Tosic, 1966). In most regions of the country, virus occurs in high incidence in *S. halepense* (Stefanac, 1967; Sutic and Tosic, 1966; Tosic and Simova, 1967). Also, the virus is seed transmitted (Tosic and Sutic, 1977) and this plays a role in virus distribution and carryover from season to season. In addition to transmission by seed, MMV-YU is transmitted very effectively by aphids (Sutic et al., 1969; Tosic and Simova, 1967; Tosic and Sutic, 1968), which probably allows for rapid dispersal in fields infested with infected johnsongrass or with corn seedlings infected through the seed.

The most frequent chain of virus spread under natural conditions for areas where *S. halepense* is abundant involves infected johnsongrass or with corn seedlings infected through the seed. This host emerges early in the spring from rhizomes, and aphids presumably transmit MMV-YU from the young infected johnsongrass plants to maize. Subsequently, MMV-YU is spread from infected to noninfected maize by viruliferous aphids. The second chain of virus spread involves MMV-YU transmission by viruliferous aphids from seed-infected maize to healthy maize and spread subsequently from the newly infected maize to healthy maize, again by aphids.

**Spread of virus during the season.** MMV-YU in Yugoslavia progressively increases on maize from early June to mid-July when infections reach the highest incidence; yet new infections occur until the end of September (Tosic and Sutic, 1974). The pattern of spread of MMV-YU during the growing season is influenced first probably by the presence of *S. halepense* as a primary source of inoculum for infection and second by an active population of aphid vectors.

**Susceptibility of maize hybrids and varieties.** During these investigations (Stakic and Savic, 1976; Sutic et al., 1969; Tosic and Misovic, 1967; Tosic et al., 1979), a big
difference in susceptibility among different maize varieties and hybrids to MMV-YU was observed. Krajinski tvrdunac, a domestic maize variety, proved very resistant to this virus, with infection rates from 0.44 to 1.88%. Novosadski zlatni zuban, another domestic maize cultivar, was more susceptible, with infection rates from 4.06 to 17.50%. Overall, rates of infection for maize hybrids ranged from 0.94 to 79.55%.

These data show that more attention should be paid to the selection of maize hybrids which are more resistant to MMV-YU.

Inheritance of resistance in maize. Inheritance of resistance to MMV-YU was studied with 21 inbred lines and 13 of their hybrid combinations (Tosic et al., 1978). The results showed that resistance of the maize hybrids to MMV-YU was never less than the resistance of the more susceptible inbred line. The resistance of hybrids was usually higher than that of the male parent or in other combinations it was intermediate. It was shown that highly resistant hybrids may be obtained by crossing highly susceptible by susceptible inbred lines. Thus, it is necessary to check combinations of all inbred lines in breeding maize hybrids resistant to MMV-YU (Tosic et al., 1978).

Effect on germination, growth, and yield of maize. Investigations have shown that MMV-YU decreased seed germination up to 19.2%, radicle length up to 7.4%, and radicle width up to 20% (Stakic and Savic, 1980). It also decreased plant height up to 18.5%, caused sterility of plants at rates up to 25.8%, and decreased yield per fertile infected plant up to 41.8% (Tosic and Misovic, 1967).

Effect on fatty acids and oil content of grain and on protein content of leaves. MMV-YU infection on maize reduced oil content of grain by as much as 30.9%, but it did not influence fatty acid ratios. In most cases, palmitic acid was slightly increased in grains from virus-infected plants (Pesic et al., 1977). Protein content decreased by about 2.8% in infected corn seedlings (Pesic et al., 1978).

Loss assessment and resistance evaluation. Since total loss caused by virus infection results from loss to increased number of sterile plants and to decreased yield of fertile plants, a method for loss assessment caused by MMV-YU in maize production fields was proposed (Tosic, 1974a). The method is based on the following equation:

\[ L = S_i \times \frac{1}{100} + (I - S_t) \times M_1 \times \frac{1}{100}, \]

where \( L \) = total loss expressed in percentage (yield without mosaic plants = 100%); \( S_i \) = percentage of sterile mosaic diseased plants; \( I \) = percentage of mosaic diseased plants; \( S_t \) = percentage of sterile mosaic diseased plants in relation to the total number of plants; and \( M_1 \) = average loss in yield per mosaic diseased plant expressed as percent.

The first component of the equation, \( S_i \times \frac{1}{100} \), concerns the sterile mosaic plants, and the second component, \( (I - S_t) \times M_1 \times \frac{1}{100} \), concerns the percentage of mosaic plants and their reduced yield.

Applying this equation with the data obtained at Zemun Polje for field experiments (Tosic and Misovic, 1967), the losses of yield with some corn hybrids and varieties due to MMV-YU infection are presented in Table 1.

In order to evaluate resistance of maize to MMV-YU based on yield loss, the following evaluation categories were proposed (Tosic, 1974a): 1 = very resistant as measured by losses up to 5%; 2 = resistant, losses 5-10%; 3 = susceptible, losses 10-30%; and 4 = very susceptible, losses more than 30%.

Other pathogens and maize mosaic virus interactions. In the course of field investigations in Yugoslavia, interactions of MMV-YU with some fungal pathogens of maize were observed. Previous mosaic virus infection increased susceptibility of maize to stalk rot caused by *Gibberella zeae* (Schw.) Lev. (Tosic et al., 1977). When *G. zeae* was inoculated artificially, this increase was small; but when natural infection occurred, a previous infection by MMV-YU increased significantly the susceptibility of maize to *G. zeae*. With almost all hybrids checked, susceptibility to *G. zeae* was increased by about 2.5 times (Tosic et al., 1977) for virus-infected plants.

Similar results were obtained in the interaction between the MMV-YU and *Helminthosporium turcicum* Pass., where MMV-YU diseased plants were 9-13% more susceptible (Panic et al., 1978).

MMV-YU infected plants were also more susceptible by an average 21.2% to *Ustilago maydis* (DC) Cda. (Ivanovic, 1979).

These results, which show an interactive relationship between the MMV-YU and several fungi, suggest that it is necessary to take into consideration virus infection of corn plants in order to evaluate properly their resistance to fungal diseases.

Cross protection between sugarcane mosaic virus, maize dwarf mosaic virus strains, and brome mosaic virus...
virus. Cross protection among MDMV and SCMV strains. Results from cross protection research showed some interference but not complete cross protection between some MDMV and SCMV strains. MDMV-B did not prevent multiplication of MDMV-A when challenge-inoculated in sweet corn but it increased the time it took to reisolate MDMV-A from the mixed infection. This effect was most pronounced when the MDMV-A challenge was done 1 or 2 days after MDMV-B inoculation (Tosic, 1981). The same effect occurred when corn plants were inoculated with MDMV-A and then challenged with SCMV-I (Tosic, 1981), in that initial infection by MDMV-A did not protect sorghum seedlings against infection by SCMV-I. However, development of necrosis caused by SCMV-I was much slower when compared to the control plant. The longest delay of necrosis was recorded with Atlas sorghum seedlings inoculated with MDMV-A 5 days after emergence and then challenge-inoculated 3 days later with SCMV-I. Necrosis in this case was delayed for nearly 3 mo (Tosic, 1981).

Interference between brome mosaic virus (BMV) and sugarcane mosaic virus. Interference between BMV and SCMV has been demonstrated by a delay in the onset of the necrosis of Atlas sorghum seedlings caused by SCMV-I. The longest delay was when challenge inoculations with SCMV-I were done 2 days after previous inoculation with BMV. In this case, complete necrosis of infected Atlas was delayed by about 1 wk (Tosic, 1980).

CONCLUSIONS

On the basis of results shown here and on my own experience, I feel that the virus strains should be grouped as follows and that this gathering of maize virologists should make a decision as to which name we should assign priority. Then we should collectively agree to use that name. The first group includes MDMV-A, SCMV-Jg, and MMV-YU which are identical and should be considered as one strain. Additionally to this group belong: a) European maize mosaic (mozaicul porumbului in Romania, carevicna mozajka in Bulgaria, and virus mozaika kukuruza in Yugoslavia), b) maize dwarf mosaic virus (karlikovaja mozaika kukuruzi in the Soviet Union and mozaik zakrzeljalog kukuruza in Yugoslavia), and c) sorghum red stripe (krasnaja polosatost sorgo in the Soviet Union, Die Rotstreifigkeit des Sorghum und das Streifenmosaik des Maises in Czechoslovakia, Striatia ruginie a Sorghulu in Romania, and Crvena prugavost sirka in Yugoslavia).

The second group includes MDMV-B and SCMV-E which are apparently identical and should be considered as one strain. The third group contains SCMV-A, -B, -D, -H, and -I, each of which differ enough to be considered as different strains.
LITERATURE CITED


Virus Diseases of Sugarcane and Maize in Egypt

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ABSTRACT


Sugarcane streak is the most prevalent virus disease found on most sugarcane (Saccharum officinarum) cultivars grown in Upper Egypt. The causal agent, serologically related to maize streak virus, was transmitted only by cuttings and by the leafhopper, Cicadulina bipunctella zeae. Sugarcane mosaic virus (SCMV) (transmitted by sap, cuttings, and aphids) was less prevalent than streak on most cultivars.

Maize streak virus disease, found on maize (Zea mays) in Egypt, was identified serologically, by electron microscopy, and by transmission of the virus with the leafhopper, C. bipunctella zeae. Occurrence of this disease was frequent on late-sown maize and on maize plants adjacent to sugarcane fields. SCMV from maize was transmitted by two aphid species, Rhopalosiphum maidis and Myzus persicae, in a nonpersistent manner.

Three unidentified virus or virus-like diseases found on maize plants in Egypt were: a) stunting, characterized by rough lower surface of leaves due to gall formation on veins; b) isometric viruslike particles with a 40 nm diam and detected by immunosorbent electron microscopy with antiserum to the African maize stripe virus; and c) long, wide, white stripes on leaves, which were sometimes completely discolored. Possible identities of these diseases are discussed, but further work is needed to investigate such possibilities.

Egypt is a highly populated country, mostly dependent on agriculture, in spite of recent and successful efforts at industrialization since the late 1950's. Population now is about 44 million, and the area of cultivated land in 1978 was 2,461,352 hectares (ha) (Anonymous, 1978). However, owing to the organized and regular irrigation system provided by the Nile River, its dams and barrages, two to three crops are cultivated per year in most areas. Thus the actual acreage of cultivated crops reached 4,699,433 ha. Sugarcane (Saccharum officinarum L.) and maize (Zea mays L.) are the two largest produced food crops in Egypt; 8,446,000 and 3,047,000 metric tons, respectively, of these two crops were produced in 1977.

Sugarcane acreage in 1978 was 104,078 ha, grown mostly in Upper Egypt (south of Cairo), particularly at Hawamdia, Kom-Ombo, and Naga-Hammadi, where most of the sugarcane factories are located. The average sugarcane yield in Egypt during 1975-1977 was 86.22 tons/ha which ranked second in the world after that for Peru (Anonymous, 1978).

Maize is the most widely used cereal crop in Egypt, followed by wheat (Triticum aestivum L.) and rice (O. sativa L.). In 1977, 3,047,000 metric tons of maize were produced locally and 591,000 tons were imported; 92% was used for human consumption, 2% for animal feed, and the rest for industry and seed.

Maize is grown mostly in Lower Egypt (the Nile Delta, north of Cairo) in two main plantings: Sifi (=Summer) sown in May to early June and Nili sown in July and August. Plantings in the Sifi are more extensive than the Nili; 589,214 ha and 207,128 ha of maize were grown in 1978, respectively. Maize yield is also much higher in the Sifi than in the Nili plantings, with yield averages of 4.574 and 3.168 metric tons/ha, respectively. Three major maize cultivars are grown: Balady (a local variety), Early American, and Hybrid. Mean yields of these cultivars in the Sifi plantings of 1978 were 4.531, 4.491, and 5.091 metric tons/ha, respectively. Maize yield in Egypt was rated twelfth in the world but first in the developing countries during 1975-1977 (Anonymous, 1978). However, average yield is still much lower than the potentials attained in developed countries.

The latest index of plant diseases in Egypt (Ziedan, 1980) listed 13 disease agents affecting sugarcane, including five fungi, five nematodes, and three viruses; the latter cause mosaic, streak, and ratoon stunting diseases. However, the ratoon stunting disease is now believed to be caused by a bacterium (Gillaspie et al., 1981). Disease agents of maize listed (Ziedan, 1980) were 15 fungi, 2 bacteria, 4 nematodes, and only 1 virus, causing mosaic. The study of virus diseases in Egypt, particularly those transmitted obligately by insects, is at an initial stage. Following is a brief account of the information available to date on identified and suspected viral diseases of sugarcane and maize in Egypt.

SUGARCANE DISEASES

Sugarcane streak. In a report to the first Conference on Sugarcane held in Egypt, Abdel-Hak (1964) stated that "streak" was one of the most important diseases affecting yield in Egypt. He indicated that this disease was the prime reason for abandoning the cultivar "Jawa 105" previously the most widely grown sugar
cane cultivar in Egypt. Symptoms of this disease are broken, narrow, chlorotic streaks or stripes on leaf blades and correspond to those described by Smith (1972) for maize streak virus (MSV) on sugarcane. Higgy (1966), studying the effect of this disease on sugarcane, found that it reduced crop and sugar yields, stalk weight, length, thickness, and number of internodes. Streak also reduced starch accumulation and chlorophyll A in leaves. Ammar et al. (1982) studied insect transmission, epidemiology, and natural occurrence of the streak disease on sugarcane cultivars grown in Upper Egypt. They transmitted the virus from naturally infected sugarcane plants to maize seedlings with nymphs and adults of the cicadellid leafhopper, *Cicadulina bipunctella zeae* China. Serological (slide-agglutination) tests using antisera to MSV and sugarcane streak virus (kindly provided by K. R. Bock, Nairobi, Kenya) gave positive reactions with crude preparations from naturally infected sugarcane streaked plants and also from experimentally infected maize plants. Thus, this disease in sugarcane is apparently caused by a strain of MSV, as Rose (1978) indicated.

Ammar et al. (1982) also examined 15 sugarcane cultivars for natural infection with streak at two locations in Upper Egypt (Naga Hammadi and Kom-ombo) during 1974 and 1975. Cultivars highly infected (85-100% infection) were CP 34/29, 384 B 62, 153 B 64, 59 B 64, 59 A 1135, and 59 A 1253; moderately infected cultivars (90-70% infection) were Co. 413, 59 A 1479, 59 A 1469, and 59 A 1478; and weakly infected cultivars (0.2-15% infection) were Hawamdia 7, Hawamdia 6, C9/54, N. Co. 310, and Co. 997. Kira et al. (1978) studied the occurrence of leafhoppers and planthoppers on sugarcane by sweep net during 1975. Population peaks of the vector, *C. bipunctella zeae*, occurred in June, July, and August, reaching 20-44 insects/100 sweeps at Kom-Ombo and 5 insects/100 sweeps at Naga Hammadi. Another *Cicadulina* species, *C. chinai* Ghauri, which has not been tested for transmission of MSV, was found in fewer numbers than *C. bipunctella zeae* on maize and sugarcane plants. These two *Cicadulina* species may have eight to nine generations per year in Egypt (Ammar, 1975, 1977). No other *Cicadulina* species was reported as occurring in Egypt.

**Sugarcane mosaic.** Abdel-Hak (1964) reported that this disease was less important on sugarcane in Egypt than the streak disease and that the causal agent was transmissible mechanically, by cuttings, and by aphids. Ammar et al. (1982) studied the natural occurrence of mosaic on sugarcane cultivars in Upper Egypt during 1974 and 1975. Cultivars highly infected with mosaic (52-83% infection) were Co. 413 and N. Co. 310; those moderately infected (33-40% infection) were 59 A 1478 and Hawamdia 7; and 11 others were weakly infected (0-20% infection). Comparing these results with those of sugarcane streak mentioned above, it is clear that the majority of sugarcane cultivars grown in Egypt are less infected with mosaic than they are with streak. Higgy (1966), studying the effect of mosaic on sugarcane, found that this disease reduced sugar yield; stalk weight, length, thickness; and number of internodes. It also reduced chlorophyll A and starch accumulation in leaves. However, losses caused by this disease were markedly reduced when the cane was planted during March or early April and also when the crop was supplied with 20 to 30 irrigations per season. Studying the properties of sugarcane mosaic virus (SCMV) in vitro, Higgy (1966) reported that the virus remained infective at 26 C for 12 hr and that it did not lose its infectivity when the infectious juice was either diluted with distilled water up to 10^-5 or exposed to 50 C for 10 min.

**MAIZE DISEASES**

**Maize streak.** Smith (1972) reported that maize streak has been recorded from many parts of Africa, even as far north as Egypt. Symptoms of this disease on maize are narrow, broken, chlorotic streaks running along the secondary veins of leaves (Fig. 1b). Ammar et al. (1982) found these symptoms on maize plants adjacent to sugarcane fields in Upper Egypt during 1975. Percentages of streaked maize plants were 0-4% in July, 11-14% in August, 77-78% in September, and 25-58% in October. The leafhopper vector, *C. bipunctella zeae*, present in both maize and sugarcane fields, may have been responsible for the spread from the sugarcane to maize plants.

In a 1981 maize virus disease survey (Ammar and A. E. Abul-Ata, unpublished) in Lower Egypt far from most sugarcane fields, occurrence of streak symptoms on maize plants was almost nil from June to September, 0.6-1.2% in October, 3-10% in November, and 6-12% in December. Thus, it is clear that in both Upper and Lower Egypt incidence of this disease in maize plants sown late in the season is greater than in early sown maize. MSV was experimentally transmitted from naturally infected maize plants to healthy maize seedlings by *C. bipunctella zeae*. Some adults of this leafhopper species collected from maize fields at Giza, Kalubiya, and Monofia Governorates during September to November 1981 were viruliferous. The virus was also transmitted by this vector to wheat seedlings. The identity of MSV disease in Egypt was further ascertained by immunosorbent electron microscopy (ISEM). Samples of maize plants naturally infected with MSV were sent by the author to Roy Woods (Rothamsted Experimental Station, England), who kindly performed this test using antisera to MSV obtained from K. R. Bock (Kenya). Isometric particles, usually found in pairs measuring 30 x 20 nm and typical of MSV, were detected in these samples (Fig. 2).
ages were 30, 50, and 75% when 2, 5, and 10 insects were used per plant, respectively. The above characteristics, in addition to serological and host range studies, indicated that this disease in maize is caused by a strain of SCMV. The virus was not seed transmitted. All tested lines and varieties of maize in Egypt were susceptible to SCMV. Crude extracts of maize plants from Egypt, showing symptoms of this disease, reacted serologically in the enzyme-linked immunosorbent assay with antiserum to MDMV strain B (Ammar, unpublished).

**Unidentified diseases.** In a recent survey of virus and viruslike diseases of maize in the Nile Delta (Ammar and A. E. Abul-Ata, unpublished) three viruslike diseases were encountered. These are characterized as follows.

a) Symptoms involved extreme dwarfing of the plant (Fig. 3) and occurrence of a rough lower surface on leaves due to the formation of elongated, pale colored galls on the secondary veins (Figs. 1d, 4). Leaves were usually darker in color and the upper leaves were shorter and somewhat stouter than those of healthy plants. Sometimes the apical part of the plant failed to emerge from the leaf underneath, giving a malformed appearance (Fig. 5). The severe stunting was observed on a high proportion of diseased plants. Many of these plants withered and died prematurely. Occurrence of

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**Figs. 1-4.** 1) Lower surface of maize leaves showing: a) no symptoms; b) symptoms of maize streak virus (cf. Fig. 8); c) mosaic symptoms; d) vein-gall symptoms, and e) chlorotic striping. 2) Viruslike particles (diam = 20 x 30 nm) found in diseased maize leaves by immunosorbent electron microscopy using antiserum to maize streak virus. 3) A severely stunted maize plant, showing field symptoms similar to those of maize wallaby ear or maize rough dwarf. 4) A closer view of maize leaves with vein-gall symptoms.
these symptoms in maize fields in the Nile Delta during 1981 was extremely low during June to August, but increased to 7-9% in September, 3-50% in October, 10-16% in November, and 6-18% in December.

The above symptoms are very similar to those described for maize wallaby ear disease (Grylls, 1975), but they also resemble some of the symptoms described for maize rough dwarf virus (MRDV) (Damsteegt, 1981). Vector species of both disease agents are found in Egypt, viz *C. bipunctella zeae* associated with maize wallaby ear and *Sogatella vibix* (Haupt) for MRDV. *S. vibix* is the most abundant delphacid planthopper found in Egypt (Ammar et al., 1977). Maize wallaby ear has been reported only from Australia and New Guinea, whereas MRDV has been reported from Europe and Israel (Damsteegt, 1981). A disease similar to maize wallaby ear has been reported in Egypt.

Figs. 5-8. 5) A maize plant showing field symptoms similar to those of maize wallaby ear disease. 6) Viruslike particles (40 nm diam) found in diseased maize leaves by immunosorbent electron microscopy using antiserum to the African maize stripe virus. 7) A maize plant showing field symptoms of stunting and chlorotic striping. 8) A field maize plant showing long, white striping on the leaves caused by an unidentified agent.
wallaby ear, named maize vein enation, was reported from India (Ahlawat and Raychaudhuri, 1976). However, the viral etiology of maize wallaby ear is still in question; some evidence suggests that it may be caused by toxic saliva of Cicadulina spp. (Boccardo et al., 1980). Obviously, further work is needed to find out whether either of these diseases occurs on maize in Egypt.

b) A maize plant showing the symptoms described above plus chlorotic streak symptoms was found in Egypt and sent to Rothamsted Experimental Station. By ISEM with antisera to maize stripe virus (MStpV), obtained from H. Y. Kulkarni in Kenya, isometric viruslike particles 40 nm in diam (Fig. 6) were observed in these samples by R. Woods (personal communication). They were similar to those of MStpV described by Kulkarni (1973). Early symptoms of the latter disease included bright yellow bands 1 cm or more in width on chlorotic leaves, apical bending, and severe stunting. Maize plants showing these symptoms (Fig. 7), except for the apical bending, were found at Giza and Kalobia Governorates (1.2-3% infected plants) during October and November 1981. Maize stripe disease, again without the apical bending, was also reported from Nigeria (Fajemisin and Shoyinka, 1977). MStpV is transmitted by the delphacid planthopper, Pererginus maidis (Ashmead). This species has not been reported from Egypt, but eight other delphacid species have been found (Ammar et al., 1977). However, the involvement of an isometric particle with maize stripe is now question- able (Gingery et al., 1981). Another African maize disease with which 40 nm isometric particles have been associated is maize mottle/chlorotic stunt (Rossal and Thottappilly, 1983). Symptoms induced by this virus, which is transmitted by Cicadulina triangula Storey, range from inconspicuous chlorosis to chlorotic and severely stunted plants. Transmission experiments with chlorotic-striped plants found in Egypt are now underway using Cicadulina and delphacid spp. collected in fields where the disease occurs.

c) Symptoms of a third suspected disease were observed on maize plants at Gharbia Governorate during September 1981 and June 1982. These symptoms were white stripes of variable width extending longitudinally almost the whole length of the leaf blades (Fig. 8). Younger leaves were sometimes completely colorless. These symptoms are somewhat similar to those of maize hoja blanca described from Venezuela (Trujillo et al., 1974). The latter disease, however, is related to maize stripe (Damsteegt, 1981; Gingery et al., 1979).

Although hampered by lack of facilities for proper identification and characterization of viruses, work is continuing to elaborate the above preliminary findings concerning the suspected virus and viruslike diseases of maize in Egypt.

LITERATURE CITED


Virus and Virulike Diseases of Maize in Morocco

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ABSTRACT


Three viruses have been identified in maize (Zea mays) in Morocco: maize dwarf mosaic virus (MDMV), barley yellow dwarf virus (BYDV), and a virus with rhadoviruslike particles similar in some respects to maize mosaic virus. In addition, plants with chlorotic ringspot symptoms, but from which no etiologic agent has been isolated, are occasionally encountered. Cucumber mosaic virus infection occurs but infrequently. MDMV occurs ubiquitously in Morocco and is the virus of greatest economic importance. Field infection rates of 70-80% have been observed. The degree of field infection appears to be correlated with the presence of infected johnsongrass (Sorghum halepense). BYDV infection is frequent in early planted maize, especially in the vicinity of barley (Hordeum vulgare) and wild grass hosts of BYDV. Rhadoviruslike particles measuring 50-60 x 220-240 nm in negatively stained leaf-dip preparations are associated with chlorotic striping, mottling, and stunting symptoms in maize. The virus occurs in Cynodon dactylon, Digitaria commutata, D. sanguinalis, and Setaria verticillata, but no insect vector has yet been identified. The relationship of this virus to previously described maize rhadoviruses has not been established.

In terms of area under cultivation, maize (Zea mays L.) is the third most important field crop in Morocco after barley (Hordeum vulgare L.) and wheat (Triticum aestivum L.). Approximately 500,000 hectares of maize are planted annually; the actual acreage in any year depends on the rainfall pattern since the bulk of the crop is grown in nonirrigated semiarid areas. In areas or years in which late occurrence of winter rains shortens the growing season for wheat and barley, there is a shift towards maize cultivation. Maize is also grown under irrigation in some areas of southern Morocco, but this represents a relatively small proportion of the total acreage. Until recently maize was produced almost entirely for human, and principally home, consumption. There has been a marked increase in the proportion of the crop used for livestock feed, from less than 1% in 1971 to approximately 50% in 1982. Although there has been increasing use of hybrid varieties of American or French origin, especially in irrigated areas, the use of such varieties is limited, and most of the acreage is planted with one of the two local types, one white- and the other yellow-seeded.

The results reported here are derived from surveys conducted throughout Morocco between 1973 and 1982.

MAIZE DWARF MOSAIC VIRUS (MDMV)

This virus was first identified in maize in Morocco in 1974 (Lockhart and Elyamani, unpublished) and has since been found in maize-growing areas throughout the country. Field symptoms include slight to severe mosaic, broken chlorotic streaks, mottle and chlorotic ring patterns (Louie and Knoke, 1981), and stunting. The local yellow and white seed types are both susceptible to MDMV infection, but the effects on plant growth and yield are not marked under average conditions of cultivation, unless infection occurs at an early growth stage. In routine surveys MDMV is identified by electron microscopy and serological testing. All MDMV isolates from maize in Morocco have reacted in SDS-gel diffusion tests with antisera to Minnesota isolates of MDMV-A and MDMV-B.

Johnsongrass [Sorghum halepense (L.) Pers.] appears to be the most important alternate host of MDMV in Morocco, and high levels of field infection (up to 80%) are correlated with proximity to MDMV-infected, perennial johnsongrass growing, for example, in citrus orchards, grapevine fields, or along roadsides. No other alternate host of MDMV has been identified, but field occurrence of the disease in relation to the nearest known location of johnsongrass sometimes suggests that other sources of MDMV exist. Cynodon dactylon (L.) Pers., the most common grass weed in and around maize fields in Morocco, has been reported to be susceptible to both MDMV-A and MDMV-B (Rosenkranz, 1980).

Although all Moroccan MDMV isolates have reacted in gel-diffusion tests with both MDMV-A and MDMV-B antisera, all isolates have not been tested for infectivity on johnsongrass, so it is not known whether any...
nonjohnsongrass isolates have been among them. The strain of sugarcane mosaic virus (SCMV) which occurs widely in the sugarcane (Saccharum officinarum L.) cultivar NCo. 310 in northern Morocco (Fischer and Lockhart, 1974) is mechanically transmissible to maize but not to johnsongrass, but there is so far no evidence to indicate that the virus is transmitted naturally from sugarcane to maize, or that sugarcane in northern Morocco acts as a virus reservoir for infection of maize.

The most important vectors of MDMV in Morocco are Rhopalosiphum padi (L.) and R. maidis (Fitch), which occur throughout the year on maize, barley, and a range of wild Gramineae. The field occurrence of MDMV in maize appears to be more strongly correlated with virus source than with vector populations. However, one isolate of MDMV from johnsongrass has been found to be non-aphid transmissible, and it is possible that MDMV may not be always readily aphid transmitted from chronically infected johnsongrass.

**BARLEY YELLOW DWARF VIRUS (BYDV)**

In recent years BYDV has become an increasingly important disease of barley, wheat, and oats (Avena sativa L.) in Morocco. During 1981-82 viruslike symptoms consisting of stunting, chlorosis, and leaf-reddening were observed in fields of early-planted maize in southern and west-central Morocco. Plants showing these symptoms produced small, deformed, and poorly filled ears. No viruslike particles were observed in leaf-dip preparations, and mechanical transmission from maize to maize was unsuccessful. When virus-free R. padi or R. maidis were allowed to feed for 2-3 days on diseased maize and then transferred to healthy test plants of Coast Black oats, the latter developed typical leaf symptoms of BYDV infection in 18-20 days at 15-20 C. The virus was transmitted by R. padi from diseased maize and Coast Black oats to healthy maize, although there were differences in the reactions of the varieties tested. Symptoms were produced on Early Xtra Sweet and the local white variety, but not on Golden Cross Bantam which was previously reported to be immune to BYDV (Stoner, 1977). Faint broken chlorotic lines at the base of the lamina similar to those described for BYDV infection in maize line F7 in France (H. Lapierre, personal communication; Lapierre and Mehrad, 1977) were also occasionally observed. In early 1982 BYDV was also found in mixed infections with MDMV in southern Morocco. The symptoms, consisting of dwarfing with yellow and red stripes, were similar to those described from mixed BYDV-MDMV infection in Italy (Bell et al., 1981).

The symptoms attributed to BYDV infection were observed more frequently in early-planted than in late-planted maize. This may be related to the source of the virus and vectors, since symptoms of BYDV infection could be invariably observed in neighboring cereal fields or wild grasses. It is also possible that symptoms of BYDV infection in maize are more apparent at lower temperatures early in the year. It is of interest that in an epiphytotic of BYDV on barley, oats, and wheat in Switzerland, maize was suggested to be the source of virus for infection of the other cereals (Gugerli and Derron, 1981).

**MAIZE RHABDOVIRUS**

In 1975 bullet-shaped viruslike particles were observed in leaf-dip preparations of diseased maize plants from southern Morocco (H. U. Fischer and Lockhart, unpublished). Symptoms consisted of chlorotic streaks, and no etiological agent could be mechanically transmitted from diseased to healthy maize. The virus was assumed to be maize mosaic virus (MMV), but since the vector of MMV, Peregrinus maidis (Ashmead), was not found, no transmission or further identification of the virus was attempted.

In 1981 and 1982, similar viruslike particles were seen in leaf dip preparations of maize plants with symptoms that included chlorotic veinbanding, general chlorosis, stunting, and faint mottling. Mixed infections with MDMV were also found in which severe dwarfing occurred and the typical foliar symptoms of MDMV predominated.

In leaf dip preparations negatively stained with either phosphotungstic acid (PTA) or ammonium molybdate, bullet-shaped particles measuring 50-60 x 220-240 nm and bacilliform particles 50-60 x 300 nm were seen. The virus has been isolated from maize in several locations in the southern Sous-Massa region and the Tadla region of central Morocco. The percentage of plants showing symptoms varied from 2-20%.

Similar rhabdoviruslike particles have been found in C. dactylon, Digitaria commutata Schult., D. sanguinalis (L.) Scop., and Setaria verticillata (L.) P.B., all showing chlorotic streak symptoms. Occurrence of the virus in maize was invariably correlated with the presence of infected C. dactylon either within or along the edges of fields. Attempts to transmit the virus mechanically from infected C. dactylon to healthy C. dactylon, maize, and S. verticillata have been unsuccessful. Typical rhabdoviruslike particles were observed in field maize plants and they developed symptoms consisting of interveinal mottling and chlorosis.

The relationship of the Moroccan maize rhabdovirus to MMV and to those described in maize in Spain (Rubio-Huertos, 1978), Iran (Izadpanah and Parvin, 1979), and elsewhere (Jackson et al., 1981) remains to be determined. Until comparative serological, morphological, histopathological, and vector transmission studies are done, the relationships between those viruses cannot be ascertained. P. maidis has not been identified in planthopper surveys conducted over several areas of Morocco in 1978-81 (N. Bencheqroun, personal communication) and has not been found on corn. Whether or not the Moroccan maize rhabdovirus is related to MMV, it appears to have a vector or vectors other than P. maidis which has been the only reported vector of MMV (Nault and Knoke, 1981). Two leafhoppers, Exitianus capicola (Stal) and Psammotettix striatellus (L.), and two delphacid planthoppers, Laodelphax striatellus (Fallen) and Toya propinguia Fieber, breed on C. dactylon and Setaria spp. and are frequently found on maize. Preliminary transmission tests with
these four species have so far been unsuccessful. *L. striatellus* is a vector of barley yellow striae mosaic virus (BYSMV), reported from Italy and France (Conti, 1969), and of wheat chlorotic streak mosaic virus, reported from France (Signoret *et al.*, 1977), while *P. striatus* is the vector of winter wheat mosaic virus (Sherban and Onishchenko, 1972). These three cereal rhabdoviruses have particles similar in size to the Moroccan maize rhabdovirus, and BYSMV has been reported to infect sweet corn (Conti, 1974). More detailed studies on vector relationships and other properties are required in order to relate the Moroccan maize rhabdovirus to these and other similar viruses of the Gramineae.

Rhabdovirus-infected *C. dactylon* occurs throughout Morocco and identical symptoms have been seen on this plant in Tunisia, suggesting that the virus may be distributed around the Mediterranean in areas other than Morocco.

**OTHER VIRUSES**

Plants showing chlorotic ring symptoms are occasionally found in irrigated maize in southern Morocco. Other symptoms include pronounced thinning of stems and narrowing of leaves. The foliar symptoms are similar to those described by Lapierre *et al.* (1976) from Bugey, France, although the chlorotic rings tend to be circular rather than elongate. Such symptoms are observed only rarely in the field. Attempts at mechanical transmission have failed, and no viruslike particles have been observed in leaf-dip preparations. The virus described in France has been shown to be related to maize white line mosaic virus reported from the U.S. and Italy (H. Lapierre, personal communication).

Cucumber mosaic virus (CMV) infection occurs occasionally in maize in southern Morocco, but such infection is rare and of minor importance. Plants infected at the seedling stage develop chevron-like chlorotic bands on lower leaves, accompanied by thickening and cracking of midribs. Lethal systemic necrosis sometimes occurs in very young plants. Natural infection of maize by CMV appears to be restricted to areas in which maize is grown in close proximity to bananas (*Musa paradisiaca* L.) or other crops in which undergrowth weeds such as *Solanum nigrum* L., *Stellaria media* (L.) Vill., and *Malva* spp. act as reservoirs of both virus and vectors.

**LITERATURE CITED**


Insect Vectors of Maize Dwarf Mosaic Virus and Maize Chlorotic Dwarf Virus

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Cooperative investigations of the ARS, USDA, and OSU, OARDC, Wooster. This paper reports the results of research only. Mention of a commercial or proprietary product in this paper does not constitute an endorsement of this product by the USDA.

ABSTRACT


The aphid vectors of maize dwarf mosaic virus (MDMV) are reviewed. Data are presented on the aphid species occurring on maize (Zea mays) in Ohio, the probable significant vectors of MDMV, and the efficiency of methods for estimating aphid populations. Although not the most efficient vectors in laboratory trials, Myzus persicae and Rhopalosiphum maidis appear to be largely responsible for maize dwarf mosaic epiphytotics in Ohio. The leafhopper vectors of maize chlorotic dwarf virus are discussed. Data are presented on host plants, virus transmission efficiency, and survival of Graminella nigrifrons. Most small grains and grasses were better hosts for G. nigrifrons in the field than maize. The highest populations of the MCDV vectors were found on Echinochloa crusgalli, Digitaria sanguinalis, and Cynodon dactylon. In the laboratory, 33% of individually tested, unmated adult G. nigrifrons transmitted MCDV on their first attempt. The transmission rate was higher in their fourth week as adults, and a lifetime transmission rate of 86% was demonstrated. Female G. nigrifrons were better vectors and survived longer than males.

APHID VECTORS OF MDMV

MDMV is transmitted by aphids in a nonpersistent manner. There is a very loose, nonstructured, and temporary association of aphid and virus. The virus may be acquired by the aphid while probing with its stylets into epidermal or parenchyma cells in the leaves of infected plants of the Graminaceae. Both virus acquisition and subsequent inoculation can occur in a few seconds or minutes, permitting the entire transmission process to be completed in a short time. The virus is confined to the aphid's mouthparts and/or foregut and is lost when the insect molts. Adult aphids remain infective for a few minutes to at most a few hours under natural conditions.

At least 25 species of aphids have tested positive as MDMV vectors (R. E. Ford, personal communication; Knoke and Louie, 1981). In these tests to determine the transmission capability of different aphid species, laboratory-reared or field-collected aphids are normally confined for a few minutes on diseased maize plants before transfer to healthy, but MDMV-susceptible,
maize test plants for a similar time period. Other vector
species have been identified by confining field-collected
specimens directly on healthy test plants which later
showed symptoms of maize dwarf mosaic (Knoke et al.,
1977).

The tasks of determining the transmission efficiency
and the probable relative importance in nature of the
various species are difficult. Different groups of aphid
species are often assayed in different laboratories by
different techniques under different sets of environmen-
tal conditions. As a result, individuals of the same
aphid species may transmit MDMV to 1-10% of the test
plants in one laboratory and to 30-50% in another. The
relative transmission efficiency of the various vector
species may also depend on the strain of MDMV used in
the transmission trials (Louie and Knoke, 1975).

The ranking of the efficiency of various species of
aphids in MDMV transmission by use of laboratory
assays is somewhat subjective and may have only an
incidental relationship to the aphid’s relative impor-
tance as a vector in nature. For example, one of the most
efficient laboratory-tested vectors (Table 1), Schizaphis
graminum (Rondani), was not found to be carrying
MDMV in an area of high maize dwarf mosaic incidence
in southern Ohio and this species was not
detected on maize in northern Ohio during July, a time
of the season when aphid populations were rapidly
increasing in that area. Only two of the five efficient
vectors, the root inhabiting Aphis maidiradicis Forbes
and the bean aphid (Aphis fabae Scopoli), have maize as
a host, but the former and Aphis craccivora Koch have
been recorded in low numbers on maize leaves (Knoke,
unpublished). Both A. craccivora and A. fabae have a
wide host range, indicating considerable adaptability
in nature.

Some of the less efficient laboratory vectors (Table 2),
such as Therioaphis maculata (Buckton), Acrythosi-
phon pisum (Harris), and Aphis gossypii Glover, have
fairly high (>10%) transmission percentages in labora-
ory trials. Myzus persicae (Sulzer), which has only a
slightly lower average transmission rate (8%), is the only
species of the five less efficient vectors that has maize as
a host.

### TABLE 1. Efficient vectors of maize dwarf mosaic virus.

<table>
<thead>
<tr>
<th>Species</th>
<th>Common name</th>
<th>Percentage transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schizaphis graminum (Rondani)</td>
<td>greenbug</td>
<td>14</td>
</tr>
<tr>
<td>Aphis maidiradicis Forbes</td>
<td>corn root aphid</td>
<td>14</td>
</tr>
<tr>
<td>Aphis craccivora Koch</td>
<td>cowpea aphid</td>
<td>29</td>
</tr>
<tr>
<td>Aphis fabae Scopoli</td>
<td>bean aphid</td>
<td>7</td>
</tr>
<tr>
<td>Hyalopterus atriplices (L.)</td>
<td>boat gall aphid</td>
<td>9</td>
</tr>
</tbody>
</table>

* Knoke and Louie, 1981.

Order of species reflects their relative efficiency in one or more trials and is based on the overall averages presented in column 3.

* Average of one or more assays in different laboratories.

### TABLE 2. Less efficient vectors of maize dwarf mosaic virus.

<table>
<thead>
<tr>
<th>Species</th>
<th>Common name</th>
<th>Percentage transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acyrthosiphon pisum (Harris)</td>
<td>pea aphid</td>
<td>12</td>
</tr>
<tr>
<td>Myzus persicae (Sulzer)</td>
<td>green peach aphid</td>
<td>8</td>
</tr>
<tr>
<td>Aphis gossypii Glover</td>
<td>cotton aphid</td>
<td>11</td>
</tr>
<tr>
<td>Therioaphis maculata (Buckton)</td>
<td>spotted alfalfa aphid</td>
<td>18</td>
</tr>
<tr>
<td>Macrosiphum avenae (F.)</td>
<td>English grain aphid</td>
<td>1</td>
</tr>
</tbody>
</table>

* Knoke and Louie, 1981.

Order of species reflects their relative efficiency in one or more trials and is based on the overall averages presented in column 3.

* Average of one or more assays in different laboratories.

### TABLE 3. Inefficient vectors of maize dwarf mosaic virus.

<table>
<thead>
<tr>
<th>Species</th>
<th>Common name</th>
<th>Percentage transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyadaphis erysimi (Kaltenbach)</td>
<td>turnip aphid</td>
<td>3</td>
</tr>
<tr>
<td>Rhopalomyzus poae (Gillette)</td>
<td>bluegrass aphid</td>
<td>12</td>
</tr>
<tr>
<td>Rhopalosiphum padi (L.)</td>
<td>oat bird-cherry aphid</td>
<td>4</td>
</tr>
<tr>
<td>Dactynolus ambrosiae (Thomas)</td>
<td>brown ambrosia aphid</td>
<td>3</td>
</tr>
<tr>
<td>Macrosiphum euphorbiae (Thomas)</td>
<td>potato aphid</td>
<td>1</td>
</tr>
<tr>
<td>Rhopalosiphum maidis (Fitch)</td>
<td>corn leaf aphid</td>
<td>4</td>
</tr>
</tbody>
</table>

* Knoke and Louie, 1981.

Order of species reflects their relative efficiency in one or more trials and is based on the overall average presented in column 3.

* Average of one or more assays in different laboratories.
a host plant. However, it is seldom collected from maize plants. The inefficient vectors (Table 3) inoculated only an average of about 4% of the test plants in laboratory trials. Within this inefficient vector group are Rhopalosiphum padi (L.) and R. maidis (Fitch), two species that have maize as a host plant and whose alate and apterous forms are common on maize in northern Ohio.

In addition to comparative transmission assays and host range, other factors must be considered in determining a species' importance in nature. The mere occurrence of a species on maize plants or the presence of high populations of a species as measured by various types of traps or collection devices in a high disease area does not necessarily guarantee that the species is important in the epidemiology of the disease. However, other things being equal (transmission efficiency, flight behavior, probing behavior, etc.), a species with the greater number of individuals is probably a more important vector in the field than a species with fewer individuals. In order to be certain that an individual aphid (or aphid species) was responsible for an infected plant in nature, we would need to record the presence of the vector on the maize plant, observe its probing activity, identify the aphid, and immediately assay it for the presence of MDMV. We would also need to protect the target field plant and assay plant from exposure to other vectors for approximately 2 wk before and 2 wk after exposure to the individual vector. The subsequent development of maize dwarf mosaic symptoms on the target field plant and on the aphid assay plant would permit vector designation. Most of the field-related aspects of the above requirements are highly impractical on any scale that would produce sufficient data for statistical analysis.

Because of the above limitations, most attempts to identify the major vectors have been confined to counting, collecting, and identifying the aphids present on field-planted maize, or relating the numbers of individual species caught in traps to the amount of MDMV inoculum pressure at a particular test site as measured by maize trap plants.

Based on the relationship of aphid species collected in yellow-pan traps to disease in maize trap plants for locations in southern and northern Ohio, six species (Table 4) were most often correlated with the occurrence of disease at both locations (Madden et al., 1982). M. persicae was the predominant species and, although it was not one of the most efficient vectors in laboratory trials (Table 2), it probably contributed most to epiphytotic in the field due to its prevalence.

Because of a similar aphid-disease association in the two field areas, R. maidis must be considered as a significant vector of MDMV in the field. However, the transmission rate with this species is very low and even though it regularly colonizes maize in Ohio, this colonization occurs primarily late in the season when dent maize is physiologically more resistant to infection and tolerant to disease. R. maidis would appear to be relatively more important in the development of epiphytotics in sweet corn in northern Ohio, where successive and frequently adjacent plantings provide both mature plants for colonization and seedling plants that are highly susceptible to infection and of low tolerance to disease.

Both A. craccivora and A. gossypii may be relatively more important in the field than their low population values would indicate, since their laboratory transmission rates are generally higher than M. persicae or R. maidis and their populations peak earlier in the season when maize is more susceptible to infection and damage.

Major drawbacks to counting and identifying aphids on maize plants are the restricted amount of time the observer can spend with individual plants and the potential influence that the maize genotype may have on the aphids occurring thereon. The limited time period for the sample may be particularly crucial. To search for aphids on top and bottom surfaces of several leaves of one corn plant may require 30 sec or only 0.05% of the plant's exposure period for 1 day. Do these 30 sec counts accurately reflect the total numbers and species of aphids present on the plant for the entire day? Many of these population estimates are often made during "normal working hours" for humans, whereas major aphid flights often occur at dusk.

Several potentially significant disadvantages exist in the use of aphid traps for estimating vector populations and judging the significance of vector species in test

<table>
<thead>
<tr>
<th>TABLE 4. Probable vectors of maize dwarf mosaic virus in Ohio.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Species</strong></td>
</tr>
<tr>
<td>------------</td>
</tr>
<tr>
<td>M. persicae</td>
</tr>
<tr>
<td>Rhopalosiphum maidis</td>
</tr>
<tr>
<td>Aphis gossypii</td>
</tr>
<tr>
<td>Aphis craccivora</td>
</tr>
<tr>
<td>Dactynotus ambrosiae</td>
</tr>
<tr>
<td>Hyalopterus ariplics</td>
</tr>
<tr>
<td><strong>Mean all aphids</strong></td>
</tr>
</tbody>
</table>

* Data based on catches in yellow-pan traps exposed near Portsmouth, Ohio, for about 14 wk per year for 10 yr.

* Data based on catches in yellow-pan traps exposed near Wooster, Ohio, for about 19 wk per year for 13 yr.
areas. These include differences in trap catches due to trap type (water pan vs. suction vs. adhesive), trap color (yellow vs. green), and trap placement (height above soil or foliage and lateral proximity to foliage).

**LEAFHOPPER VECTORS OF MCDV**

MCDV is transmitted by leafhoppers in a semipersistent manner (Nault et al., 1973). Adults and nymphs can transmit the isometric virus particle immediately after acquisition, nymphs become noninoculative after molting, and adults remain viruliferous for about 2 days. The transmission efficiencies of vectors are improved by increasing the acquisition-access period (AAP), whereas a decrease in temperature increases the time a vector remains inoculative (Nault, 1977).

Known vector species include the blackfaced leafhopper, *Graminella nigrifrons* (Forbes), the lesser *Lawn* leafhopper, *G. sonora* (Ball), and the gray lawn leafhopper, *Exitianus exictiosus* (Uhler) (Nault and Knoke, 1981). Laboratory transmission trials with the above vectors revealed a transmission efficiency ratio of 1.00:18.0:23, with about one-third of the most efficient *G. nigrifrons* individuals transmitting MCDV in any one trial. Based on reports of geographical distributions (Gustin and Stoner, 1968; Kramer, 1967) and relative populations at various test sites (Douglas et al., 1966; Knoke, unpublished; Pitre and Hepner, 1967), it is probable that *G. nigrifrons* is also the most effective vector of MCDV in nature. Maize chlorotic dwarf is endemic in areas with overlapping distributions of *G. nigrifrons* and johnsongrass (*Sorghum halepense* (L.) Pers.), the overwintering host for the virus (Gordon and Nault, 1977; Nault et al., 1973). Reported nonvectors included *Dalbulus* spp., *Baldulus* sp., *Macrosteles fascifrons* (Stål), and *Stirellus bicolor* Van Duzee (L. R. Nault, personal communication; Nault and Knoke, 1981).

**MATERIALS AND METHODS**

Data from some of the methods we have used to estimate aphid vector populations and from studies related to the transmission efficiency and host plants of *G. nigrifrons* in Ohio follow.

**Aphid population estimates.** Aphid populations in and around a maize planting were estimated by two methods: hand counting of aphids found on maize leaves, and counting of aphids captured in yellow-pan water traps positioned between the replications of planted maize. Two dent corn hybrids (W9xOh51A and Pioneer Brand 3179), the dent corn inbred Oh28, and Jubilee sweet corn were hand planted on June 21 in a Latin square design of four 6.1 m rows per genotype for each of four replications. Twenty-five seeds were planted in each row, rows were 76 cm apart, and replications were separated by 4.6 m of bare soil. When plants were 30-40 cm tall, all the aphids were counted on and collected from the top five leaves of a 10-plant sample in one of the two center rows of each genotype. Collected aphids were placed in a vial of 70% alcohol and returned to the laboratory for identification. Counts and collections were made on nine dates starting July 11 and ending on August 12, when some plants were tasseling and aphid numbers were increasing rapidly.

During this same 33-day time span, aphids were also removed thrice weekly from yellow-pan water traps described previously (Knoke et al., 1974). The upper edges of the traps were positioned 61 cm above the soil; each was supported by a metal bracket and located over bare soil, equidistant from the north and south edges of the replications. Collected aphids were preserved for later counting and identification.

**Graminella nigrifrons populations on grains and grasses.** Fifteen grains and grasses were hand planted on May 7 as four replicates in a randomized complete block design in soil areas 1.2 by 6.1 m per grass host. The small grass seeds were distributed on the soil surface at recommended rates and the soil was then raked to cover the seed. The maize was planted with a hand planter at about 100 kernels per square meter (about 20 times the recommended seeding rate). A 0.6 m wide bare soil area surrounded each seeded area and a 2 m wide strip of barley (*Hordeum vulgare* L.) separated each replicate and surrounded the plot area.

Leafhoppers were sampled at approximately 2-wk intervals with a D-Vac suction sampler (Dietrick, 1961), passing the 930 cm$^2$ sampling head just above the leaves for 12.2 m in each plot. Forage ratings were calculated from values obtained by estimating the surface area covered by plant growth, estimating the amount of green leaf tissue present, and measuring the plant height.

Leafhopper count data were transformed by log X + 1 (where X is the number of leafhoppers) and analyzed by analysis of variance. Separation of transformed means was accomplished by Duncan’s New Multiple Range Test but actual means are listed in tables.

**Survival of and transmission by adult leafhopper vectors.** *G. nigrifrons* adults were tested individually for their ability to transmit MCDV over a lifespan of 6 wk. Insects were reared on barley host plants as described previously (Nault et al., 1973). Individuals, as late instar nymphs, were isolated on barley in 5.5 x 4.5 x 2 cm plastic, leaf cages ventilated top and bottom with a dacron-organandy covered 2 cm diam hole. A 0.5 x 3 cm area of plastic on the ends of the hinged-lid cage was replaced by foam rubber to permit the entrance and exit of host leaf tissue. Cages were held above host plants growing in 10 cm diam plastic pots by a rubber band stretched around the cage and a 30 cm pot label. A small, corked entrance hole permitted the introduction of the leafhopper with a glass aspirator.

Newly emerged adults were selected for MCDV transmission testing as four replications of 25 males and 25 females per replication. Each week, individuals were confined for a 2-day AAP on a MCDV-infected, 21-day-old Oh28 maize seedling inoculated by viruliferous leafhoppers 10 days earlier. The leafhoppers were then transferred individually to a second cage and confined on the youngest two leaves of a healthy, 14-day-old Oh28 maize seedling for an inoculation-access period (IAP) of about 2 days. Following this they were transferred to a cage containing several intact barley leaves and confined for about 3 days. This 2:2:3 day
cycle was continued until each insect died (maximum 6 wk). All host plants with caged insects were held in a rearing room at 25 ± 3°C, 50–70% RH, and 16 hr light per 24 hr. Seedlings exposed in the IAP were held in a greenhouse and observed 3 wk after exposure for detection of MCDV by appearance of diagnostic symptoms (Louie and Knoke, 1981).

RESULTS AND DISCUSSION

Aphid population estimates. The average aphid population on the upper five leaves of four maize genotypes for nine sample dates (a 32-day time period from about mid-July to mid-August) was greater on Wf9xOh51A than on Pioneer Brand 3179, whereas the sweet corn and dent corn inbred genotypes had intermediate numbers (Table 5). Significant differences between genotypes were apparent on six of the nine sample dates. More aphids were found on Wf9xOh51A than on Pioneer Brand 3179 on four dates (July 23, August 1, 4, and 12), and equal numbers were found on both hybrids on two dates (July 11 and 25). More aphids were found on Oh28 than on the other three genotypes on July 25; the reverse occurred on July 11. It is apparent that a 5-leaf by 10-plant sample is sufficient to detect differences in aphid populations on different maize genotypes on one sample date. However, counts must be made on more than one and probably several dates before valid aphid resistance or susceptibility can be identified in the genotypes.

Using yellow-pan traps and a sampling period covering the approximate time that aphids were counted on maize leaves, significantly more aphids were captured in the trap located in the center of the planting than in traps at the other four positions (Table 6). Further, more aphids were trapped adjacent to the east edge of the planting than adjacent to the west edge. Clearly, trap position in relation to surrounding vegetation influences the quantity of aphids trapped.

It is not possible with the data obtained in this study to evaluate the above two sample methods for accuracy in determining the actual aphid population in an experimental area. The hand count method permitted the detection of an average 14.1 aphids per plant per sample date. However, with only a few seconds to possibly 1-2 min spent looking for aphids on the leaves of each plant, this technique may not adequately provide representative numbers and species of aphids present on the plants for the entire 1440 min of the sample date. The number of aphids collected in yellow-pan traps averaged 45.7 per trap per day. A trap of this type collects aphids mainly during daylight, and traps large numbers of alate forms only if positioned above a contrasting, dark background. Does it trap a greater number or a lesser number of aphids than would normally be frequenting maize plants in adjacent plantings?

Populations of aphids in yellow-pan traps appear to fluctuate more on adjacent sample dates than populations observed on maize leaves (Fig. 1). The passage of a weather cold front with more than 5 cm of rain during July 21-22 drastically reduced the number of aphids found in the yellow-pan traps but had little influence on the counts of aphids on the maize leaves. Populations on maize leaves increased 20-40 times from July 22 to early August because alate forms began to colonize the plants and produced many nymphs. During this same period, only a 2-4 fold population increase occurred in the yellow pan traps. Since they were positioned a short distance from the maize plants, traps would rarely capture apterous forms.

The species identification of the collected aphids also revealed differences between the two sample methods

<table>
<thead>
<tr>
<th>Table 5: Aphid populations on maize leaves. *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
</tr>
<tr>
<td>Wf9xOh51A</td>
</tr>
<tr>
<td>Pioneer Brand 3179</td>
</tr>
<tr>
<td>Jubilee</td>
</tr>
<tr>
<td>Oh28</td>
</tr>
</tbody>
</table>

* Data are means of four replications of 10 plants per genotype for nine sample dates between July 11 and August 12, 1980. Means followed by the same letter are not significantly different (P = .05) based on Duncan's New Multiple Range Test.

<table>
<thead>
<tr>
<th>Table 6: Effect of trap placement on aphid catch. *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trap location</td>
</tr>
<tr>
<td>East</td>
</tr>
<tr>
<td>East-Center</td>
</tr>
<tr>
<td>Center</td>
</tr>
<tr>
<td>West-Center</td>
</tr>
<tr>
<td>West</td>
</tr>
</tbody>
</table>

* One 30.5 cm² yellow-pan trap was positioned 61 cm above soil and aphids were collected for 36 days. Means followed by the same letter are not significantly different (P = .05) according to Duncan's New Multiple Range Test.

Fig. 1. Average population of aphids in five yellow-pan traps (for 2-3 day periods per data point) compared with the average population on maize leaves (160 plant samples per data point) near Wooster, Ohio.
TABLE 7. Aphids found on maize plants and in yellow-pan traps near maize planting in northern Ohio in 1980.

<table>
<thead>
<tr>
<th>Species</th>
<th>Percentage of total found*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>On maize plants</td>
</tr>
<tr>
<td>Rhopalosiphum maidis</td>
<td>66.0</td>
</tr>
<tr>
<td>Macrosiphum euphorbiae</td>
<td>22.1</td>
</tr>
<tr>
<td>R. padi</td>
<td>9.1</td>
</tr>
<tr>
<td>M. avenae</td>
<td>1.0</td>
</tr>
<tr>
<td>Capitophorus elaegni (del Guercio)</td>
<td>0.7</td>
</tr>
<tr>
<td>Therioaphis muculata</td>
<td>0.2</td>
</tr>
<tr>
<td>My. as persicae</td>
<td>0.2</td>
</tr>
<tr>
<td>Aphis maidiradici</td>
<td>0.1</td>
</tr>
<tr>
<td>A. gossypii</td>
<td>0.1</td>
</tr>
<tr>
<td>A. crucivora</td>
<td>0.1</td>
</tr>
<tr>
<td>C. hippophaes (Walker)</td>
<td>0</td>
</tr>
<tr>
<td>Dactynotus ambrosiae</td>
<td>0</td>
</tr>
<tr>
<td>Hyadaphis erysmi</td>
<td>0</td>
</tr>
<tr>
<td>Acythosiphon pisum</td>
<td>0</td>
</tr>
<tr>
<td>Hyalopterus atriplicis</td>
<td>0</td>
</tr>
<tr>
<td>Others</td>
<td>0.4</td>
</tr>
</tbody>
</table>

* Sample size for identification purposes: on maize, 2716; in yellow-pan traps, 1000.

Graminella nigrifrons populations on grains and grasses. The use of a D-vac on the grain and grass plots yielded a total of more than 32,000 leafhoppers from five sample dates (Table 8). Of these, about 34% were G. nigrifrons, 38% M. fascifrons, and 28% Empoasca spp. or other leafhoppers. In this area, G. nigrifrons apparently had two seasonal population peaks, one in June and a second in late July-early August, while the M. fascifrons population sharply peaked only in mid-July. On maize about 70% of the leafhoppers were Empoasca spp., 25% were M. fascifrons, and only about 5% were G. nigrifrons or other species. Therefore, most leafhoppers captured on maize were not the MCDV vector.

Most small grains and grasses were better hosts than maize for G. nigrifrons (Table 9). Significantly more G. nigrifrons were found on more than one-half the grass species than on maize on each of five sampling dates. At the end of July there was about twice as much green plant material (forage rating) in the maize plot than in the johnsongrass plot and both had at least 16 times more green forage for leafhoppers than the ryegrass (Lolium perenne L.) planting. However, G. nigrifrons populations were highest in the ryegrass plots and relative populations were much higher in this plot than in...
the maize or johnsongrass plots. It is likely that johnsongrass and ryegrass contribute substantially to the maize chlorotic dwarf epiphytotics in southern Ohio. Ryegrass growing in fields, waste places, stream banks, and fence rows provides breeding places for large numbers of leafhoppers. Johnsongrass growing in and around maize fields, particularly in lowland areas, provides not only breeding places for vectors but also a source of MCDV. That G. nigrifrons prefers these two grasses over maize suggests that there would be considerable leafhopper movement in maize fields and therefore many chances to inoculate maize and seedling johnsongrass plants with MCDV.

In this study the small grains also generally supported more G. nigrifrons than maize supported. However, since small grains mature relatively early in the season, they would support fewer leafhoppers as the season progresses. The average G. nigrifrons population on these crops was equal to the plot mean on June 27 (41 vs. 40), but was only about one-half the mean on July 29 (25 vs. 51) and eventually supported less than 25% the mean number of leafhoppers in the entire plot on August 27 (4 vs. 18).

Most G. nigrifrons were found on three weed grasses common in the maize chlorotic dwarf disease area. Barnyardgrass [Echinochloa crusgalli (L.) Beauv.], crabgrass [Digitaria sanguinalis (L.) Scop.], and barnyardgrass [Cynodon dactylon (L.) Pers.] supported an average of 41 times more MCDV vectors than maize. Barnyardgrass, crabgrass, and giant foxtail (Setaria faberi Herrm.), a common weed in maize fields, are annual grasses susceptible to MCDV (Nault et al., 1976). They therefore may contribute substantial numbers of vectors and virus-infected source material for secondary virus spread in maize fields. Three perennial forages, orchardgrass (Dactylis glomerata L.), meadow fescue (Festuca pratensis Huds.), and timothy (Phleum pratense L.), may also provide additional breeding sites

<table>
<thead>
<tr>
<th>Sample date</th>
<th>Graminella nigrifrons</th>
<th>Macrostele fascifrons</th>
<th>Empoasca spp.</th>
<th>Others</th>
<th>Percent of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>June 27</td>
<td>2383</td>
<td>1737</td>
<td>880</td>
<td>457</td>
<td>16.9</td>
</tr>
<tr>
<td>July 14</td>
<td>1546</td>
<td>885</td>
<td>716</td>
<td>313</td>
<td>35.5</td>
</tr>
<tr>
<td>July 29</td>
<td>3242</td>
<td>974</td>
<td>263</td>
<td>2049</td>
<td>20.2</td>
</tr>
<tr>
<td>Aug. 14</td>
<td>2668</td>
<td>506</td>
<td>368</td>
<td>1871</td>
<td>16.8</td>
</tr>
<tr>
<td>Aug. 27</td>
<td>1100</td>
<td>277</td>
<td>119</td>
<td>1899</td>
<td>10.5</td>
</tr>
<tr>
<td>Percent of total</td>
<td>33.9</td>
<td>38.4</td>
<td>7.3</td>
<td>20.4</td>
<td></td>
</tr>
</tbody>
</table>

---

**TABLE 8. Total number leafhoppers captured on 15 grasses with D-vac suction sampler at Wooster, Ohio.**

**TABLE 9. Populations of Graminella nigrifrons on various grass hosts at Wooster, Ohio.**

<table>
<thead>
<tr>
<th>Host genus and species</th>
<th>Common name</th>
<th>Mean forage rating on July 31*</th>
<th>Mean number per plot on</th>
<th>G. nigrifrons</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>June 27</td>
<td>July 29</td>
<td>Aug. 27</td>
</tr>
<tr>
<td><strong>Echinochloa crusgalli (L.) Beauv.</strong></td>
<td>barnyardgrass</td>
<td>9</td>
<td>45 A</td>
<td>157 A</td>
</tr>
<tr>
<td><strong>Digitaria sanguinalis (L.) Scop.</strong></td>
<td>crabgrass</td>
<td>18</td>
<td>52 A</td>
<td>159 AB</td>
</tr>
<tr>
<td><strong>Cynodon dactylon (L.) Pers.</strong></td>
<td>bermedgrass</td>
<td>15</td>
<td>60 A</td>
<td>118 AB</td>
</tr>
<tr>
<td><strong>Lolium perenne L.</strong></td>
<td>ryegrass</td>
<td>3</td>
<td>57 A</td>
<td>52 DE</td>
</tr>
<tr>
<td><strong>Setaria faberi Herrm.</strong></td>
<td>giant foxtail</td>
<td>42</td>
<td>36 A</td>
<td>88 A-C</td>
</tr>
<tr>
<td><strong>Secale cereale L.</strong></td>
<td>rye</td>
<td>6</td>
<td>41 A</td>
<td>41 C-E</td>
</tr>
<tr>
<td><strong>Hordeum vulgare L.</strong></td>
<td>barley</td>
<td>3</td>
<td>55 A</td>
<td>27 D-F</td>
</tr>
<tr>
<td><strong>Sorghum halepense (L.) Pers.</strong></td>
<td>johnsongrass</td>
<td>48</td>
<td>40 A</td>
<td>58 B-D</td>
</tr>
<tr>
<td><strong>Dactylis glomerata L.</strong></td>
<td>orchardgrass</td>
<td>9</td>
<td>39 A</td>
<td>20 D-G</td>
</tr>
<tr>
<td><strong>Festuca pratensis Huds.</strong></td>
<td>meadow fescue</td>
<td>9</td>
<td>41 A</td>
<td>26 D-G</td>
</tr>
<tr>
<td><strong>Phleum pratense L.</strong></td>
<td>timothy</td>
<td>3</td>
<td>35 A</td>
<td>16 E-G</td>
</tr>
<tr>
<td><strong>Avena sativa L.</strong></td>
<td>oats</td>
<td>3</td>
<td>35 A</td>
<td>23 D-G</td>
</tr>
<tr>
<td><strong>Bromus inermis Leyss.</strong></td>
<td>smooth brome</td>
<td>3</td>
<td>27 A</td>
<td>7 G</td>
</tr>
<tr>
<td><strong>Trisetum aestivum L.</strong></td>
<td>wheat</td>
<td>1</td>
<td>32 A</td>
<td>10 FG</td>
</tr>
<tr>
<td><strong>Zea mays L.</strong></td>
<td>maize</td>
<td>100</td>
<td>1 B</td>
<td>7 G</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td></td>
<td>40</td>
<td>54</td>
<td>18</td>
</tr>
</tbody>
</table>

---

* Visual estimation of amount of green plant material in plot.
* For each date, means followed by the same letter are not significantly different (P = .05) based on Duncan's New Multiple Range Test.
* June 27, July 14 and 29, and August 14 and 27.
* Forage rating of maize × Number leafhoppers on other host (July 29)  
Forage rating of other host × Number leafhoppers on maize (July 29)
for *G. nigrifrons*. Seasonal populations of vectors on these grasses were similar to populations on johnsongrass or about 12 times the populations on maize.

By limiting the stands of these weed hosts and forage grasses in and around maize fields, *G. nigrifrons* populations and the incidence of maize chlorotic dwarf could be considerably reduced. Since johnsongrass is the only grass that is both an MCDV host and a perennial, eliminating this overwintering host of MCDV from areas in and around maize fields could prevent or at least drastically limit maize chlorotic dwarf epiphytotics (Knoke et al., 1983).

Survival and transmission of MCDV by adult vectors. In tests of unmated *G. nigrifrons* adults for their ability to transmit MCDV following consecutive 48 hr AAP and IAP on maize, only 33% of the individuals transmitted MCDV during their first week as adults (Table 10). This increased to a maximum of 50% at 4 wk of age and resulted in a 6-wk accumulated transmission rate of about 86% for individuals allowed one attempt at transmitting MCDV each week. With the high lifetime transmission rate of this *G. nigrifrons* population and with a minimal 1 hr combined AAP and IAP (Nault et al., 1973), it is probable that most if not all individuals would transmit MCDV under natural conditions in maize-johnsongrass areas, since they may have many more than six opportunities to acquire virus and inoculate susceptible maize.

In this study of caged and unmated individuals, however, more than 39% failed to transmit MCDV with just over an average of two attempts per individual (Table 11). Of the 10 leafhoppers that transmitted MCDV at least once, the average rate was 46.2%. The 10 leafhoppers that survived and were tested each week for 6 wk had a 76.7% transmission rate. Nine percent of the vectors failed to transmit the first three times they were tested but then did transmit during subsequent attempts.

The median survival time and total percentage transmitting virus were calculated for each replicate and sex. A strong relationship (R² = 79%) existed between survival time and transmission (Fig. 3). For females, the median survival time and transmission rate values of 4.5 wk and 71.3% were significantly greater (P

---

**TABLE 10. Age of adult *Graminella nigrifrons* as related to transmission of maize chlorotic dwarf virus.**

<table>
<thead>
<tr>
<th>Age at first inoculation (wk)</th>
<th>Number Tested</th>
<th>Percentage Positive Accumulation*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>166</td>
<td>55</td>
</tr>
<tr>
<td>2</td>
<td>75</td>
<td>17</td>
</tr>
<tr>
<td>3</td>
<td>48</td>
<td>14</td>
</tr>
<tr>
<td>4</td>
<td>26</td>
<td>13</td>
</tr>
<tr>
<td>5</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>1</td>
</tr>
</tbody>
</table>

* The weekly percentage accumulation (PA) values are based on the current week's percentage positive (PP) value and the previous week's PA value as follows: PA(i,6) = PA(i,5) + (PP(i,5) x (100 - PA(i,5))).

---

**TABLE 11. Transmission of maize chlorotic dwarf virus following weekly transfers of adult *Graminella nigrifrons.***

<table>
<thead>
<tr>
<th>Number of times leafhopper transmitted</th>
<th>Leafhoppers per class</th>
<th>Total inoculations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>0</td>
<td>65</td>
<td>39.2</td>
</tr>
<tr>
<td>1</td>
<td>43</td>
<td>25.9</td>
</tr>
<tr>
<td>2</td>
<td>28</td>
<td>16.9</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>12.0</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>4.2</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>1.2</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>0.6</td>
</tr>
<tr>
<td>Total</td>
<td>166</td>
<td>100</td>
</tr>
</tbody>
</table>

* Two days on maize source plant, 2 days on maize test plant, and 3 days on barley food host.
than comparable values for males of 1.9 wk and 44.8%.

A chi-square test was used to determine if individual transmissions for 1 wk were correlated with transmissions the following week. For females, significant correlations occurred only for wk 1 and 2 and wk 4 and 5; for males the correlation was significant only for wk 2 and 3. Therefore, for most G. nigrifrons adults, success in transmitting MCDV on one attempt was usually not related to success on previous or subsequent attempts.

**LITERATURE CITED**


Maize White Line Mosaic and Maize Subtle Mosaic Viruses

Raymond Louie, J. K. Knoke, D. T. Gordon, and R. E. Gingery

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The authors acknowledge the technical assistance of J. J. Abt, R. J. Anderson, A. Juan Rubink, and A. M. Stofer.

ABSTRACT


Maize white line mosaic virus (MWLMV) and maize subtle mosaic virus (MSMV) are both soil-borne. Presently, MWLMV has been found infecting corn (Zea mays) in eight states in the northeastern and north central USA, whereas MSMV has been found only in Ohio. MWLMV causes distinct white lines and severe mosaic patterns on leaves of diseased maize, whereas MSMV causes only mild mosaic symptoms. MSMV is mechanically transmissible and MWLMV is not. Vectors of both viruses are unknown, but seed transmission has been reported for MWLMV. MWLMV has a spherical particle ca. 35 nm in diam and MSMV a flexuous particle of variable length. No disease control measures are currently available, but Benlate was reported to control soil transmission of MWLMV.

Before 1976, most of the known viruses of maize (Zea mays L.) were vectored by aphids, leafhoppers, or plant-hoppers (Nault and Knoke, 1981). Since then, three soil-borne maize viruses have been isolated. The first was maize chlorotic mottle virus (MCMV) reported in Kansas in 1976 (Niblett and Claflin, 1978) and in Nebraska in 1977 (Wysong et al., 1978). The U.S. isolate is serologically related (Niblett and Claflin, 1978) to MCMV described in 1973 from Peru (Hebert and Castillo, 1973).

The other two more recently discovered soil-borne viruses are maize white line mosaic virus (MWLMV), first observed in New York in 1979 (Boothroyd and Israel, 1980), and maize subtle mosaic virus (MSMV) found in Ohio in 1975 (Louie, unpublished). This report reviews information on MWLMV and MSMV. Information on MCMV is presented by Uyemoto (1983).

MAIZE WHITE LINE MOSAIC VIRUS

Distribution. Maize white line mosaic has been reported from Massachusetts (J. D. MacKenzie, personal communication), Michigan (Gordon and B. P. Singh, unpublished), Maine and New Hampshire (B. M. Bryce, personal communication), New York (Boothroyd and Israel, 1980), Ohio (Louie et al., 1982), Vermont (Gotlieb and Liese, 1980), and Wisconsin (de Zoeten et al., 1980). The number of states reporting the occurrence of maize white line mosaic has not increased in the last 2 yr. However, the number of counties within these states where maize white line mosaic has been found has increased. This may indicate increased survey activity rather than an actual increased incidence. For example, the known distribution of maize white line mosaic in Ohio during 1982 increased from three to seven northern counties following an intensive survey.

MWLMV isolates from Wisconsin and Vermont are serologically indistinguishable from each other (de Zoeten et al., 1980); this was a somewhat unexpected finding since reports of maize white line mosaic indicate occurrences in isolated pockets within fields, counties, or states. However, symptomless infections in maize are common (Louie et al., 1983) and actual disease distribution may be more extensive than symptoms would indicate. Another possible explanation for isolates being similar is that the virus is more stable within the soil.

Symptomatology. In the initial stages of disease, small, discrete, chlorotic spots are seen on leaves. Later, severe mosaic and mottling usually appear (Boothroyd and Israel, 1980), although recovery from the disease (de Zoeten et al., 1980) and symptomless infections (Louie et al., 1983) are reported. Discrete chlorotic white lines, primarily within the veinal tissue, are distinctive and characteristic of the disease. Variability in mosaic and mottle patterns is common, including the occasional appearance of dark-green, blotchy spots against a cream-colored or yellow background.

No obvious root, leaf, or flower malformations have been reported. Stunted plants are common. In some sweet corn fields in Ohio where plant tops were removed prior to hand harvesting, the stunted, diseased plants were easily observed from a distance because they...
were the only ones with tassels due to delayed maturity at the time of detasseling.

Kernel and ear production are also adversely affected. Yield loss in field plants ranges from 28-34% (Boothroyd and Israel, 1980) up to 45% (Louie et al., 1982). In sweet corn, losses approach 100% because the under-sized and poorly filled ears are not marketable.

Virus characteristics. MWLMV has a 35 nm diam spherical particle with a buoyant density of 1.33-1.35 g/ml CsCl and a single protein subunit of 32,000 ± 1600 daltons molecular weight (Boothroyd and Israel, 1980; de Zoeten et al., 1980; Louie et al., 1982). The virus extinction coefficient at 260 nm is 3.9 cm²/g. The genome is a single-stranded RNA with a base composition of U = 24.3%, G = 30.0%, A = 19.7%, and C = 26.0% (de Zoeten et al., 1980).

MWL MV has been detected in pollen, anthers, silks, whole kernels, and roots as well as leaves of infected plants (Boothroyd and Israel, 1980; Louie et al., 1982). Possible seed transmission has been reported by Boothroyd and Israel (1980), but de Zoeten et al. (1980) and Louie et al. (1982) were unable to demonstrate seed transmission. No maize inbred or hybrid tested has appeared resistant to MWLMV infection, but some sweet corn hybrids have been more tolerant than others to yield loss (T. A. Zitter, personal communication). In addition to maize, MWLMV has been reported in naturally infected Digitaria sanguinalis (L.) Scop., Panicum dichotomiflorum Michx., Setaria faberi Herrm. (Louie et al., 1982), and S. viridis (L.) Beauv. (de Zoeten et al., 1980).

Virus transmission. MWLMV has not been transmitted by mechanical inoculation or by leafhoppers or aphids. Transmission to maize seedlings under greenhouse conditions was most successful when seeds were planted in soil collected from the vicinity of infected plants. Transmission was also achieved in greenhouse soil infested with infected roots (Louie et al., 1982) and with chopped, infected leaves (T. A. Zitter, personal communication). The fungi, Polymyxa graminum Led. and Olpidium spp., were suggested as possible vectors (C. W. Boothroyd, personal communication), but this has not been demonstrated.

Disease control. Except for the previously mentioned testing of sweet corn, no evaluations or breeding of maize lines for resistance have been done. A major obstacle to studies on disease control has been the lack of an efficient method of inoculation. Reliance on natural infection under field conditions is unsatisfactory because of low disease incidence and frequent symptomless infections. Evaluations in the greenhouse also are limited by similar difficulties. However, some progress can be made by testing superior entries for disease escape or symptomless infection. Chemical control with Benlate was reported (Louie et al., 1982).

MAIZE SUBTLE MOSAIC VIRUS

MSMV was first observed in maize plants growing in MWLMV-infested field-collected soil tested in the greenhouse. The first MSMV isolates induced very mild mosaic symptoms. By subculturing areas of leaf tissues with more severe symptoms, an isolate causing obvious mosaic symptoms was selected. Like MWL-MV, MSMV is apparently transmitted by an unknown soil-inhabiting vector, but unlike MWLMV, MSMV is mechanically transmissible. Gingery (unpublished) showed a serological relationship between MSMV and maize dwarf mosaic virus, strain A, in microprecipitin and immunosorbent electron microscopy tests. This relationship was not confirmed by the double antibody sandwich enzyme-linked immunosorbent assay (Louie, unpublished). MSMV is has been found in maize growing in seven Ohio soil samples contaminated with MWLMV and has not been reported to occur outside of Ohio. This may reflect difficulty in detecting MSMV because of mild symptoms and masking of symptoms by other viruses. Furthermore, host range tests and serological assays for other maize viruses would not detect coinfections with MSMV. Chance observation of infections involving only MSMV presently appears to be the only means for MSMV detection.

We are studying the virus particle characteristics, disease distribution, and vector identity. We also plan to screen inbreds and hybrids for disease resistance and tolerance.

LITERATURE CITED


Corn Lethal Necrosis: Disease Symptoms, Control, and Epidemiology Considerations

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ABSTRACT


Maize chlorotic mottle virus (MCMV), a vital component of the corn lethal necrosis (CLN) disease complex, is beetle transmitted and is persistent in continuously cropped corn (Zea mays) fields. Evidence accumulated to date suggests a soil-borne phase for MCMV and that viruliferous beetle larvae initiate virus infections in young corn plants. Crop rotation will effectively control MCMV. Overwintering sources for MCMV are infected crop residues and for maize dwarf mosaic virus strain B (MDMV-B), the principal second virus in the disease complex, the winter annual grass Bromus tectorum. The role of soil moisture in the epidemiology of both MCMV and MDMV-B is discussed.

Corn lethal necrosis is a disease caused by two viruses acting in concert in a common corn (Zea mays L.) plant. By definition, the virus complexes producing corn lethal necrosis include maize chlorotic mottle virus (MCMV), which is beetle vectored (Jensen, 1979; Nault et al., 1978), and either maize dwarf mosaic virus (MDMV) or wheat streak mosaic virus (WSMV) (Niblett and Claflin, 1978). Although three viruses are involved in the disease complex, the research thrust at Kansas State University has been primarily on MCMV. This is because MDMV and WSMV were already endemic in the Corn Belt states and much information was already available. Also, corn breeding efforts had contributed greatly to the development and release of MDMV and WSMV resistant and/or tolerant hybrids and inbreds.

In contrast, pertinent biological and epidemiological data for MCMV were lacking and the 1976 corn lethal necrosis epidemic indicated the need for such information. Hence, a corn virus research program was initiated in Kansas. The following is a review of the progress made since late 1977.

SYMPTOMATOLOGY AND CORN YIELDS

In 1978 and 1979, corn plots were established to determine the effect of different inoculation times with single and double virus inocula on disease symptoms and grain yields. Prior studies involving natural field occurrences of the disease found inconsistent effects. For example, during the 1976 epidemic several affected corn fields contained plants with bright chlorosis followed by leaf necrosis and plant stunting. Corn yields for these plants were low. In neighboring fields, however, plants of normal height showed severe leaf necrosis of the terminal growth, but corn yields appeared little affected. Virus assays of all symptomatic leaves confirmed an association of MCMV with principally MDMV.

In a study to elucidate these differences in symptomatology, strain A of MDMV was used with a Kansas culture of MCMV. Virus inocula were sprayed onto terminal leaves of three corn hybrids at 3-, 7-, and 14-leaf growth stages. Test results showed that leaf chlorosis and necrosis and reduced plant height and/or corn yields were pronounced when infections, particularly by MCMV + MDMV, occurred at or before the seven-leaf stage. For double virus inoculations at the 14-leaf stage, terminal growth continued and plants attained a height comparable to noninoculated controls, even though the growth exhibited severe leaf necrosis. Corn yields of two hybrids differed significantly from 14-leaf non-diseased controls (P = 0.05) (Uyemoto et al., 1981).

EPIDEMIOLOGY

From 1978 to 1981, potted plant distributions, seed and/or corn plant collections were made as needed throughout each year (Bockelman et al., 1982; Uyemoto, unpublished). Seeds or kernels were harvested from MCMV-infected weeds and corn plants and tested for seedborne-virus transmission (Bockelman et al., 1982). Similarly, corn residues and soil samples were collected at various times of the year and tested for virus content and soilborne-virus transmission (Hutchens, 1981; Uyemoto, unpublished).

The findings are summarized as follows: a) MCMV infections occurred only in potted bait plants distributed at or after mid-season, i.e., during the active feeding period of adult western corn rootworm beetles (Diabrotica virgifera LeConte), the primary vector spe-
cies in Kansas. b) Winter annual and perennial grasses were free of MCMV, even though greenhouse tests showed that many native grasses were MCMV suspects. c) Initial MCMV infections in field-grown corn plants were routinely detected in late June and prior to emergence from soil of adult western corn rootworm beetles. d) MCMV was not detected in germinated seeds of weeds or corn. e) Corn residues collected in October (roots) or April (pith tissues) contained infectious MCMV. f) No virus transmissions occurred when soils were devoid of insect larvae.

The lack of an apparent weed reservoir for the virus, coupled with virus recovery from corn residues and detection of MCMV-infected corn plants in late June, suggested to us that MCMV may be soilborne, via feeding activities of western corn rootworm larvae. Presumably they could have acquired virus from corn residues and then transmitted it to roots of rapidly growing corn plants. Based on an egg maturation temperature threshold of 52°F and an accumulated thermal number of ca. 400, larval hatches from overwintered eggs of the western corn rootworm should proceed rapidly by mid-June (Musick and Fairchild, 1971; Wilde, 1971). If larval virus acquisitions and transmissions occurred then, mosaic symptoms in the foliage should appear as noted above by late June. In the 1982 survey, MCMV was again detected on June 25.

**DISEASE CONTROL**

Although virus transmission has been established for larvae of several beetle-vectored plant viruses (e.g., cocksfoot mottle virus [Serjeant, 1967]; phleum mottle virus [Catherall, 1970]; turnip crinkle virus [Tollings and Stone, 1972]; and turnip yellow mosaic virus [Markham and Smith, 1949]), their role in virus disease epidemiology does not appear significant. Many of these viruses can overwinter in perennial host species, are seedborne in one or more plant species, and can survive in adult beetles (Walters et al., 1972). However, since evidence to date suggests that none of these properties applies to the survival and reestablishment of MCMV in newly planted corn fields, the soilborne hypothesis appears plausible for MCMV epidemiology. If so, this should permit the utilization of cultural practices (e.g., crop rotation) for virus control. With the possibility that crop rotation might control MCMV by destroying infected corn residues through decomposition and/or affecting egg laying behavior of corn rootworm beetles, we established in 1979 an eight replicated corn and sorghum [Sorghum bicolor (L.) Moench] plot within a grower’s corn field (Phillips et al., 1982). In the succeeding 2 yr, the field was planted entirely to corn and corn plants were sampled and assayed for MCMV on a weekly basis.

In the first year, incidence of MCMV was 17.5% and 0% in corn and sorghum, respectively. In the second year, MCMV was detected on June 25 when plants were about 8 wk old. Periodically through that summer we sampled all symptomatic corn plants. In subplots cropped continuously to corn, virus incidence rose from 1.6% to 12.2%. However, subplots planted to sorghum and then corn contained an initial 0% to a final 0.6% MCMV level. Values between cropping sequences differed significantly (P = 0.05). Rootworm egg numbers were 0.5/100 cc soil for the continuous corn subplots and 1.3/100 cc soil for rotated subplots (Phillips et al., 1982). The egg laying behavior of female beetles in the sorghum subplots was not altered in our test plot, presumably due to its size (4.5 m x 15.24 m) and immediate proximity to the commercial corn planting.

In the third year, MCMV was detected on June 21 and final virus incidence in the two cropping sequences was 37% for the continuous corn subplots and 22% for the sorghum-corn-corn rotation. These percentages were significantly different (P = 0.05).

As an alternative to crop rotation, we incorporated crop residues into the soil after harvest in an attempt to hasten tissue decomposition and we also removed stalk-crowns; these treatments and a control treatment were done in replicated trials. In the ensuing cropping season, we found the incidences of MCMV among all treatments were indicating that these practices were ineffective for controlling MCMV infections (Uyemoto, 1983).

Under our test conditions, excellent control of MCMV was mediated only by crop rotation. However, to ensure a similar degree of control as noted in the first corn planting following sorghum, it is advisable to alternate cropping sequence on a regular basis. When corn is replanted, MCMV may be reintroduced via viruliferous adult beetles arising from neighboring corn fields and become reestablished on that site.

**OTHER EPIDEMIOLOGICAL CONSIDERATIONS**

In corn lethal necrosis areas of north-central Kansas, we have observed that certain fields, but not some adjoining corn fields, continually exhibit early season MCMV infections. A possible explanation for this difference was derived from soil maps, where a virus-soil moisture relationship was suggested. In MCMV endemic fields, the soil type is silt to silty clay-loam with an available water capacity (AWC) rating of 0.56 cm 2.54 cm soil. Nonendemic fields contain sandy loam soils and an AWC of 0.36 cm. Hence, it is conceivable that high water holding capacity soils provide the necessary moisture regime required for overwintering of infectious MCMV in corn residue and/or maintaining viability of insect eggs. At this time it is noteworthy to mention that recent laboratory findings showed that diseased tissue dehydrates, a concomitant reduction and loss of infectivity occurred in certain tissues (Table 1).

To date, corn lethal necrosis outbreaks have occurred in 1976 (Niblett and Claffin, 1978), 1978 (Uyemoto et al., 1980), and 1980 (Uyemoto, unpublished). However, in the intervening years, MCMV was frequently detected in corn samples collected in previously diseased fields (Uyemoto, unpublished). The seemingly ever presence of and pivotal position held by MCMV in the corn lethal necrosis complex indicated us that other factors contributing to MDMV epidemiology were likely.
responsible in determining the severity of corn lethal necrosis outbreaks.

In Kansas, MDMV strain B, not MDMV-A, was routinely recovered with MCMV from corn lethal necrosis diseased plants (Uyemoto et al., 1980). Since MDMV-B does not infect johnsongrass [Sorghum halepense (L.) Pers.] we embarked on a search for its alternate weed host. Based on accumulated findings, downy chess (Bromus tectorum L.) is probably the bridging host between corn plantings (see Bockelman et al., 1982). B. tectorum, a winter annual, germinates readily in late summer-early fall when moisture is adequate. In corn lethal necrosis-nonepidemic years, the recorded precipitation were above normal, and weed beds were established along field edges and other uncultivated areas. Symptomatic plants were found in those weed beds (during fall to spring surveys) and these contained MDMV-B (Uyemoto, unpublished). Virus was presumably transmitted there by viruliferous aphids arising from maturing corn plants. If these correlations prove to be meaningful, moisture may be a critical component in the epidemiology of both viruses.

**LITERATURE CITED**


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**TABLE 1. Maize chlorotic mottle virus: Effect of desiccation on infectivity.**

<table>
<thead>
<tr>
<th>Tissue source</th>
<th>Number of samples</th>
<th>Fresh tissue sero-/bio-assays</th>
<th>Air dried for:</th>
<th>2-16 mo. bioassay</th>
</tr>
</thead>
<tbody>
<tr>
<td>root</td>
<td>9</td>
<td>pos/9+++/e</td>
<td>neg/neg</td>
<td>-d</td>
</tr>
<tr>
<td>root*</td>
<td>6</td>
<td>pos/6+++</td>
<td>neg/neg</td>
<td>-</td>
</tr>
<tr>
<td>pith*</td>
<td>6</td>
<td>pos/6+++</td>
<td>neg/3+</td>
<td>-</td>
</tr>
<tr>
<td>husk</td>
<td>3</td>
<td>pos/3+++/g</td>
<td>neg/neg</td>
<td>neg</td>
</tr>
<tr>
<td>(yellow mottled)</td>
<td>3</td>
<td>pos/3+++/g</td>
<td>neg/3+</td>
<td>3+++</td>
</tr>
<tr>
<td>(yellow mottled)</td>
<td>3</td>
<td>pos/3+++/g</td>
<td>neg/3+</td>
<td>3+++</td>
</tr>
<tr>
<td>husk (green)</td>
<td>4</td>
<td>neg/g</td>
<td>neg/neg</td>
<td>neg</td>
</tr>
</tbody>
</table>

* Seroassay by agar gel diffusion plates and MCMV antiserum, pos = positive and neg = negative. Bioassay hosts included corn (N28Ht) and sorghum (Asgrow Bagoff).

+ Corn tissues stored in paper bags for 0.7 to 16 months at ambient temperature.

++ = all corn plants infected, + = one-third or less of inoculated plants showed symptoms.

— is not tested.

* Paired samples, i.e., root and pith tissues removed from same corn plants.

† MCMV infections of corn as ay plants confirmed by serology.

§ Maize dwarf mosaic virus strain B was detected in all samples by assay on sorghum and in serological tests.
ABSTRACT


The distributions, life cycles, host relationships, and mobility of Diabrotica species are reviewed in an historical and evolutionary context, with emphasis on the species associated with maize (Zea mays). The geographic ranges of Diabrotica in North America are determined by overwintering ability and ranges of host plants or host plant communities. The fucata group species are multivoltine (more than one generation per year) and overwinter as adults; they do not survive where freezing temperatures occur. Species which survive where subfreezing temperatures occur are all in the virgifera species group and overwinter as eggs. Larvae feed on roots and adults feed on pollen, floral parts, and in some cases foliage. In some cases larvae and adults feed on different species of plants, and therefore host complexes are sometimes required to complete the life cycle. Species of the fucata group are polyphagous; as larvae they are known to feed on plants from eight different families. Adults feed on an even greater variety of plants. By contrast, larvae of the virgifera group are essentially restricted to certain grasses and adults tend to feed on the flower parts or foliage of larval host plants. Differences among the species in habitat and geographic mobility are described and discussed.

DISTRIBUTION

That the genus is primarily neotropical is reflected in the number (in parentheses) of recognized species present in representative political divisions: Canada (4), USA (7), Mexico (31), Panama (35), Colombia (39), Brazil (136), Peru (38), Bolivia (29), Argentina (16), and Chile (5). For the tropical species, for which diversity is the greatest, our knowledge is weakest; thus, our viewpoint largely concerns temperate climate species. Those species which overwinter in temperate North America are all in the virgifera group (Krysan, 1982). Indeed, the virgifera group is disproportionately represented in the temperate climate; of the 25 species in the group, five occur in temperate North America (Table 1).

By contrast, the fucata group, with 298 species, has only two species, the banded cucumber beetle (BCB) (D. baleata LeConte) and the southern corn rootworm (SCR) (D. undecimpunctata howardi Barber), with any interaction with the temperate climate. The northern range of the BCB (Smith, 1966) is limited to areas where hard freezes seldom occur. Since about 1900, the range of the BCB has expanded dramatically eastward and westward from southern Texas, presumably due to agricultural practices (Smith, 1966). The ability of the BCB to expand its range is clear and hosts suitable to this highly polyphagous species occur throughout North America; inability to survive in subfreezing temperatures (Saba, 1970) clearly limits the range.

The SCR also challenges the temperate climate. The species overwinters in the Gulf Coastal and southern
**TABLE 1.** *Diabrotica* species associated with maize.

<table>
<thead>
<tr>
<th>Species</th>
<th>Locale</th>
<th>Stage reported</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>adelpha Harold</td>
<td>Guatemala</td>
<td>X X</td>
<td>Melhus et al., 1954</td>
</tr>
<tr>
<td>balteata LeConte</td>
<td>U.S., Mexico, Guatemala, Costa Rica, Colombia</td>
<td>X X</td>
<td>Marsh, 1910; Melhus et al., 1954; Young, 1961</td>
</tr>
<tr>
<td>cristata (Harris)</td>
<td>U.S.</td>
<td>X</td>
<td>Weisenborn and Krysan, 1982</td>
</tr>
<tr>
<td>decempunctata sicuanaica</td>
<td>Bechyne</td>
<td>X X</td>
<td>Krysan and Branson, unpublished</td>
</tr>
<tr>
<td>lepida (Say)</td>
<td>Guatemala</td>
<td>X</td>
<td>Melhus et al., 1954</td>
</tr>
<tr>
<td>bartheri Smith and Lawrence</td>
<td>Guatemala</td>
<td>X X</td>
<td>Chiang, 1973; Tyler &amp; Ellis, 1974</td>
</tr>
<tr>
<td>nigrofasciata Jacoby</td>
<td>Guatemala</td>
<td>X</td>
<td>Melhus et al., 1954</td>
</tr>
<tr>
<td>pronnea Harold</td>
<td>Guatemala</td>
<td>X X</td>
<td>Melhus et al., 1954</td>
</tr>
<tr>
<td>rufomaculata Jacoby</td>
<td>Guatemala</td>
<td>X</td>
<td>Melhus et al., 1954</td>
</tr>
<tr>
<td>sexmaculata Baly</td>
<td>Guatemala</td>
<td>X</td>
<td>Melhus et al., 1954</td>
</tr>
<tr>
<td>speciosa speciosa Germar</td>
<td>Brazil</td>
<td>X X</td>
<td>Christensen, 1943</td>
</tr>
<tr>
<td>speciosa vigens (Erich)</td>
<td>Peru</td>
<td>X X</td>
<td>Krysan &amp; Branson, unpublished</td>
</tr>
<tr>
<td>speciosa vigens decolor</td>
<td>Peru</td>
<td>X</td>
<td>Wille, 1952</td>
</tr>
<tr>
<td>tibialis Jacoby</td>
<td>Guatemala</td>
<td>X</td>
<td>Melhus et al., 1954</td>
</tr>
<tr>
<td>tricolor Jacoby</td>
<td>Guatemala</td>
<td>X</td>
<td>Melhus et al., 1954</td>
</tr>
<tr>
<td>undecimpunctata undecimpunctata Mannerheim</td>
<td>Western U.S.</td>
<td>X X</td>
<td>Michelbacher et al., 1943</td>
</tr>
<tr>
<td>undecimpunctata howardi Barber</td>
<td>Eastern U.S.</td>
<td>X X</td>
<td>Arant, 1929</td>
</tr>
<tr>
<td>virgifera virgifera LeConte</td>
<td>U.S. and northwest Mexico</td>
<td>X X</td>
<td>Krysan et al., 1980</td>
</tr>
<tr>
<td>virgifera zeae Krysan and Smith</td>
<td>Oklahoma to Central America</td>
<td>X X</td>
<td>Krysan et al., 1980</td>
</tr>
<tr>
<td>viridula auct (as longicornis n. ssp.)</td>
<td>Guatemala</td>
<td>X</td>
<td>Melhus et al., 1954</td>
</tr>
<tr>
<td>viridula auct</td>
<td>Colombia, Costa Rica</td>
<td>X X</td>
<td>Harries, 1975; Risch, 1979</td>
</tr>
</tbody>
</table>

* So rare that its presence is trivial.

**TABLE 2.** Geographic ranges of selected *Diabrotica*.

<table>
<thead>
<tr>
<th>Taxon (acronym)</th>
<th>Range</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>fuscata group</td>
<td>D. undecimpunctata undecimpunctata (WSCB)</td>
<td>Smith (1966)</td>
</tr>
<tr>
<td></td>
<td>D. undecimpunctata howardi (SCR)</td>
<td>Smith (1966)</td>
</tr>
<tr>
<td></td>
<td>D. balteata (BCB)</td>
<td>Smith (1966)</td>
</tr>
<tr>
<td></td>
<td>D. tibialis</td>
<td>Smith (1966)</td>
</tr>
<tr>
<td>virgifera group</td>
<td>D. cristata (Harris)</td>
<td>Smith (1966)</td>
</tr>
<tr>
<td></td>
<td>D. lemniscata LeConte</td>
<td>Smith (1966)</td>
</tr>
<tr>
<td></td>
<td>D. longicornis (Say)</td>
<td>Krysan et al. (1983)</td>
</tr>
<tr>
<td></td>
<td>D. barbari (NCR)</td>
<td>Krysan et al. (1983)</td>
</tr>
<tr>
<td></td>
<td>D. virgifera virgifera (WCR)</td>
<td>Krysan et al. (1980)</td>
</tr>
<tr>
<td></td>
<td>D. virgifera zeae (MCR)</td>
<td>Krysan et al. (1980)</td>
</tr>
</tbody>
</table>
areas of the USA where subfreezing temperatures do not occur (Arant, 1929); during the warm season it migrates into most areas of eastern North America. It has been found as far north as Hudson Bay in Canada (Smith, 1966). Thus, the geographic distribution of the fucata group species must be viewed dynamically, varying from summer to winter and year to year depending on weather conditions.

The virgifera group species have several biogeographic characteristics open to biological and evolutionary interpretation. There is evidence of range expansions related to agricultural practices for the two major U.S. maize pest Diabrotica, the western corn rootworm (WCR) (D. virgifera virgifera LeConte) and the northern corn rootworm (NCR) (D. barberi Smith and Lawrence). The ranges of selected Diabrotica are summarized in Table 2.

Before 1940, the WCR was found in the USA only in areas adjacent to the eastern slope of the Rocky Mountains; early collection dates include western Kansas in 1865 (LeConte, 1858), New Mexico before 1893 as D. filicornis Horn (Horn, 1893), Colorado in 1909 (Gillette, 1912), and western South Dakota in the 1920's (Kantack, 1965). The WCR was first detected as a pest of maize in Colorado in 1909 (Gillette, 1912); it was unnoticed for several years and then reappeared as a pest on irrigated maize in Kansas and Nebraska in the 1940's. Beginning in the late 1940's, perhaps as a result of cultural practices, the WCR rapidly expanded its range across the Corn Belt until it now occurs as far east as West Virginia, Pennsylvania, and New York. The range of the WCR is apparently limited to the range of maize, so it probably has occupied temperate North America no longer than maize, i.e., about 1000 yr (Branson and Krysan, 1981).

The NCR first appeared as a pest of maize in the 1860's in Illinois and Missouri, and Webster (1908) documented the geographic expansion of the range of the species as a pest in the eastern USA in the late 1800's. Webster considered the NCR to be a prairie species and concluded that a population in Illinois or Missouri changed its host affinities to maize and thence expanded its range. That view is confounded by museum holdings of specimens, which we identify as the NCR, that were collected from those eastern areas long before reports of the insect as a pest (Krysan et al., 1982). Webster's idea that the NCR was an inhabitant of North American prairies before the introduction of maize is supported by our recent studies on biogeography, mechanisms of dormancy, and reproductive isolation in the D. longicornis complex (Branson and Krysan, 1981; Krysan, 1982; Krysan et al., unpublished).

Adults of D. longicornis (Say) are intimately associated with the native plant Cucurbita foetidissima H.B.K. where that plant occurs in relict prairies or montane grassland systems in the central and southwestern USA, respectively. Indeed, the northeastern limits of the range of D. longicornis correspond approximately with that of C. foetidissima (Bemis et al., 1973).

D. cristata (Harris) occurs in relict prairies over much of the USA east of the Rocky Mountains and D. lemniscata LeConte is found only in montane grasslands in the southwestern USA and northern Mexico. No virgifera group species occur on the west coast of the USA.

Our lack of knowledge of the ecological requirements of tropical species prevents speculation on factors that limit the ranges of those species, but some relationships to elevation have been detected. Melhus et al. ('54) noted that in Guatemala the BCB was the only species of Diabrotica seen on the Pacific Coastal Plain and "D. porracea Harold was the last species to disappear in cornfields above about 6500 feet" (= ca. 2000 m). In the Cuzco Department of Peru, changes in species composition relative to elevation are striking; elevations from 600 to 1800 m yielded 17 species of Diabrotica. Only D. speciosa vigens (Erichson) was found in the range of 2600 to 3000 m elevation. D. s. vigens, which disappeared from collections above 3200 m, was joined at 5000 m by D. decempunctata sicuanaica Bechlyne. The latter species was found at the highest elevation (3500 m) of any Diabrotica (Krysan, Branson, and R. F. W. Schroeder, unpublished).

Several factors appear to be responsible for limiting the ranges of these species. As mentioned above, none of the species of the virgifera group occurs on the west coast of the USA, suggesting that the mountains are a barrier. However, since 1970 the WCR has come to occupy the Great Basin of Utah and Idaho. The ranges of the WCR, the NCR, D. longicornis, and perhaps D. cristata and D. lemniscata are determined by the ranges of their host plants or host plant complexes. The overwintering range of the SCR and the northern limit of the range of the BCB are determined by climate. Factors setting the southern limits of these species are unknown.

LIFE CYCLES

The life cycles of the North American Diabrotica conform to taxonomic groupings; those species in the fucata group are multivoltine (more than one generation per year) and overwinter as adults, whereas those species in the virgifera group are univoltine (one generation per year) and overwinter as eggs. In Argentina D. speciosa (fucata group) is multivoltine and overwinters as an adult (Christensen, 1943). Eggs of D. viridula auct from Peru hatched in about 10 days at 20 C (Krysan, unpublished), which suggests it is not like the North American virgifera group species which all appear to have an obligatory egg diapause (Krysan, 1982). We are aware of no other information on life cycles of South American Diabrotica.

The SCR in Alabama has three generations per year and overwinters in reproductive diapause (Arant, 1929); the regulation of diapause has not been studied but the seasonality suggests photoperiod is probably involved. The BCB has no apparent diapause (Saba, 1970) and undergoes six generations per year in Texas (Marsh, 1910) and Mexico (Young, 1961). Adult populations fluctuate greatly, but they are present year-round.

The obligatory egg diapause in the virgifera group species prevails and imposes univoltinism even in those geographic areas of the southern USA and Mexico...
where *fucata* group species are multivoltine (Krysan, 1982). Given the seasonal realities of temperate North America, the obligatory egg diapause confers absolute univoltinism because development cannot occur in the cold of winter. Based on laboratory studies of eggs from populations of *D. virgifera zeae* Krysan and Smith [Mexican corn rootworm (MCK)], collected in the wet/dry subtropics of Mexico, Krysan et al. (1977) speculated that egg diapause in this subspecies of *D. virgifera* is adaptive by synchronizing egg dormancy with the dry season. Field studies by Branson et al. (unpublished) confirmed that once diapause in the egg is completed, the amount of moisture present in the soil determines the end of dormancy in the eggs. However, given variability in diapause duration in eggs, vagility (capacity to compete in its environment), and variable cropping practices (e.g., irrigation versus nonirrigation), all stages are present over most of the year in any one area. Once the conditions of spring are favorable for growth and development (warmth in temperate zones and moisture in subtropical and tropical zones), egg dormancy terminates, hatching occurs, and the larvae feed on growing roots. In the USA, adults emerge during mid- to late summer and lay diapause eggs.

Branson and Krysan (1981) have suggested that the life cycle strategies are related to the larval host ranges of the taxa involved, i.e., the polyphagous species are multivoltine and those restricted to certain grasses are univoltine. Univoltinism in the *virgifera* group is believed to have evolved as an adaptation to alternating wet and dry seasons of central Mexico (Krysan, 1982).

**HOST PLANT RELATIONSHIPS**

The ability of *Diabrotica* adults to exploit many different plants is reflected in the number of crops which beetles in the genus attack; our survey of the literature (the articles are too numerous to cite individually) lists 61 crops. Larvae, for which such observations are much more difficult to obtain, have been reported to feed on plants in eight different families (Table 3). Despite this bewildering diversity, patterns of heuristic value can be discerned.

The host relationships of *Diabrotica* will be reviewed with the recognition that insect/plant interactions are dynamic, in both phylogenetic and ontogenetic arenas. For example, in ontogeny some species are at some times “specialists” and at other times “generalists”, depending on the stage of development or its age. In the evolutionary sense some insect herbivore/host interactions appear to have been acquired in geological time, often through co-evolution, whereas other host relationships have evolved more recently in response to more immediate ecological and biogeographical constraints or opportunities (Strong, 1979). Host relationships in *Diabrotica* will be considered at these several levels from a dynamic perspective.

Metcalf (1979) has concluded that the Diabroticites, and perhaps *Aulacophora* of the Old World, share a co-evolutionary relationship with Cucurbitaceae. The antiquity of the relationship for *Diabrotica* is indicated by the fact that beetles from five genera of Diabroticites feed on Cucurbitaceae (Krysan, unpublished; Metcalf, 1979). Central to this association are the cucurbitacins, a group of secondary plant chemicals which for most insects are repulsive or indeed toxic, but which are arrestance or feeding stimulants for many species of the Diabroticites and *Aulacophora* (Metcalf, 1979). This ancient evolutionary association with the Cucurbita provides a background against which one may view changes in host relations.

<table>
<thead>
<tr>
<th>Species</th>
<th>Plant family</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>adelpha</em></td>
<td>Gramineae</td>
<td>Melhus et al., 1954</td>
</tr>
<tr>
<td><em>baltieata</em></td>
<td>Gramineae</td>
<td>Marsh, 1910</td>
</tr>
<tr>
<td></td>
<td>Convulvulaceae</td>
<td>Pitre and Kantack, 1962</td>
</tr>
<tr>
<td></td>
<td>Leguminosae</td>
<td>Marsh, 1910</td>
</tr>
<tr>
<td><em>speciosa speciosa</em></td>
<td>Gramineae</td>
<td>Christensen, 1943</td>
</tr>
<tr>
<td></td>
<td>Leguminosae</td>
<td>Christensen, 1943</td>
</tr>
<tr>
<td><em>speciosa vigens</em></td>
<td>Gramineae</td>
<td>Krysan &amp; Branson, unpublished</td>
</tr>
<tr>
<td></td>
<td>Leguminosae</td>
<td>Krysan &amp; Branson, unpublished</td>
</tr>
<tr>
<td></td>
<td>Cucurbitaceae</td>
<td>Wille, 1952</td>
</tr>
<tr>
<td><em>undecimpunctata undeimpunctata</em></td>
<td>Gramineae</td>
<td>Michelbacher et al., 1943</td>
</tr>
<tr>
<td></td>
<td>Cucurbitaceae</td>
<td>Michelbacher et al., 1943</td>
</tr>
<tr>
<td></td>
<td>Leguminosae</td>
<td>Michelbacher et al., 1943</td>
</tr>
<tr>
<td></td>
<td>Solanaceae</td>
<td>Baker, 1928</td>
</tr>
<tr>
<td></td>
<td>Polygonaceae</td>
<td>Rockwood &amp; Chamberlin, 1943</td>
</tr>
<tr>
<td><em>undecimpunctata howardi</em></td>
<td>Gramineae</td>
<td>Arant, 1929</td>
</tr>
<tr>
<td></td>
<td>Cucurbitaceae</td>
<td>Arant, 1929</td>
</tr>
<tr>
<td></td>
<td>Leguminosae</td>
<td>Arant, 1929</td>
</tr>
<tr>
<td></td>
<td>Solanaceae</td>
<td>Thomas, 1943</td>
</tr>
<tr>
<td></td>
<td>Compositae</td>
<td>Chittenden, 1905</td>
</tr>
<tr>
<td></td>
<td>Cyperaceae</td>
<td>Chittenden, 1905</td>
</tr>
<tr>
<td></td>
<td>Convulvulaceae</td>
<td>Cuthbert &amp; Davis, 1971</td>
</tr>
</tbody>
</table>
Some Diabroticides are almost totally committed to cucurbits throughout development; e.g., Acalymma vitattum (Fab.) larvae feed only on cucurbits and adults prefer cucurbits, feeding on other plants in the vicinity of cucurbits only during those times of the year (e.g., before and after adult hibernation) when cucurbit plants are not satisfactory (Houser and Balduf, 1925). Thus A. vitattum needs cucurbits to complete its life cycle. We are aware of no species of Diabrotica committed to cucurbits at both larval and adult stages. However, all the species of Diabrotica so far tested have a tendency to eat cucurbitacins (Metcalfe, 1979). Despite the omnipresence of an appetite for cucurbitacins, some species associate with cucurbits and some do not.

The Diabrotica known to be polyphagous at both larval and adult stages are D. undecimpunctata undes-cimpunctata Mannerheim [western spotted cucumber beetle (WSCB)], a subspecies of the SCR, SCR, BCB, D. speciosa speciosa Germar, and D. speciosa vigens. The known larval host ranges of these species include eight plant families (Table 3). The adults feed on such a tremendous variety of plants that to prepare a list would be futile. Sell (1916) reported that the SCR feeds on some 280 plant species and Webster (1913) considered a host list to be more interesting for the plants it did not contain. Similar conclusions can be drawn about the BCB (Saba, 1970), the WSCB (Michelbacher et al., 1943), and D. speciosa (Christensen, 1943). These species obviously exploit many plants other than cucurbits and apparently many populations go through several generations without an individual ever feeding on a cucurbit.

For the virgifera group, we have information available on larval hosts for North American species only; they feed almost exclusively on the roots of certain grasses (reviewed by Branson and Krysan, 1981). The one exception is the report by Branson et al. (unpublished) of D. virgifera zea feeding on roots of a sedge, Cyperus macrocephalus Liebm.

There are striking differences in the virgifera group in the relationship of adult stage to Cucurbitaceae. Among the species in the USA, only D. longicornis appears to have an intractable association with Cucurbitaceae species. Adults are collected on the flowers of C. foetidissima in relict prairie ecosystems in the Great Plains or montane grasslands of the southwest (Krysan et al., unpublished). Exhaustive searches for D. longicornis adults on other plants were without success, except for occasional specimens collected on domestic cucurbits in rural gardens (Krysan, unpublished). Indeed, the range of D. longicornis (Krysan et al., unpublished) extends no farther north and east than does the range of C. foetidissima (Bemis et al., 1978), which suggests an obligatory dependence on C. foetidissima. However, it is not collected from C. foetidissima in the deserts of the southwest.

The NCR (D. barberi) tends not to associate with cucurbits as an adult, a trait which probably contributes to its reproductive isolation from D. longicornis (Krysan et al., unpublished). Larvae of the NCR feed on the roots of several grasses, most successfully on maize (Branson and Ortman, 1971) from which it is most commonly collected. Most NCR larvae feed on the roots of maize and adults feed on pollen, silks, and ear tips until those plant parts become dry. The NCR then abandons maize plants and feeds on the pollen and floral parts of a variety of plants which might be collectively termed "prairie forbs" (Ludwig and Hill, 1975); indeed, it has been classed as a "generalist" (Messina and Root, 1980).

Presumably C. foetidissima is the ancestral host of adults of the D. longicornis complex, and it appears that NCR adults switched from feeding on C. foetidissima to other prairie forbs. Such a change would have allowed the range of the NCR to occupy, as it does, the northern and eastern USA and southern Canada.

Another virgifera group species, D. cristata (Harris), occupies relict prairies of the USA and northern Mexico east of the Rocky Mountains. The larvae apparently feed on the roots of native perennial grasses and the adults feed on pollen of a great variety of prairie forbs (Weisenborn and Krysan, 1980). The prairie ecosystem, with roots of perennial grasses and an admixture of forbs which flower in mid-to late summer, provides larval and adult food in a single system. D. cristata is only occasionally collected from cucurbits (Krysan, unpublished). The NCR and D. cristata have similar adult hosts.

Larvae of the WCR feed on roots of maize and the adults, like the NCR, feed on silks, pollen, and tender ears of maize. However, once those parts of the plant are dry, WCR adults feed on the foliage of maize. Unlike the NCR, the WCR is not found feeding on the flowers of prairie forbs (Branson and Krysan, 1981). Despite this commitment to maize, the WCR retains a predilection that only a miniscule portion of the WCR beetle population ever feeds on cucurbits. In our opinion, the WCR is an ecological monophag and the taste for cucurbitacins is vestigial.

The association of Diabrotica with Cucurbitaceae appears to stem from ancient associations developed by co-evolutionary processes, whereas the host relationships of the virgifera group in North America seem to be products of more recent biogeographical and ecological phenomena. For the WCR, taste for cucurbitacins is apparently ecologically irrelevant. For D. longicornis, an association with cucurbits as adults may be obligatory, whereas for the NCR there may be selection against feeding on cucurbits related to reproductive isolation from D. longicornis (Krysan et al., unpublished).

Given the clear differences between larval and adult host plants among some of the Diabrotica, it is intuitively obvious that oviposition cues must exist to insure that the eggs are placed within range of a host for the poorly mobile, indiscriminate, first instar larvae (Branson, 1982). This becomes especially complex with those species in the virgifera group which are univoltine in
the egg stage. The relationship of feeding behavior to oviposition has been reviewed by Branson and Krysan (1981). Little is known about the subtleties of this key step between the generations.

The manner in which Diabrotica populations are affected by host and non-host plants in monocultural and polycultural cropping systems in the tropics has been reported (Altieri et al., 1978; Risch, 1979, 1980). In the wet/dry highlands of Jalisco, Mexico, Branson and J. Reyes (unpublished) found adult MCR and D. porrectae in a field of Zea diploperennis Ilits, Doebly, and Guzman and found in laboratory studies that MCR larvae feed on the roots of this species. These observations place these known pests of maize with a presumed near ancestor of maize. Furthermore, Branson et al. (unpublished) found that larvae of the MCR fed on and adults emerged from several weedy grasses and a sedge near ancestor of maize. The MCR, which is the widespread rootworm pest of maize in Mexico (Krysan et al., 1980), would be a reasonable candidate as a vector of viruses among maize and related grassy plants. This possibility is further highlighted with the recent discovery of MCMV in the Mexican Central Plateau (D. T. Gordon, personal communication).

**DISPERSAL**

There is considerable variation among the Diabrotica in the tendency to move among host plants and between different ecological and geographic areas. The most striking pattern of movement is the yearly northward migration of SCR beetles from overwintering ranges in the southern USA, as mentioned previously (Smith and Allen, 1982). The collection of SCR beetles (but not the BCB) in October on oil platforms in the Gulf of Mexico 160 km at sea indicated an autumnal migration to the south (Baust et al., 1981). Beetles of the SCR which have overwintered migrate northward in the spring (Smith and Allen, 1982) and feed and oviposit on maize. Such beetles are rarely numerous enough to cause damage, but they could carry plant or insect pathogens from southern reservoirs. In the southern USA the BCB shares with the SCR many host plants but there is no evidence that the BCB ever migrates.

There is documentation of a pre-and post-dormancy migration of WSCB, between lowlands and foothills in California, probably in response to availability of food (Smith and Michelbacher, 1949). In other cases, selection of overwintering habitats by WSCB adults is less definite (Rockwood and Chamberlin, 1943).

Given the enormous variety of host plants of fucata group adults, and the variation in the presence and palatability of these hosts during the season, frequent and rapid movements between plants occur (Rockwood and Chamberlin, 1943).

Within the virgifera group species, the NCR and WCR have clear differences with respect to long and short range movements. Locally, WCR beetles are generally found on maize or in cucumber flowers near maize. Late in the season in corn growing areas, the NCR is found on flowering forbs in a variety of ecosystems such as weed patches, gardens, small grain stubble, and remnant native prairies. Witkowski and Owens (1979) detected different movement patterns of WCR and NCR beetles between maize plots of different plant maturity. The WCR tends to be found in maize fields throughout its life, whereas the NCR is found in these fields only in the earlier part of its life. Differences in adult movements apparently account for the fact that the NCR (but not the WCR) frequently occurs as a pest of maize in fields not planted to maize the previous year (Hill and Mayo, 1980).

Since beetles of the WCR tend to move from more mature to less mature maize (Hill and Mayo, 1974), one would expect considerable inter-field movement in areas where season length and cultural practices result in the presence of maize at a variety of maturities for any one time (e.g., highlands of Mexico) (Branson et al., unpublished).

The WCR and NCR appear to differ also in long distance movements. The rapid range expansion of the WCR in the USA demonstrates that founder populations move some 70 miles per year; therefore, beetles emerging from a given field could conceivably contribute to a WCR gene pool some 70 miles away the next year. Apparent migratory female WCR, but not NCR, have been found by Turpin (in Balsbaugh, 1980).

Despite the conclusion of Webster (1938) that the NCR spread rapidly east to Ohio from Illinois in the 1900's, there is some evidence that the NCR has low vagility. Krysan et al. (1983) have documented extensive geographic variability in color of beetles in the eastern USA. Hamilton (1965) reported that resistance in the NCR to the insecticide aldrin was confined to isolated geographic areas. This geographic variability of aldrin resistance was found again in 1976 and 1981 between populations only about 40 miles apart (Sutter, personal communication). Studies underway on isozymes show there is less intraspecies and intra-population variability in the WCR than in the NCR (I. C. McDonald and Krysan, unpublished).

**LITERATURE CITED**


Occurrence and Control of Maize Streak Virus in Maize in Kenya

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Appreciation is extended to K. R. Bock for inspiration and information from the Crop Virology Research Project at the Kenya Agriculture Research Institute, Muguga, Kenya. I also thank C. K. Bungey et al. for providing unpublished data from a field survey on maize streak virus in Kenya. I am especially grateful to B. K. Gakonyo for permission to travel and present this manuscript in Wooster, Ohio, USA. Last but not least, I express my appreciation to the Ohio Agricultural Research and Development Center and U.S. Agency for International Development for the invitation and for funding my travel to Wooster to attend this second International Maize Virus Disease Colloquium and Workshop.

ABSTRACT


Isolated occurrences of maize streak virus (MSV) were observed in an irrigated maize (Zea mays) nursery at an altitude of 1890 m in Kitale in 1980. Previously in 1973, MSV field surveys conducted countrywide revealed a general incidence of MSV in bimodal rainfall areas of Kenya. Breeding for resistance is under way at the Kenya Agriculture Research Institute (KARI) at Muguga. No resistant commercial maize hybrids or varieties have been released yet in Kenya. Better cultural practices are the only simple, inexpensive means for control of MSV at present.

Maize (Zea mays L.) is the major food crop and is considered an economical source of calories in Kenya. It is the basic staple diet of the majority of people in Kenya.

To overcome any food shortage in the country, the government of Kenya, under the leadership of His Excellency President Daniel Arap Moi, has drawn up a comprehensive National Food Policy (Anonymous, 1981). "One of the major objectives" clearly pointed out in the paper "is the production of enough maize to meet an ever increasing demand for food stemming from a rapidly expanding population." The Kenya Seed Company (KSC) has been directed to ensure that adequate seed supplies, particularly for maize, are available to all maize growing areas before the start of every season. This necessitates production of basic or foundation maize seed for all the hybrids and composites at the KSC experimental farm located at Endebess in the Kitale area. A need to isolate maize lines by distance in order to avoid simultaneous flowering presented problems in efficient land utilization. However, since the climate of Kenya permits year-round maize production, KSC considered trying a continuous maize nursery in 1980 in which flowering of different lines occurred at different times. This practice was successfully tried by Brewbaker (1979) in his development of a "Continuous Breeding Nursery" in Hawaii. A continuous maize nursery was initiated by KSC in April 1980.
Fig. 1. A field sketch map of maize breeding block (bird's eye view). Maize was sown at 10 plants per row, with 77 rows per range and a total of 18 ranges. Diseased plant stand count as indicated by the dots was observed just after maize had flowered. Figure is not to scale of research plot.
Table 1. Occurrence of maize streak (MS) in surveys of maize fields in three districts of two Kenyan provinces during 1973.*

<table>
<thead>
<tr>
<th>District</th>
<th>Range of infection (% MS)</th>
<th>Range in altitude (m)</th>
<th>Maize cultivar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kirinyaga in</td>
<td>1-50</td>
<td>1200-1700</td>
<td>Katumani CB</td>
</tr>
<tr>
<td>Central Province</td>
<td>more in lower altitude sites</td>
<td></td>
<td>H 512 H 613</td>
</tr>
<tr>
<td>Kiambu in</td>
<td>4-90</td>
<td>1400-2300</td>
<td>Katumani CB</td>
</tr>
<tr>
<td>Central Province</td>
<td></td>
<td></td>
<td>H 512 H 613</td>
</tr>
<tr>
<td>Siaya in</td>
<td>2-75</td>
<td>1200</td>
<td></td>
</tr>
<tr>
<td>Nyanza Province</td>
<td></td>
<td></td>
<td>H 511 H 632 H 613</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Local maize</td>
</tr>
</tbody>
</table>

* Unpublished data of N. S. Sindu and C. K. Bungey, National Agricultural Laboratories, Nairobi.

Breedling for resistance has been undertaken on and off at Kenya Agriculture Research Institute (KARI), beginning in the 1930's with Storey's work. Storey worked on resistance derived from a South African source and although he produced lines by selective inbreeding, which apparently bred true for resistance, subsequent attempts to incorporate these into East African maize were not successful (Storey and Howland, 1967a, b). These resistant lines were not maintained and the Storey material was lost.

**RESULTS AND DISCUSSION**

According to Bock (1980), maize streak is of less economic significance in Kenya than in some parts of neighboring countries. In Kenya MSV fluctuates very widely from year to year and often the disease appears to be almost sporadic in occurrence. The incidence rarely exceeds 10%, although incidence can be much higher in individual plots (N. S. Sindu and C. K. Bungey, personal communication). Field screening cannot therefore be used because of the unpredictable and generally low incidence of MSV in Kenya. Results would

Table 2. Incidence of maize streak virus diseased plants in resistant (R), susceptible (S), and crosses of R and S cultivars. Trial conducted at Karen location* near Nairobi City in 1981.**

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Stand count</th>
<th>No. diseased plants</th>
<th>Percent diseased</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inbred A x Reunion S4 (S x R)</td>
<td>83</td>
<td>1</td>
<td>1.20</td>
</tr>
<tr>
<td>H 511 x Reunion S4 (S x R)</td>
<td>56</td>
<td>1</td>
<td>1.80</td>
</tr>
<tr>
<td>H 511 (S)</td>
<td>98</td>
<td>34</td>
<td>34.8</td>
</tr>
<tr>
<td>Inbred A (S)</td>
<td>53</td>
<td>22</td>
<td>41.5</td>
</tr>
<tr>
<td>Reunion S4 (R)</td>
<td>19</td>
<td>0</td>
<td>0.00</td>
</tr>
</tbody>
</table>

* This location provided MSV in epidemic proportion.

** Unpublished data of R. Rasiah.

The produced crosses of Reunion S4 (H 511 x Reunion S4 and Inbred A x Reunion S4) were tested in an isolated severe MSV epidemic area in Karen location. The two hybrids were sown in a plot adjacent to diseased maize plot. H 511, inbred A, and Reunion S4 were used as controls. The disease incidence in the plot was recorded 2 months after sowing. At this location maize plants would be at 75 cm - 100 cm high, 2 months after planting. This would be a period just before flowering.
be unreliable and the exercise would waste resources and time. All studies must, therefore, be done under controlled conditions in the glasshouse, as presently done at KARI, Muguga. However, K. R. Bock and R. Rasatah (personal communication) exposed their new maize crosses in a heavily affected area in Karen near Nairobi and have reported tolerance in the hybrids developed (Table 2).

For high value maize crops, such as foundation seed or sweet corn for fresh-green maize markets, chemical control by application of systemic insecticides (although expensive) could be tried. B. Anastasiadis (personal communication) relates some experience of successful chemical control using carbofuran as 10% granules at 1.5-2.0 kg active ingredient. The granules applied at seeding of the maize have controlled *C. mbila* in southern Africa.

**CONCLUSIONS AND RECOMMENDATIONS**

Development of maize hybrids and cultivars resistant to MSV would be welcomed by farmers in low and medium altitudes in Kenya. Breeding for resistance should be given some priority and such a program should be incorporated into a long-term national maize breeding program. Better cultural practices should be followed by all maize growers. There are numerous benefits in planting early, and using early maturing maize hybrids or varieties would go a long way to help make a definite break between seasons, especially in bimodal areas. Closed seasons should be observed rigorously.

For sophisticated farmers, use of carbofuran or equivalent could be tried not only to control the vector of MSV, but also to control other damaging pests, *i.e.*, stalk borers *Busseola fusca*, *Chillo* spp., *Sesamea* spp., and others common in maize growing areas. The treatment would be expensive but would pay off through raising high value, higher yielding but susceptible hybrids or cultivars. A concerted effort by all, including farmers, extensionists, pathologists, agricultural financial officials, seedmen, breeders, entomologists, and local and international organizations would help Kenya eradicate, or at least minimize, the occurrence of maize streak disease which at times is a worrisome problem for some of our esteemed small scale farmers.

**LITERATURE CITED**


The Status of Maize Virus Diseases in Zimbabwe

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Appreciation is expressed to C. J. Grimmer for assistance in preparation of this manuscript.

ABSTRACT


Virus diseases of maize (Zea mays) have never posed a serious problem in Zimbabwe. Maize streak virus is epiphytic but not considered a severe problem since cultural and chemical control measures have proved successful. Streak incidence has increased in recent years as a result of expansion of irrigation and winter

Historically, virus diseases of maize (Zea mays L.) have never been considered a major problem in Zimbabwe and maize streak virus (MSV) is the only virus recorded in the published list of maize diseases. It is probable that a number of other viruses exist but have not been identified due to a lack of research in this field. The scant attention to maize viruses is probably attributable to the fact that the maize industry has never considered them economically important. Changing agricultural systems, however, may introduce factors which increase prevalence of diseases and it would be prudent to initiate research on maize virus diseases.

MAIZE PRODUCTION IN ZIMBABWE

Zimbabwe is a landlocked country located approximately between the latitudes 16 S and 23 S. Most of the land lies between 600m (lowveld) and 1500m (highveld) altitude. Mean annual rainfall varies between 400mm (lowveld) and 1000 mm (highveld). Most of the rainfall occurs between November and March and seasonal fluctuations are considerable. Since only 20% of the land area of the highveld receives reliable rainfall, only a small proportion of the country is suited to dryland maize production.

Maize was introduced into the southern half of Africa in the mid-seventeenth century. The crop was accepted rapidly by the native tribes who came to rely on it as a staple diet despite primitive production methods. Today, of the total area planted to maize in Zimbabwe, the greater proportion is farmed by peasants and small-scale growers. The yields are characteristically low when compared to the large scale commercial plantings (Table 1), since the peasant and small-scale sector is fraught with many production problems.

All maize not used by the grower must be marketed through a statutory quasi-governmental body, the Grain Marketing Board. The bulk of maize available for processing, export, and local consumption is produced by the commercial farming sector (Table 2). At present Zimbabwean governmental policy is to increase the productivity in the peasant areas; such a minor "green revolution" would significantly alter the present agricultural structure and new pest/disease complexes may arise. A sophisticated seed maize industry exists and several hybrid cultivars are marketed. "SR 52", a potential high-yield cultivar, and "R201", a shorter season cultivar a lapted for marginal areas, are the most widely used.

The most important diseases are the fungal cob rots caused by Diplodia maydis (Berk.) Sacc. and Fusarium graminearum Schwabe. Other fungal diseases, Helminthosporium turcicum Pass. and Puccinia sorghi Schw., are present but do not present a serious problem

<table>
<thead>
<tr>
<th>Type of production</th>
<th>Area (ha)</th>
<th>Production (t)</th>
<th>Yield (kg/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial/large scale</td>
<td>212,450*</td>
<td>1,008,136</td>
<td>4,726</td>
</tr>
<tr>
<td>Peasant/small scale</td>
<td>738,400</td>
<td>514,800</td>
<td>695</td>
</tr>
</tbody>
</table>

* Mean for production from 1976 to 1980 (Tattersfield, 1982).

<table>
<thead>
<tr>
<th>Intake</th>
<th>Amount (t)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>2,002,000</td>
</tr>
<tr>
<td>Peasant/small scale</td>
<td>362,000</td>
</tr>
<tr>
<td>Commercial/large scale</td>
<td>1,646,000</td>
</tr>
<tr>
<td>Internal consumption</td>
<td>635,000</td>
</tr>
</tbody>
</table>

* Data from Mombeshora, 1982.
due to climatic factors and resistance levels in the major cultivars.

**VIRUS DISEASES**

**Maize streak virus. History and importance.** MSV was first recorded on maize in 1928 (Rothwell, 1979). Thereafter, until 1951, infection was virtually confined to weed grasses. Its reappearance and increase since 1951 can be correlated to the introduction and subsequent expansion of irrigation in Zimbabwe (Rothwell, 1979). Economics dictate that farmers investing in irrigation equipment need to grow two crops per year to justify the capital expenditure. This, in conjunction with a drive for self-sufficiency in wheat (*Triticum aestivum* L.) production, has led to the development of a winter wheat industry. The presence of irrigated cereals during the dry winter months has altered the population dynamics of MSV vectors (*Cicadulina* spp.) and therefore increased the potential for MSV disease in maize.

**Symptoms and etiology.** The symptoms and etiology of MSV have been well documented (Damsteegt, 1981; Gorter 1955; Herd 1956; McClean, 1947; Rothwell, 1979; Van Renburg and Kuhn 1977) and no striking divergence from these recordings has been observed. The effect of MSV varies according to age of the plant when infection first occurs (Rothwell, 1979). In extreme instances, stunting and poor yield or barrenness can result (Gorter, 1955; Rose 1978).

MSV can also affect the winter wheat crop. In this case infection results in stunted plants, leaf markings similar to those on maize, increased tillering, and twisting of the leaf blades. Most infection takes place early in the development of the wheat crop and such plants do not produce any grain (Rothwell, 1979).

Typical streak symptoms also are often seen on grasses surrounding streak-infected maize (Rose, 1978).

Storey (1928) demonstrated that *Cicadulina mbila* (Naude) transmitted MSV. In Zimbabwe, *C. mbila*, *C. parazae* Ghauri, and *C. storeyi* China have been shown to be vectors of the virus (Rose, 1974). *C. similis* China, *C. triangula* Ruppel, and *C. vescula* Kuppel are also known to occur (Rose, 1978). Storey (1928) described in detail the process of transmission.


Rose (1978) found that *Cicadulina* spp. were capable of breeding in most of the cereal crops and annual weed grasses common in cultivated lands, but notably on *Digitaria velutina* Beauv., *E. indica*, *S. homonyma*, and *U. panicoides* and on perennial grasses, especially in irrigated pastures.

The severity of MSV is affected by the preceding rainy season and the proximity of irrigation. Under natural conditions, *Cicadulina* spp. produce two or three generations during the period from November to March. In years when there is little rain between March and June, leafhoppers disperse from the drying grasses, reaching a peak in April/May. These long distance leafhopper flights may coincide with the winter wheat crop. However, there are no accurate records of yield loss and disease incidence and severity on wheat crops. Where there is no winter irrigation, the majority of *Cicadulina* spp. die during the winter months. Irrigation allows breeding to commence earlier and four generations are possible during the summer months. Similarly, late rains or irrigation during the period March to June can allow development of a fifth generation. Hence, there is a good correlation between the total amount of rain in March-June and the size of flights in July-September. This correlation can be used to predict years in which the likelihood of MSV infection is increased (Rose, 1973).

Late (September) *Cicadulina* flights can cause streak in early planted (August) maize. Crops planted once the flight period is over generally escape infection and therefore, under normal conditions the main part of the crop, which is planted in late October or November, is not subjected to the principal leafhopper flight. Standard control recommendations are to clear or spray a 10 m swath around the perimeter of the crop. This prevents the short distance migration of leafhoppers onto the maize (Rose, 1978). When 5% infection is found at the early stage of crop growth or one leafhopper is seen on every three plants, Rose (1978) recommends applying a systemic insecticide such as 0.1% dimethoate and then roguing diseased plants; this treatment must be repeated 3 wk later. When maize is likely to be subjected to a high incidence of streak (for example, green maize from seed planted in August), control is necessary up to 7 wk after emergence. Carbosulfan at the rate of 20g/100m row gives complete control up to 4 wk (Van Rensburg and Walters, 1978), but the levels of disease in commercial plantings seldom justify this expense. Infection after the 7th wk, although causing visual symptoms, results in negligible yield loss (Guthrie, 1978; Van Rensburg, 1981).

MSV appears to be more prevalent in stressed crops. J. F. Douce (personal communication) observed a higher incidence on soil with a low pH.

Because MSV in Zimbabwe has increased in recent years, a program to breed for streak-resistant varieties has been initiated, although only as a low priority. Other factors such as drought resistance and lodging are considered more important. Sources of streak resistance have been introduced from the International Institute of Tropical Agriculture (IITA) in Nigeria and Potchefstroom in South Africa. Initial investigations show that these sources exhibit the same resistance under Zimbabwean conditions. Since Zimbabwe's national maize breeding program is hybrid oriented, resistance will be introduced into existing elite lines by backcrossing and later two or three generations of hybrid progeny. Reciprocal recurrent selection will be employed to improve these sources of resistance.
elite inbred lines. The sources which have been used are IB32, IB32 x Lakey, and J20705T0 (R. C. Olver, personal communication).

Initial observations show that the range of germplasm being used commercially and privately in Zimbabwe is susceptible to streak. On a severity rating from 1-5 as used at Potchefstroom, most of the Zimbabwe commercial hybrids have a mean rating of 4.5 compared with a 1.8 rating of the resistant varieties released in South Africa (M. J. Caulfield, personal communication). There appears, however, to be a strong negative correlation between streak resistance and yield. One of the major problems in screening for streak resistance is that of vector preference for specific cultivars of maize.

UNIDENTIFIED VIRUSLIKE DISEASES
Pellucid spot. Symptoms of the maize disease known as pellucid spot or leopard spot are often observed in Zimbabwe. Sensitivity to the disorder varies with variety. Tanner (1973) has found that application of zinc in the pre-planting fertilizer markedly decreases pellucid spot symptoms.

"White maize". Interest in this problem of white maize was revived last season after total failure of a field and approximately 40% loss of yield in the Chibitrial herd, a variety. He concluded that a virus disease caused the loss of yield in the Chibitrial herd.

VIRUS DISEASE
SUSPECTED OF BEING PRESENT
Maize mottle virus. In a recent visit a representative of IITA in Nigeria thought he recognized maize mottle virus (MMotV) in some of the maize breeding trials on the Harare Research Station. The symptoms pointed out were quite prevalent. Since vectors of MMotV are Cicadulina spp., it would be possible for this virus to occur in Zimbabwe (Storey, 1937).

OTHER VIRUS DISEASES THAT MAY OCCUR
It is possible that a number of other virus diseases may occur on maize in Zimbabwe, although the symptoms have not been observed. They include maize dwarf mosaic virus (MDMV) since the alternate host, Sorghum halepense (L.) Pers. and the vectors [Rhopalosiphum maidis (Fitch), Schizaphis graminum (Rondani), and Myzus persicae (Sulzer)] occur in Zimbabwe. Similarly, the vector of maize mosaic virus (MMV) and maize stripe virus (MStpV), Peregrinus maidis Ashm., also exists although not commonly, as does the alternate host R. cochinchenensis. Aphids that potentially could transmit virus and which occur in Zimbabwe are: Acrithosiphon pismum (Harris), Aphis gossypii Glover, Brevicoryne brassicae (L.), and Rhopalosiphum padi (L.). If viruses such as these (MDMV, MMV, and MStpV) are present in Zimbabwe, their occurrence will probably be very limited because vector populations are low and distribution of the alternate hosts isolated. Unless future conditions favor dramatic increases in numbers of these vectors, it is likely that the incidence of the viruses they transmit will remain relatively low.

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Maize Chlorotic Stunt in Africa:  
A Manifestation of Maize Mottle Virus?

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ABSTRACT


Results of investigations are presented that led to the elucidation of the viral etiology of a severe chlorotic stunt disease of maize (Zea mays) in Nigeria and elsewhere in West Africa. The virus has a spherical particle of approximately 40 nm diam and, like maize streak virus, was persistently transmitted by Cicadulina triangula. In its early stages the disease strongly resembled maize mottle, which was identified and described as a distinct but transient virus disease by Storey in East Africa in 1937. The virus of maize mottle was transmitted by Cicadulina spp. In Nigeria the presumed maize mottle disease is also mostly transient in local and adapted maize. However, it causes chlorotic stunt on most exotic materials and for this reason is of great importance in the breeding program at the International Institute of Tropical Agriculture. Since Storey’s maize mottle virus is most likely involved and the non-transient chlorotic stunt apparently is a result of exotic genotypic susceptibility, the name maize mottle/chlorotic stunt is proposed for this virus disease of West Africa.

During the early stages (1975-76) of the maize streak virus (MSV) resistance breeding program at the International Institute of Tropical Agriculture (IITA) in Ibadan, Nigeria, chlorotic and severely stunted maize (Zea mays L.) plants were often observed in experimental fields (I. W. Buddenhagen, personal communication). This phenomenon appeared to be particularly common among breeding materials that had been artificially infested with MSV-carrying leafhoppers (Cicadulina triangula Storey), both in the field and in screenhouses (Rossel et al., 1980; E. J. Soto and I. W. Buddenhagen, personal communication).

A similar or identical disease was observed in 1979 in Sao Tome, where IITA is involved in a collaborative program on crop improvement and diversification (Rossel, unpublished). Its common occurrence at IITA, particularly among exotic materials and in fields artificially infested with viruliferous leafhoppers, was a strong indication that a disease transmitted by C. triangula was involved. The possibility that the disease was a hitherto unknown manifestation of MSV was not ruled out.

As early as 1937 in East Africa, Storey described a viruslike disease transmitted by Cicadulina spp. which also were vectors of MSV (Storey, 1928; 1932; 1937). He described it as maize mottle virus (MMotV) and gave the following description of the transient symptoms caused on maize. “An affected maize seedling under greenhouse conditions at Amani showed a transitory mottling of the young unfolding leaf. The effect was one of a rather diffuse blotching of normal green tissue upon a pale green background, with the darker areas tending to follow the main veins. Indeed, the symptoms are more suggestive of a nutritional deficiency than of a virus infection, but experimental evidence has certainly eliminated that cause. The pattern of streak is of an entirely different character. The mottling is evident only during the early phase following infection. The young mottled leaves are not as rigid as those of a healthy or streak-diseased plant and may fail to support themselves in the normal, nearly upright position.”

Like Storey’s description of maize mottle, early symptoms of chlorotic stunt observed at IITA strongly resembled the characteristic transient mottle symptoms, including the weak and silky appearance of the young whorl leaves. To no lesser extent, however, the chlorotic stunt syndrome, which followed these initial transient mottle symptoms, resembled severe nutritional deficiency. However, its scattered occurrence in the field and its high incidence among exotic materials strongly indicated that a communicable disease was involved.

C. triangula colonies used in the MSV resistance screening, besides being viruliferous with MSV, proved able to induce the mottle and chlorotic stunt symptoms. Attempts were made to eliminate the suspected disease agent, including traces of MSV, from the colonies to rule out the possibility that a specific feeding effect or a MSV-induced syndrome was involved. However, except for eliminating MSV from the colony, initially all attempts to achieve this goal by initiating disease agent-free colonies from eggs failed (Rossel et al., 1980). The prime objective of further studies was to obtain cultures of C. triangula which were unable to induce the disease symptoms in order to elucidate the transmissible nature of the disease agent and to isolate and/or characterize it.
MATERIALS AND METHODS

Leafhopper rearing. C. triangula subcultures, derived from the mass rearing cultures of IITA and used for MSV resistance screening, were reared on MSV-resistant maize lines in an attempt to eliminate traces of MSV from these cultures.

Sorghum [Sorghum bicolor (L.) Moench] and millet [Pennisetum typhoides (Burm.) Stapf & C. E. Hubb.], two traditional and original graminaceous crop species from the savannah regions of Africa, were tested for susceptibility to MSV and to the agent of maize mottle/chlorotic stunt (MMCSA) and for their ability to sustain C. triangula. It was observed that this leafhopper species could breed both on crop species and that no streak symptoms developed on the plants upon which they were reared. Therefore, subcultures were reared on millet and sorghum. Leafhoppers from these subcultures were subsequently tested for MSV and MMCSA by feeding them on an exotic maize variety (U.V.) used as a susceptible control in the MSV resistance screening. This variety was found to be also highly susceptible to MMCSA.

After establishing that the leafhopper subculture on millet did not induce maize mottle/chlorotic stunt (MMCS) and that MSV had been eliminated, transmission studies were conducted with this subculture. This was done both with MMCSA and MSV in order to establish that the subculture selected on millet was still able to transmit these two pathogens. Also, persistence studies were conducted in order to elucidate the transmission characteristics of MMCSA.

All transmission studies were performed by confining batches of approximately 15 leafhoppers each in gauze-covered plastic vials of 2.5 x 8 cm. These vials had been provided with a narrow side slit through which virus source plant and subsequently test plant leaves were inserted for acquisition and inoculation feedings, respectively.

Leafhopper subcultures were checked for the presence of both MSV and MMCSA by means of introducing batches of susceptible (U.V.) maize plants into the cages and leaving them for 24 hr. They were then removed, sprayed with an insecticide, and monitored for disease.

Purification. The following purification procedure was used. Young, infected leaves of maize, inoculated 3-4 wk earlier using viruliferous leafhoppers, were homogenized in a Waring blender with a 0.1 M phosphate buffer, pH 7.4, containing 0.1% 2-mercaptoethanol (2 ml/g of tissue). Cold chloroform (1 ml/11 g of tissue) was added and the mixture was homogenized for 1 min.

The aqueous phase was separated by centrifugation at 6000 g for 10 min. The pellets were discarded and virus in the supernatant fraction was precipitated by adding 0.2 M NaCl and 10% polyethylene glycol (MW 6000). The virus was further purified by two cycles of differential centrifugation (12,000 g for 10 min and 88,000 g for 3 hr). The pellets from the high speed centrifugation were suspended in 0.01 M phosphate buffer, pH 7.0. After a low speed centrifugation, the virus suspension was layered on a sucrose density gradient (10-40% sucrose in a 0.01 M phosphate buffer, pH 7.0) and centrifuged for 3 hr at 25,000 rpm in a Beckman SW 27 rotor. The gradients were fractionated with an ISCO fractionator, the virus zone recovered, and virus concentrated by centrifugation. These preparations were used for electron microscopy and infectivity tests.

RESULTS

Transmission of maize mottle/chlorotic stunt agent. C. triangula leafhoppers from the MSV-contaminated mass-rearing colonies and subcultured from eggs laid on MSV-resistant maize lines still transmitted traces of MSV. These subcultures also induced typical MMCS symptoms in susceptible maize lines, whereas no such symptoms were observed on the MSV-resistant maize on which these leafhoppers were reared. Subcultures reared on millet, however, proved to be completely free of MSV. In addition, leafhoppers from this culture did not induce MMCS symptoms in susceptible maize lines. Furthermore, it was observed that millet readily sustained this Cicadulina species with populations building up to high densities. A subculture established on sorghum also thrived and tests of this colony for MSV were negative. Thus, although these leafhoppers also proved to be completely free of MSV, they were still able to transmit the agent which induced MMCS symptoms in susceptible maize.

After feeding leafhoppers from the millet-reared colony on MMCSA diseased maize, it could be shown that the Ms...CSA was readily acquired during a 2 hr acquisition feeding period. With these leafhoppers, 100% infection was obtained in groups of plants of susceptible maize lines exposed in succession to viruliferous leafhoppers for 24 hr over a 2 wk period; most leafhoppers were dead at the end of 2 wk (14th group) and the experiment was terminated. In more detailed studies it was found that after a 3 hr virus acquisition, batches of 15 leafhoppers started to transmit the disease...
agent in another 3 hr. Thus, transmission was obtained beginning 6 hr after the start of the virus acquisition period.

Purification of maize mottle/chlorotic stunt agent. Attempts were made to isolate a virus through purification from artificially inoculated maize plants. Fractions collected from the centrifuged sucrose density-gradient columns were examined under the electron microscope after staining with 2% uranyl acetate, pH 4.5. Numerous virus particles were present in a band from the centrifuged gradient column. The particles were spherical, about 40 nm in diam (Fig. 1). The virus fraction was assayed for infectivity by a membrane feeding method using virus-free leafhoppers. The leaffoppers transmitted the virus and the maize seedlings showed typical symptoms of both mottle and chlorotic stunt in all 8 plants that had become infected out of 10 inoculated by this means.

DISCUSSION

*C. triangula*, vector of MSV in Nigeria, was shown also to transmit the agent of maize chlorotic stunt. The agent appeared to be a virus apparently unrelated to MSV. However, chlorotic stunt has practically no characteristics of a virus disease and is best described as due to "poor growth" resulting from a serious nitrogen deficiency. At IITA, the disease was recognized because its occurrence was particularly evident among exotic materials being tested for adaptability to African lowland humid conditions. Most obvious of all, however, was its occurrence among the materials which were being screened for resistance to MSV by means of artificial infestation and inoculation with viruliferous leaffoppers in the field.

Ultimate evidence leading to the conclusion that a distinct, *C. triangula*-vectored agent was involved was obtained after initial difficulties in establishing disease agent-free leaffopper colonies were overcome. At first, MSV could not be eliminated from the leaffopper cultures since IITA's MSV-resistant maize lines, although highly resistant to MSV, still occasionally developed a few chlorotic stripes. From these infected plants new MSV cultures readily became established.

In early stages maize chlorotic stunt strongly resembled symptoms induced by MMotV as described by Storey as early as 1937. There are no further reports of this disease as far as we know. Maize mottle, however, was described as a transient disease. Since maize grown by Nigerian farmers as well as that at IITA showed mostly transient mottle symptoms, the conclusion was reached that MMotV was most likely involved in maize chlorotic stunt. As such, the virus represents a genuine disease agent for exotic, non-adapted, and highly susceptible genotypes.

Indeed, the disease poses a serious problem for the maize breeding program at IITA, which deals with exotic and non-adapted materials. On the other hand, screening for and selection of resistance to this disease is greatly facilitated by the fact that the vector of this disease agent is the same as for MSV, which has similar transmission characteristics. In the maize improvement program these diseases can be handled simultaneously. This explains why IITA's MSV-resistant varieties also proved to be highly tolerant to MMCSA, although there had been no conscious selection for resistance to MMCSA. Considering that a new and potentially serious disease of maize in Africa is involved, it is felt that the name, maize mottle/chlorotic stunt, be adopted to refer to both Storey's transient mottle disease and chlorotic stunt; apparently both syndromes are induced by the same virus. Unless further evidence demonstrates otherwise, the virus particles shown to be associated with this *Cicadulina*-vectored virus are thought to induce both the transient maize mottle as well as the persistent chlorotic stunt symptoms.

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Viruses Affecting Maize in South Africa

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ABSTRACT


Maize streak is the most prevalent virus disease in South Africa. Severe yield losses can be avoided by using resistant maize (Zea mays) cultivars and applying systemic insecticides to the soil. Sugarcane mosaic virus (SCMV) isolates related to maize dwarf mosaic virus strain B occur in maize, sorghum (Sorghum bicolor), and sugarcane (Saccharum officinarum). SCMV isolates are spread by common grain aphids including Diuraphis noxia, a new invader aphid in South Africa infesting mainly wheat (Triticum aestivum). Brome mosaic virus and barley stripe mosaic virus are found occasionally in maize. A seed-transmitted, unidentified icosahedral virus has been found in maize seedlings.

Maize (Zea mays L.) is South Africa's largest agricultural crop. In 1980, a crop of 10,794 metric tons was produced on approximately 4.5 million hectares. Although the total production is a relatively small component of the world production, South Africa is an important maize exporter, ranking as the third largest.

Portuguese traders brought maize to their trading posts on the east coast of southern Africa during the 16th Century where its cultivation soon became more popular among African tribes than the traditional grain sorghum (Sorghum bicolor (L.) Moench). Gradually the crop spread to other tribes farther inland.

The Dutch colonists were also familiar with maize. The first seed reached the Cape in 1658 from New Guinea. Thus, maize has been in cultivation for several hundred years, but only during the 20th Century has production been large-scale.

MAIZE STREAK VIRUS

Maize streak virus (MSV) is the oldest virus disease on record in South Africa. Fuller (1901) wrote the following: "The disorder of the mealie plant, locally described as 'Mealie Blight', 'Mealie Yellows', or 'Striped Leaf Disease', belongs to a group of plant troubles arising from obscure causes, concerning which plant physiologists are but slowly arriving at definite conclusions." His conclusions did not include possible viral causation of the disease. However, his observation that low-lying coastal areas of Natal were better suited than the higher inland tablelands for disease incidence was an inadvertent pointer to the geographic preferences of the leafhopper vector of MSV. Storey (1924, 1925) was the first to show that the leafhopper, Cicadulina mbila (Naude), was the vector of the virus. Subsequent work on virus-vector relationships has been reviewed by Rose (1978). The Cicadulina species occurring in southern Africa are C. mbila, C. storeyi China, C. para-zeae Ghauri, C. niger Ghauri, and an unidentified species. Only the first three transmit MSV (Van Rensburg, 1976).

Today, maize streak is still the most prevalent virus disease occurring in maize in South Africa. However, the potential threat that MSV presented to maize growers has been largely diminished by introducing resistance genes into commercial cultivars. Studies at the Summer Grain Centre, Department of Agriculture, Potchefstroom, showed that some field control was possible with pesticides. For instance, crop losses due to MSV are not correlated with the number of infected plants, but rather with the developmental stage of the plant at the time of infection. Therefore, seedling infection has a severe effect on yield, whereas infection just prior to tassel emergence has no effect. Yield losses can be limited to a minimum if infection is prevented for up to 6 wk after emergence (van Rensburg and Kuhn, 1977). The application of Carbofuran (a systemic insecticide) to the planting furrow at a rate of 0.2 g active ingredient/m was effective in suppressing infection with MSV (Drinkwater et al., 1979).

Many natural grasses serve as alternate hosts for MSV. Wheat (Triticum aestivum L.) is also an excellent host. In some wheat cultivars the virus causes characteristic streaking of leaves accompanied by severe dwarfing, excessive tillering, and yellowing (Gorter, 1947; von Wechmar, 1967). Winter wheat and maize are cultivated successively but on different lands often situated in close proximity to each other. The survival of leafhoppers is thus assured by migration from drying plants to alternate hosts. Leafhoppers can migrate over distances of 100 km or more (van Rensburg, 1976).

SUGARCANE MOSAIC VIRUS

The occurrence of sugarcane mosaic virus (SCMV) is widespread in South Africa. Von Wechmar (1967) and von Wechmar and Hahn (1967) identified and des-
cribed SCMV isolates obtained from sorghum, sugarcane (Saccharum officinarum L.), and maize. Serological comparison of these isolates to an SCMV isolate obtained from Dijkstra and Grancini (1960) showed a close relationship when tested by the tube precipitin test. From 1979 to the present, serological relationship studies of the earlier isolates and various new isolates were continued using the sensitive enzyme-linked immunosorbent assay (ELISA). In one such study it was found that two isolates of SCMV (viz. SCMV-G63 and SCMV-376) were closely related but not identical to a maize isolate (SCMV-Win) (D. S. Erasmus, personal communication). In another study SCMV isolates obtained from the provinces of Natal (SCMV-Bar), Transvaal (SCMV-K), and the Orange Free State (SCMV-Win), all originating from infected maize, were antigenically similar and related to SCMV-isolate 4975 obtained from A. G. Gillaspie, Jr., Beltsville, Maryland, USA. The South African isolates reacted with anti-maize dwarf mosaic virus strain B serum (MDMV-B) but not with anti-MDMV strain A serum; both antisera were obtained from D. T. Gordon, The Ohio State University, Wooster, Ohio. Fig. 1 illustrates these relationships (Chauhan et al., 1982).

Other criteria used for virus identification were length of flexuous particles (750-789 nm ± 10 nm), mechanical and aphid-transmissibility (von Wechmar and Hahn, 1967), and protein molecular weights which fall in the range determined by Gough and Shukla (1981) for Australian isolates. Coat protein molecular weights of the S. African maize isolates ranged from 37.5 to 42 kilodaltons (R. Chauhan, personal communication).

**BROME MOSAIC VIRUS**

The symptomatology of natural SCMV and brome mosaic virus (BMV) infections in young maize are indistinguishable; serology and/or electron microscopy are necessary to differentiate the two causal viruses. BMV can either cause a mild mosaic or a lethal necrosis in maize seedlings (von Wechmar and van Regenmortel, 1966), depending on the concentration of the inoculum and also on the cultivar. Although BMV occurs endemically in natural grasses and in wheat in some maize-producing regions, serious infections have not been observed. Natural dual infection of BMV and SCMV are probably the result of aphid transmission of both viruses. Local SCMV-isolates are readily transmitted by Rhopalosiphum padi (L.) (von Wechmar and Hahn, 1967), R. maidis (Fitch), and the new invader aphid, Diuraphis noxia Mordvilko. The latter aphid is prevalent in wheat fields but will also colonize maize for short periods. BMV is also readily transmitted by D. noxia and other grain aphids (von Wechmar and Rybicki, 1981).

**OTHER VIRUSES**

Barley stripe mosaic virus has been isolated from field-collected maize plants exhibiting abnormal growth and yellowing. The virus was identified serologically and by particle morphology.

An unidentified spherical virus has been isolated from abnormal appearing maize seedlings, with indications that it was seedborne. Young seedlings exhibited distinctive light green, wide, and narrow stripes along the leaf blade. The seedlot originated from a grower who had observed similar abnormalities in his field-grown plants. Serological tests for BMV were negative. The virus was sap-transmissible.

In conclusion, this brief overview does not reflect a complete picture of maize viruses in South Africa but rather a preliminary survey of a subject that is largely untouched.

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Purification and Serology of a South African Isolate of Maize Streak Virus

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ABSTRACT


Isolation of maize streak virus (MSV) from leafhopper-inoculated maize (Zea mays) seedlings is described. The advantages of using sodium acetate rather than phosphate buffer extraction and the effect of calcium accumulation in MSV-infected maize and its subsequent effect on purification and serological tests are discussed. MSV in crude plant sap and in concentrated, purified extracts can be detected by Ouchterlony double-diffusion gel precipitin assay, immune-electron microscopy assay, and sandwich enzyme-linked immunosorbent assay (ELISA). MSV was also detected in dry leaf tissue and in single leafhoppers by ELISA.

Maize streak virus (MSV) is obligately transmitted by the leafhopper Cicadulina mbila Naude (Storey, 1936) and is endemic in all maize (Zea mays L.) producing areas of South Africa. The virus occurs at low concentration in maize plants, especially with increasing plant age. The virus particles are geminate, 20 x 30 nm in size, have a single coat protein with molecular weight (MW) of ca. 2.8 x 10^4 daltons, and contain a single-stranded circular DNA genome with a MW of ca. 7.1 x 10^6 daltons (Bock et al. 1974; Harrison et al., 1977).

Our purification schedule for MSV evolved from the observation that MSV virions are relatively resistant to low pH. Accordingly, a protocol similar to that used for brome mosaic virus (von Wechmar and van Regenmortel, 1968) was adopted.

MATERIALS AND METHODS

Two-week-old maize seedlings (cv. Goudveld) were infected by exposure for several days to viruliferous leafhoppers and then incubated for 14 days in a plant growth room at 24 C. The freshly harvested plants were homogenized with an equal weight/volume (w/v) of 0.1M sodium acetate buffer, pH 4.8 (Miller and Golder, 1950), at room temperature. The pH of the homogenate was immediately adjusted from approximately pH 6.2 back to pH 4.8 with 10% glacial acetic acid. Precipitated host plant components were removed by low speed centrifugation and the virions were pelleted by ultracentrifugation (35,000 rpm, 150 min, Beckman Type 35 rotor). After resuspension in 0.05M phosphate buffer, pH 7.5, virions were further purified by one or more cycles of differential centrifugation.

RESULTS AND DISCUSSION

Electron microscopy of purified virion preparations was only possible using 2% (w/v) aqueous uranyl acetate (UA) at pH 5.0, as phosphotungstate at pH 7.0 or higher resulted in particle disruption. The UA-stained grids clearly revealed the presence of characteristic geminate particles of MSV (Fig. 1).

![Fig. 1. Purified maize streak virus particles negatively stained with uranyl acetate. The size bar represents 100 nm.](image-url)
By immune electron microscopy, clumping of virions in infected sap was detected with antiserum diluted up to 1/2048 (Fig. 2) and positive particle decoration (Milne and Luisoni, 1977) with antiserum diluted up to 1/32. Double-diffusion gel precipitin (Ouchterlony) tests with infected sap were unsuccessful in physiologically buffered saline agar due to heavy precipitation of calcium phosphate. Use of borate/saline, pH 8.0, or sodium-acetate/saline, pH 5.0, buffered gels avoided this problem and enabled antiserum titers of 1/64 to be obtained. The range of optimal proportions where visible MSV-antibody precipitin bands formed was very narrow and could easily be overlooked if insufficient dilution combinations were tested.

A calcium analysis of infected sap by atomic absorption spectroscopy showed that there was an 80% increase in Ca²⁺ in infected sap relative to healthy control sap [M. J. Orren (Dept. Geochemistry, University of Cape Town), personal communication]. This finding prompted the development of an alternative purification procedure, using 5 mM ethylene diamine tetra-acetic acid (EDTA) and 1 mM cysteine in the 0.1M acetate extraction buffer; however, yields were not markedly increased over the simpler procedure described in the Materials and Methods (J. K. Struthers, personal communication).

The presence of elevated Ca²⁺ concentrations in infected plants could explain low yields of MSV obtained by us and other workers when phosphate buffers were used in the initial steps of clarification. Our experience is that the calcium phosphate precipitate formed during homogenization “traps” virions which are then lost in the clarification process (von Wechmar, unpublished).

Preparation of our MSV isolate with sodium acetate buffer and re-acidification of extracts to pH 4.8 resulted in a four-fold increase in yield of isolated virus when compared with the method described by Bock et al. (1974). The method is relatively quick and simple. The advantage of the low pH-clarification procedure is that the majority of host components are speedily denatured.

![Fig. 2. Purified maize streak virus clumped with homologous antibody at a 1/64 dilution and stained with uranyl acetate. The size bar represents 100 nm.](image1)

![Fig. 3. Typical symptoms of maize streak virus on an electro-infected (Pelson and von Wechmar, 1980) maize seedling, cv. Goudveld.](image2)
and are easily removed during low-speed centrifugation. The infectivity of a preparation of MSV made with this method was checked by electro-endosmotic infection (Polson and von Wechmar, 1980). Appearance of typical MSV symptoms was noted in several of the plants tested (Fig. 3).

Double antibody sandwich ELISA (Clark and Adams, 1977) detected MSV in single, field-collected leaves, single leafhoppers, and even in dehydrated plant material.

In light of the current interest in gemini viruses as potential eucaryotic cloning vectors, we hope that this article will be of some help to workers new to this virus.

**LITERATURE CITED**


Maize Mosaic Virus and Other Maize Virus Diseases in the Islands of the Western Indian Ocean

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ABSTRACT


The cultivation of maize (Zea mays) has increased in recent years in the islands of the Western Indian Ocean region, especially in Mauritius where an important program of agricultural diversification is being implemented with the view of achieving self sufficiency in food requirements. The presence of six viruses, namely maize mosaic, maize streak, maize chlorotic stripe, maize dwarf mosaic, and sugarcane mosaic, have been observed. Three of the viruses, maize mosaic, maize stripe, and maize chlorotic stripe, are transmitted by the planthopper, Peregrinus maidis, while maize streak virus (MSV) is vectored by the leafhopper, Cicadulina mbila, and sugarcane mosaic and maize dwarf mosaic viruses are aphid-borne. MSV is considered to be the most important in Mauritius and Rodrigues, while in Reunion maize mosaic virus (MMV) is the most prevalent. MMV has been the most extensively studied virus and three strains, designated MMV-Fine (MMV-F), MMV-Coarse (MMV-C), and MMV-Broken (MMV-B), have been identified, while numerous host-adapted strains of MSV have been sorted out. A maize breeding program has produced hybrids resistant to MMV and MSV in Mauritius, while in Reunion cultivar Revolution or hybrids issuing from it, which are resistant to MSV, are cultivated.

The causal agent of maize stripe has yet to be identified, and the pathogenicity of the 45 nm isometric particles associated with maize chlorotic stripe virus has to be proved. Studies have revealed that the disease referred to as maize line is in fact caused by MMV-B. No maize-infecting mycoplasma or spiroplasma has been identified in the region.

Maize (Zea mays L.) has been grown for more than 250 yr in the islands of Mauritius, Reunion, Rodrigues, and Madagascar, which are situated in the Western Indian Ocean region. However, except in Rodrigues, maize is not a staple of the diet in those islands. In Mauritius and Reunion, sugarcane (Saccharum officinarum L.) has been the backbone of the economy, while in Madagascar the economy is more diversified and in Rodrigues maize is the most widely grown crop.

In order to obviate the dangers associated with monoculture and with a view of achieving self sufficiency in food requirements, an important program of agricultural diversification was launched in Mauritius in the 1960’s. Emphasis is placed on crops that could either be intercropped with sugarcane or grown in fallow lands between two cane rotations. Maize is one of the several crops that are suitable for development in this way. The local maize cultivar, which is resistant or tolerant to the main diseases and pests present, has proved unsuitable for cultivation in sugarcane fields because of its erratic yield, marked tendency to lodge, excessive height, abundant foliage, and long growth cycle (140 days). When maize is intercropped with sugarcane, a significant reduction in sugar yield results.

Foreign dwarf hybrids with suitable agronomic characteristics proved susceptible to endemic diseases and pests. Their cultivation led to epidemics of virus diseases long known in the island and other virus diseases became apparent for the first time. A research program was therefore initiated at the Mauritius Sugar Industry Research Institute to identify and assess the economic importance of maize virus diseases (Anonymous, 1974, 1975, 1976, 1977, 1978, 1979, 1980, 1981; Autrey, 1980; Autrey and Ricaud, 1982; Ricaud and Felix, 1976, 1978a,b). This program was conducted in conjunction with a hybridization scheme aimed at blending the resistance of the local maize cultivar with the desirable agronomic characters of the imported hybrids. Since maize and sugarcane are graminaceous plants with several diseases in common and the vectors
of some of these disease pathogens feed on both crops, it was feared that intercropping could result in the increase of such diseases, especially in sugarcane.

MAIZE VIRUSES IDENTIFIED IN THE WESTERN INDIAN OCEAN REGION

The following viruses of maize have been identified in the four islands: maize streak virus (MSV), maize mosaic virus (MMV), maize stripe virus (MStpV), maize line virus (MLV), maize dwarf mosaic virus (MDMV), maize chlorotic stripe virus (MCSV), and sugarcane mosaic virus (SCMV). The vector of MSV is the leaf-hopper Cicadulina mbila Naude, while MMV, MStpV, the so-called MLV, and MCSV are transmitted by Perigrinus maidis (Ashmead). SCMV and MDMV are aphid-borne.

MAIZE MOSAIC VIRUS

**Distribution.** This disease was first reported in Mauritius (as stripe) by Shepherd (1929) who, like Stahl (1927) and Priode (Briton-Jones, 1933) in the Caribbean Islands, observed three syndromes in the field. Shepherd found plants with three patterns of striping, namely very fine stripes, coarse stripes, and broad chlorotic bands. Ricaud and Felix (1976) confirmed Shepherd's observations. After Kulkarni (1973) showed the existence of two virus diseases (MLV and MStpV) caused by apparently isometric particles, Ricaud and Felix (1976) concluded, on the basis of transmission work and evidence from electron microscopic examinations of the association of rhabdovirus particles with the disease, that the fine striping corresponded to MMV. As a result of transmission studies and positive serological reactions with Kulkarni's antisera, they also showed that the coarse striping and the broad bands were due to MLV and MStpV, respectively.

Autrey (1980), on the basis of various criteria, identified three different strains of MMV which he designated as MMV-Fine (MMV-F), MMV-Coarse (MMV-C), and MMV-Broken (MMV-B), and he produced evidence that what had been called MLV by Kulkarni (1973) was in fact MMV-B. The first two strains corresponded to what had been described as MMV-raya fina and MMV-raya gruesa by Lastra (1977). In Reunion, Etienne and Rat (1972) identified maize stripe on the basis of transmission and symptoms and Guthrie (1977) suggested that the disease could be MLV. The disease described by Etienne and Rat (1972) was that caused by MMV-C. Autrey (1980) has identified the three strains in Reunion. In Madagascar, C.Ricaud (personal communication) observed MMV-F on the east coast and the disease was diagnosed by serological tests (Autrey, 1980). In Rodrigues, despite extensive surveys in 1980 and 1981, Autrey (unpublished) did not observe the presence of MMV.

**Symptoms.** The complete syndromes associated with the strains of MMV have been reported by Autrey (1980). In the field the three distinct striping patterns (Fig. 1) have been observed and described as follows. Symptoms of the first pattern (MMV-F) are fine yellow stripes very close to each other and running all along the leaf lamina on most leaves except the lower ones, where small chlorotic spots can be seen between the stripes. On the upper leaves, the stripes cover the whole surface of the lamina, giving the latter a yellow appearance. On these leaves, the stripes cover the whole surface of the lamina, giving the latter a yellow appearance. Symptoms of the second pattern (MMV-C) are coarse yellow stripes running parallel to the veins and separated by green areas on all the leaves except the two or three lower ones, on which the pattern of striping is identical to that on the lower leaves as described for MMV-F. Along these coarse stripes, brown necrotic localized spots can be observed. Finally, symptoms of the third pattern (MMV-B) are discontinuous yellow

![Fig. 1. Symptoms of maize mosaic virus (MMV) strains in field-collected maize plants. MMV-F (left), MMV-C (middle), and MMV-B (right).](image-url)
stripes of variable length and separated by wide green areas, especially on the uppermost leaves. In plants showing these symptoms, the basal leaves again show the same fine striping as in the first and second patterns described above. This striping evolves into the coarse stripe pattern on the intermediate leaves and, eventually, into the discontinuous pattern.

In the glasshouse when plants are inoculated in the coleoptile stage, the symptoms of the three symptom types appear on the fourth leaf as fine stripes and remain similar until the sixth leaf. For MMV-F, the pattern of fine striping persists throughout the whole life of the plant, giving 22 stripes/cm across the lamina of a fully developed leaf of a mature plant. For MMV-C and MMV-B on the seventh leaf, the main veins show continuous chlorosis, while along the smaller veins in between the chlorosis is discontinuous and appears as yellow spots or streaks. Beginning with the eighth leaf, chlorosis starts to be restricted to the main veins, and on the ninth leaf the main veins are yellow, giving two to four stripes per cm across the lamina. This pattern is retained on all subsequent leaves for MMV-C. For MMV-B from the tenth leaf onwards, the stripes become discontinuous and there appears to be a gradual phasing out of the chlorosis. Eventually only a few short yellow stripes are seen while the rest of the lamina is completely green. When plants are infected late in the cycle, the distinctive striping is visible on the leaves surrounding the ears.

Virus-vector relationships. The planthopper, *P. maidis*, is the only insect known to transmit the three types of MMV. It is often abundant on maize, clustering in the leaf axils and under the leaf sheaths, but it is not an efficient vector, as determined with insects from field and greenhouse populations on both maize and *Sorghum verticilliflorum* (Stud.) Stapf. Nymphs appeared to be more efficient vectors than adults. Starving has no significant effect on transmission of MMV-F and MMV-B. There was an indication, however, that it lowered the efficiency of transmission of glasshouse cultures of MMV-C but not of field-collected cultures (Autrey, 1980). The virus was acquired in less than 15 min but effective acquisition took 24 hr. The latent period in the vector was 9 days for MMV-F and 12 days for MMV-B and MMV-C. The virus could be transmitted in feeding periods of 15 min but transmission was more effective after 24 hr. Studies have revealed that MMV-F and MMV-C were very similar in behavior and differed from MMV-B, which appears to have less affinity with its insect vector (Autrey, 1980).

Virus-host plant relationships. The latent period of the strains in maize hybrid LG 11 varied with ambient conditions and was shorter in warm than cool seasons. Efficiency of transmission increased in the warm season and this was related to the incidence of the disease in the field (Autrey, 1980). The length of the latent period, irrespective of the strain, was shorter in short-cycle genotypes than in cultivars with long growth cycles, as in the local Mauritian one. The latent period also varied with the stage of growth of the plant. In hybrid LG 11, it was in general shortest for MMV-F and longest for MMV-B, irrespective of whether the plant was inoculated in the coleoptile stage or 10-20 days after emergence. In general, the virus-host plant relationships confirm the different behavior between the strains, MMV-F and MMV-C appearing more virulent than MMV-B (Autrey, 1980).

Mechanical, dodder, and seed transmission. Attempts at transmitting the virus mechanically either by rubbing or inoculation with a hypodermic needle by the method of Harpaz (1959) were unsuccessful for the three strains. Two dodder species, *Cuscuta campestris* Yuncker and *C. reflexa* Roxb., failed to establish themselves on maize plants, although the latter species can parasitize a graminaceous plant, *Bambusa multiplex* R. E. Aeusch. Seed transmission was attempted with the three strains by using ears collected in the field from plants infected early, midway and late in the growth cycle. From 12,683 seeds sown, no plant was found with symptoms of the disease (Autrey, 1980), confirming earlier results obtained by C. Ricaud and S. Felix (personal communication) with 1,000 seeds from plants infected with MMV-F.

Host range. MMV-F and what was believed to be MLV were observed in the field in *S. verticilliflorum* and were transmitted from maize to *S. verticilliflorum* and vice versa by Ricaud and Felix (1976). Subsequently, a large number of tropical and temperate cereal species, sugarcane hybrids, and grasses, some of these known to be the hosts of other plant rhabdoviruses, were inoculated with the three strains of MMV (Autrey, 1980). Among the tropical and temperate grasses, only *S. verticilliflorum* and *Rottboellia exaltata* L. became infected and showed symptoms typical of the three strains. The latent periods in *S. verticilliflorum* and *R. exaltata* were, respectively, 13 and 26, 15 and 37, and 15 and 42 days for MMV-F, MMV-C, and MMV-B. Of all the other species inoculated, only spring barley (*Hordeum vulgare* L.) became infected and symptoms were similar to those caused by the strains in maize. In the field the three syndromes were found in *S. verticilliflorum* in Mauritius and in *R. exaltata* in Reunion (Autrey, 1980). MMV was found to have a very limited host range and this was in agreement with the findings in other countries where the virus has been reported (Herold, 1972).

Epidemiology. In Mauritius the three strains of MMV are prevalent in many locations in the east, west, and southwest of the island where there is no break in the crop cycle during the year. In other sectors of the island, the disease is rarely present or is totally absent. The exact incidence was determined in 13 consecutive plantings of the local cultivar in the east of Mauritius, which revealed that MMV-F was far more important than MMV-C and MMV-B and that the disease was more severe in the warm season with a second peak in the cool season (Fig. 2). In imported hybrids, the incidence of MMV either in full stand or intercropped with sugarcane has been found to be low, reaching a maximum of 5.9% in 1976 in hybrid United 530 in the east of Mauritius (Anonymous, 1977; Autrey and Ricaud, 1982).
The population of *P. maidis* was found to be higher in the eastern and northern sectors of the island than elsewhere. The planthopper population was higher in the warm than in the cool season, especially in November when a large number of nymphs of various instars were encountered (Autrey, 1980). It is not believed that *S. verticilliflorum* plays an important role in the carryover and severity of the disease, particularly since transmission from it to maize is inefficient. Continuous cropping is the main factor responsible for the high incidence of the disease in maize.

In Reunion the exact incidence of MMV in the field has not been determined, but it appears that the three strains are more prevalent on local cultivars than in Mauritius (Autrey, *unpublished*).

In the field, both in the local cultivar and in the imported hybrids, Autrey (1980) observed that MSV masked symptoms of initial MMV infection. On very

**TABLE 1.** Effects of the three isolates of maize mosaic virus (MMV) on growth and yield of maize hybrid LG 11 inoculated at the coleoptile stage under glasshouse conditions.

<table>
<thead>
<tr>
<th>Infecting virus strain/statistical parameter</th>
<th>Plant parameters*</th>
<th>Cob parameters*</th>
<th>Dry weight of seeds*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Height (cm)</td>
<td>Stalk diameter (mm)</td>
<td>Leaf area (mm²)</td>
</tr>
<tr>
<td>MMV-Fine</td>
<td>78.3 a</td>
<td>14.0 a</td>
<td>280.0 a</td>
</tr>
<tr>
<td>MMV-Coarse</td>
<td>88.6 a</td>
<td>15.4 ab</td>
<td>418.5 b</td>
</tr>
<tr>
<td>MMV-Broken</td>
<td>101.5 b</td>
<td>16.4 b</td>
<td>451.8 b</td>
</tr>
<tr>
<td>Healthy control</td>
<td>143.6 c</td>
<td>22.8 c</td>
<td>617.1 c</td>
</tr>
<tr>
<td>SE</td>
<td>4.5</td>
<td>0.6</td>
<td>19.5</td>
</tr>
<tr>
<td>CV %</td>
<td>5.3</td>
<td>4.5</td>
<td>5.4</td>
</tr>
</tbody>
</table>

* Data expressed per plant per plot. Mean of three replicates.

* Duncan Multiple Range test. Means followed by the same letter do not differ significantly from each other at *P* = 0.05 level.
TABLE 2. Effects of maize mosaic virus-fine isolate inoculated at two stages in the growth cycle of maize hybrid LG II.

<table>
<thead>
<tr>
<th>Infecting virus/ statistical parameter</th>
<th>Plant parameters*</th>
<th>Cob parameters*</th>
<th>Dry weight of seeds*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Height (cm)</td>
<td>Fresh weight (g)</td>
<td>Dry weight (g)</td>
</tr>
<tr>
<td>MMV-Fine Stage I</td>
<td>97.1 a</td>
<td>307.9 a</td>
<td>165.9 a</td>
</tr>
<tr>
<td>MMV-Fine Stage II</td>
<td>117.8 b</td>
<td>356.0 ab</td>
<td>181.2 a</td>
</tr>
<tr>
<td>Healthy control</td>
<td>133.9 b</td>
<td>453.6 b</td>
<td>250.0 b</td>
</tr>
<tr>
<td>SE</td>
<td>7.3</td>
<td>50.9</td>
<td>11.1</td>
</tr>
<tr>
<td>CV %</td>
<td>7.7</td>
<td>16.8</td>
<td>6.8</td>
</tr>
</tbody>
</table>

* Data expressed per plant per plot. Mean of three replicates.

b Duncan Multiple Range test. Means followed by the same letter do not differ significantly from each other at P = 0.05 level.

TABLE 3. Effects of maize mosaic virus-fine strain on growth and yield of the local maize (Zea mays L.) variety under field conditions.

<table>
<thead>
<tr>
<th>Infecting virus/ statistical parameter</th>
<th>Plant parameters*</th>
<th>Cob parameters*</th>
<th>Dry weight of seeds*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Height (cm)</td>
<td>Stalk diameter (mm)</td>
<td>Fresh weight (g)</td>
</tr>
<tr>
<td>MMV-Fine</td>
<td>148.0 a</td>
<td>21.8 a</td>
<td>364.0 a</td>
</tr>
<tr>
<td>Healthy control</td>
<td>174.8 b</td>
<td>23.8 a</td>
<td>510.0 b</td>
</tr>
<tr>
<td>SE</td>
<td>6.9</td>
<td>1.3</td>
<td>39.9</td>
</tr>
<tr>
<td>CV %</td>
<td>7.1</td>
<td>9.4</td>
<td>14.7</td>
</tr>
</tbody>
</table>

* Data expressed per plant per plot. Mean of three replicates.

b Duncan Multiple Range test. Means followed by the same letter do not differ significantly from each other at P = 0.05 level.

rare occasions in local cultivars only, symptoms of MMV masked those of MSV, in instances where sequential infection occurred in plants with mild symptoms of MSV. Co-infections of MMV-F and MMV-C were also observed in the local variety but not with MMV-B. In glasshouse and field trials with imported hybrids, it was found that MSV protected against the three strains of MMV but not vice versa and that inoculation of MSV to MMV-infected plants proved lethal (Autrey, 1980). Cross-protection tests between the three syndromes of MMV in the glasshouse revealed that MMV-B could protect against the two other strains and MMV-C could do so against MMV-F, whereas the latter could not protect against the two other strains (Autrey, 1980). These results would be expected if it is assumed that MMV-B is the mildest of the three strains. That no additive damaging effect was observed in these tests indicated that the three strains are closely related.

Yield loss assessment. In glasshouse trials the three strains adversely affected growth (Fig. 3) and yield (Table 1) when plants of hybrid LG 11 were inoculated in the coleoptile stage (Autrey, 1980). The effects of MMV-F were more severe, owing probably to the adverse effects of the striping on the photosynthetic area. When the fine strain was inoculated in the same hybrid at two stages in the crop cycle, namely coleoptile and 30 days after emergence, height and fresh weight of plants were affected significantly only when the virus was introduced in the coleoptile stage; fresh weight and dry weight of cobs and dry weight of seeds were affected at both stages of inoculation (Table 2). The results obtained in the glasshouse were confirmed in hybrid LG 11.

For the local cultivar under field conditions it was only possible to assess reliably the effects of MMV-F since the numbers of plants infected with MMV-C and MMV-B were too small in the experimental plots. MMV-F reduced significantly height and fresh weight as well as yield (Table 3). Significant linear relationships between stages of infection and both height of plant and yield were obtained for MMV-F (Fig. 4), and the data showed a tendency for an increasing effect of

Fig. 4. Relationship between growth (left) and yield (right) to the stage of infection in the local maize variety infected with maize mosaic virus-Fine. Data are expressed on a per plant per plot basis.
TABLE 4. Susceptibility of maize hybrids to maize mosaic virus strains fine, coarse, and broken under glasshouse conditions.

<table>
<thead>
<tr>
<th>Hybrids</th>
<th>MMV-Fine (x)</th>
<th>MMV-Coarse (y)</th>
<th>MMV-Broken (z)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Observed</td>
</tr>
<tr>
<td>M 5 x R 14</td>
<td>90.00</td>
<td>45.00</td>
<td>56.79</td>
</tr>
<tr>
<td>M 15 x R 14</td>
<td>90.00</td>
<td>45.00</td>
<td>90.00</td>
</tr>
<tr>
<td>R 18 x DeKalb XL 24</td>
<td>90.00</td>
<td>90.00</td>
<td>63.43</td>
</tr>
<tr>
<td>R 22 x DeKalb XL 24</td>
<td>90.00</td>
<td>90.00</td>
<td>63.43</td>
</tr>
<tr>
<td>R 22 x United 530</td>
<td>56.79</td>
<td>71.57</td>
<td>26.57</td>
</tr>
<tr>
<td>R 22 x United 530</td>
<td>56.79</td>
<td>50.77</td>
<td>26.57</td>
</tr>
<tr>
<td>M 14 x R 14</td>
<td>50.77</td>
<td>50.77</td>
<td>26.57</td>
</tr>
<tr>
<td>Topcross 1</td>
<td>53.21</td>
<td>45.00</td>
<td>0</td>
</tr>
<tr>
<td>Local x R 14</td>
<td>26.57</td>
<td>39.23</td>
<td>0</td>
</tr>
<tr>
<td>Mean</td>
<td>59.08</td>
<td>52.73</td>
<td>33.21</td>
</tr>
</tbody>
</table>

*Expressed as arcsine (sqrt %) infected plants. 10 plants of each hybrid inoculated with each MMV isolate.

Multiple correlation analysis

\[ z = a + bx + cy \]

- \[ a = 6.993 \]
- \[ b = 1.028 \] \[ R = 0.831 \] (R is significant at \( P = 0.01 \) level)
- \[ c = -0.552 \]

with the herbicide Glyphosate; the use of systemic insecticides Carbofuran and Omethoate does not result in reduction in infection levels. Emphasis was placed on research for resistant genotypes. Screening of a large number of hybrids from Europe and Africa, of pure lines from local cultivars in Mauritius and Rodrigues, and of progeny from back crosses of these pure lines as well as crosses between the latter and foreign hybrids was carried out by Autrey (1980). All foreign hybrids (250) proved susceptible to the three strains while pure lines of the local variety and hybrids issuing from them failed to become infected even after a second or third exposure to insects.

A group of 10 selected hybrids used in four tests showed an array of reactions to the three strains. Disease susceptibility was generally highest with MMV-F and least with MMV-B (Table 4). A correlation between reaction in the glasshouse and in the field was obtained for MMV-F as well as for MMV as a whole (Fig. 5), showing the validity of the glasshouse test (Autrey, 1980). In these tests it was possible to obtain a hybrid highly resistant to MMV and four moderately resistant, two slightly susceptible, and three highly susceptible hybrids (Autrey, 1980). The highly resistant hybrid, M 23 x R 14, will be bulked for use on a large scale if it has other desirable agronomic characteristics.

**Purification.** The purification procedure used by Autrey (1980) is detailed in Fig. 6. The virus was purified by homogenizing one part of infected maize leaves in four parts of 0.2M phosphate buffer, pH 9.2, containing 0.05% thioglycollic acid. After straining through cheesecloth and centrifuging at low speed, the supernatant was treated with 0.5% decolorizing charcoal for 30 sec and filtered through a Celite pad (Standard Super Gel). The virus particles were pelleted by centrifugation in a Beckman Type 30 rotor at 60,000 g for 15 min.

The disease in relation to the stages at which infection occurs (Autrey, 1980). It was also found that the effect on yield was more severe than that on height.

**Control.** In Mauritius control of MMV is carried out by successful destruction of the weed *S. verticilliflorum*.
Infected maize leaves
Homogenized with 4 vol. 0.2M Na₂HPO₄ + 0.05% thioglycollic acid and squeezed through cheesecloth

Extract
Centrifugation 1,000 g/1 min

Pellet (discarded)
Supernatant

Added 0.005 g/ml decolorizing charcoal and shaken for 30 sec
Filtration through pad of Celite (standard supercel) in Buchner funnel
Filtrate
Centrifugation 22,000 rpm/15 min (Spinco R 30 rotor)

Pellet
Resuspended in 0.01M phosphate buffer, pH 7.6, for 1 hr at 4°C
Centrifugation 2,000 g/2 min

Pellet (discarded)
Supernatant
Concentrated virus

Applied to calcium phosphate gel column equilibrated with 0.01M phosphate buffer, pH 7.6
Virus recovered in fraction following void volume
Centrifugation 22,000 rpm/15 min (Spinco R 30 rotor)

Pellet
Resuspended in 0.01M phosphate buffer, pH 7.7, layered on 10-40% sucrose gradients (Spinco SW 25.1 rotor)
Centrifugation 24,000 rpm/45 min
Virus zone recovered
Suspended in 0.01M phosphate buffer, pH 7.0
Centrifugation 22,000 rpm/15 min (Spinco R 30 rotor)

Supernatant (discarded)
Pellet
Resuspended in required buffer

Fig. 6. Procedure to purify maize mosaic virus by different centrifugation, column chromatography, and sucrose density gradient centrifugation.
After resuspending in 0.01M phosphate buffer, pH 7.6, the virus was applied to a column of calcium phosphate gel equilibrated with the same buffer. After recovery from the column, the particles were again pelleted and resuspended in 0.01M buffer before being centrifuged on 10-40% sucrose gradient at 90,000 g for 45 min.

Pure virus preparations were obtained by removing the light scattering zones (Fig. 7) and centrifuging at 60,000 g for 15 min. The yield was found to be highest with MMV-F and least with MMV-B, and yield from S. verticilliflorum was higher than from maize. The virus particles from the three strains did not differ in size. They were found to contain RNA, to be sensitive to lipophylic solvents, and to be highly unstable at room temperature. Planthoppers injected with purified preparations transmitted the virus but the insects failed to acquire virus particles from such preparations when fed through a Parafilm membrane. The sedimentation coefficient of MMV-F and MMV-C was found to be 820S and that of MMV-B, was 835S (Autrey, 1980).

Serology. Antisera prepared by injection of purified preparation in rabbits were found to be highly specific and did not react with antigen from healthy plants and those infected with MSV, MDMV, MSStpV (Fig. 8) (Autrey, 1980), and MCSV (Autrey, unpublished). The titers for MMV-F, MMV-C, and MMV-B were 1/32, 1/32, and 1/64, respectively, with crude sap from infected maize plants. The method used is demonstrated for MMV-B antiserum in Fig. 9. The rhabdovirus proved to be poorly immunogenic and a second series of antisera prepared with particles fixed with formaldehyde gave identical results.

The three strains proved serologically identical in a large number of immunodiffusion tests (Fig. 10). Precipitation lines with antigens from maize, S. verticilliflorum, and barley fused completely. The three strains from Reunion (Fig. 11) and the fine strain from Madagascar proved serologically identical to the three strains existing in Mauritius. The MMV antigen in Mauritius reacted positively with an antiserum to MMV-raya tina prepared by Lastra (1977) in Venezuela and tests showed that this antiserum was not as highly specific as the ones prepared in Mauritius (Autrey, 1980).

With the ELISA technique, the virus could be diagnosed in crude sap up to a dilution of 1:20,000 (Autrey, 1980).

**MAIZE STREAK VIRUS**

Distribution. MSV occurs throughout the four islands and in East Africa. It is considered to be the most important virus disease in Mauritius (Ricaud and Felix, 1976) and Rodrigues (Autrey, unpublished; C. Ricaud, personal communication). In Mauritius as with MMV, MSV is most prevalent in the east, west, and southwest, but elsewhere plantations are rarely free from it. In Rodrigues it is more prevalent in the central part of the island where 100% infection is not uncommon, while in the coastal part infection may range from 100% to a negligible level. The disease is quite common in Reunion but exact data on its incidence are lacking.

Symptoms. In plants inoculated in the coleoptile stage, MSV induces white to yellow spots 4-5 days after
Fig. 8. Serological test to demonstrate specificity of the antiserum to three strains of maize mosaic virus (MMV). F, C, B-antigen to MMV-Fine, -Coarse, -Broken, respectively. H = healthy antigen; S = maize streak virus; D = maize dwarf mosaic virus; Ct = control (saline water).

Fig. 9. Determination of titer of antiserum (Bas) to maize mosaic virus (MMV) against its homologous antigen and antigens of MMV-Coarse (C) and MMV-Fine (F).

Fig. 10. Determination of serological relationships between the three strains of maize mosaic virus (MMV) by double diffusion in agar gel. F, C, B-antigen to MMV-Fine, -Coarse, and -Broken, respectively; H = healthy antigen; Ct = control (saline water); Fas, Cas, and Bas = antiserum to MMV-Fine, -Coarse, and -Broken, respectively.

Fig. 11. Serological test with maize mosaic virus (MMV) antigens from Reunion and Mauritius with antiserum to maize mosaic virus-Fine (Fas). Fm, Fr = MMV-Fine from Mauritius and Reunion; Cm, Cr = MMV-Coarse from Mauritius and Reunion; Bm, Br = MMV-Broken from Mauritius and Reunion.
inoculation. The spots elongate and fuse to give 2 mm wide streaks of varying length. The disease does not form stripes like MMV. In highly susceptible varieties the whole leaf lamina may become nearly chlorotic while in resistant ones the number of spots may be greatly reduced to a few on a fully developed leaf (Fig. 12). Infected plants of susceptible cultivars are dwarfed and very often enations are visible on the midrib (Fig. 12). The local Mauritian maize cultivar shows high resistance to the disease and in the field it is common to observe plants which have recovered from infection (Autrey, unpublished; Ricaud and Felix, 1976).

Virus-vector-host plant relationships. The leafhopper, C. mbila, was first discovered in Mauritius in 1972 (J. R. Williams and H. Dove, personal communication). The virus is transmitted very efficiently by the leafhopper, C. mbila, (C. Ricaud and S. Felix, personal communication), but detailed studies on the interrelationships between virus-vector and host plant have not been carried out. Another leafhopper, C. triangula Ruppel, failed to transmit MSV in glasshouse tests (J. R. Williams and H. Dove, personal communication).

Host range. MSV has the widest host range among the viruses of maize reported in the area. The following species have been found with streak symptoms in the field by Ricaud and Felix (1976, 1978a,b): Brachiaria eruciformis (J. E. Smith) Griseb., B. reptans Gard and C. E. Hubb., Cenchrus echinatus L., Coix lachryma-jobi L., Digitaria didactyla Willd., D. horizontalis Willd., D. timorensis (Kunth) Balansa, Panicum maximum Jacq., Paspalum conjugatum Berguis, and Saccharum hybrids. Several host-adapted strains of MSV have been found in these species and only B. eruciformis, B. reptans, C. echinatus, and C. lachryma-jobi play a role in the epidemiology of MSV in maize (Ricaud and Felix, 1976). Isolates from these four graminaceous hosts induced symptoms similar to the maize strain when inoculated in various hosts and MSV can be readily acquired from and transmitted to them (Ricaud and Felix, 1978a).

Epidemiology. Although MSV was first recorded in Mauritius years ago (Shepherd, 1924), its exact incidence in the local variety was apparently never determined. In 1974 100% infection was found in plantings made in the west of Mauritius in the warm season while in the dry cool season infection was less than 5% (Anonymous, 1975; Ricaud and Felix, 1976). In imported hybrids intercropped with sugarcane in the western and eastern sectors, 40-50% infection was found on Anjou 360 and United 530 (Anonymous, 1977; Ricaud and Felix, 1979). In plantings made close to scrub land, high infection levels have been found. In other plantings far from scrub land, infection is limited and does not exceed 2-5% (Autrey, unpublished; Ricaud and Felix, 1976). However, with continuous cropping up to 100% MSV infection was found in a planting of hybrid United 530 made alongside a 6 wk earlier planting of the same hybrid in which infection was low (Autrey and Ricaud, 1982).

The incidence of MSV and MMV was determined in 13 successive monthly plantings of the local cultivar in the east of Mauritius (Autrey, 1980). Infection by MSV was at a peak in March and October plantings at the beginning of the cool-dry and warm-wet seasons, respectively, while for MMV peak infection occurred in the January planting (Fig. 13). The incidence of MMV was higher than MSV, except in March, April, and October. In general, spread of MSV was rapid early in the vegetative cycle, while for MMV maximum spread was in the middle of the vegetative cycle (Autrey, 1980; Autrey and Ricaud, 1982).

The factors affecting disease incidence, spread, and carryover are the alternate hosts, vector populations,
and maize cropping. Because a large number of perennial and annual weeds harbor MSV, epidemics occur frequently in imported hybrids which are highly susceptible. The annual alternate hosts, which grow throughout the year, help to bridge the gap between two successive maize crops. The severity of epidemics of MSV depends also on the proximity to scrub land, since in plantings established in these areas there is extensive and rapid spread of MSV by leafhoppers which have acquired the virus from perennial reservoirs. In this case disease buildup is linear with time. With the same conditions in the local cultivar, disease development shows a marked lag phase due to greater resistance to infection (Autrey and Ricaud, 1982). When planting is made away from hill slopes and scrub land, disease buildup is usually slow but when a second crop follows in the immediate vicinity, disease buildup is exponential and in such conditions 100% infection has been observed (Autrey and Ricaud, 1982).

In Rodrigues the high incidence of MSV in the central part of the island is due to continuous cropping and abundant vector populations which exceed those usually encountered in Mauritius (Autrey, unpublished). In Reunion, cultivation of cultivar Revolution and its progenies has helped to reduce losses due to MSV.

Control. In Mauritius, C. Ricaud and S. Felix (personal communication) screened a large number of foreign hybrids in the glasshouse and in the field for resistance to MSV and they all proved susceptible. These two workers found genes for resistance in pure lines issuing from the local cultivar and in hybrids between these pure lines and imported genotypes. Autrey (1980) found genes for resistance in M $3$ x R 14 hybrids issuing from pure lines of Mauritius and Rodrigues. No correlation was found between resistance to MMV and MSV (Table 5). Hybrid M $25$ x R 14 was highly resistant to MMV but susceptible to MSV.

Among 113 lines and hybrids produced in Mauritius and screened in the glasshouse, the following results (the number of lines and hybrids for each category are in parentheses) were obtained: highly resistant (29),...
resistant (17), moderately resistant (15), slightly susceptible (11), susceptible (21), and highly susceptible (20) (Anonymous, 1981; Autrey, unpublished). In Rodrigues in four trials carried out in 1980, two hybrids issuing from crosses between pure lines of Mauritius and those of Rodrigues proved highly susceptible (Anonymous, 1981; Autrey, unpublished). As mentioned earlier, in Reunion the resistant cultivar Revolution is being used to control MSV carryover.

Purification, serology and histopathology. Despite extensive attempts by C. Ricaud (personal communication) and Autrey (unpublished), it has not been possible to obtain purified preparations of MSV by using the method of Bock et al. (1974). It is believed that the leaf material used is not sufficiently rich in virus particles, despite the severity of symptoms, to allow a reasonable yield to be obtained.

Antigens in crude sap and in partially purified preparations from maize and various hosts reacted positively with an antiserum to MSV supplied by K. R. Bock and the presence of the disease was diagnosed by immunodiffusion tests (Ricaud and Felix, 1976).

In ultra-thin sections of maize leaves, the crystalline nuclear inclusions described by Bock et al. (1974) and made of virus particles have been observed by Autrey (unpublished).

MAIZE STRIPE VIRUS

The condition described as maize stripe by Kulkarni (1973) was probably first observed by Shepherd (1929) in Mauritius. Later, Ricaud and Felix (1976) and Autrey (unpublished) found plants with the syndrome of the disease, i.e., fine striping on lower leaves evolving quickly into broad chlorotic bands and goose-neck bending of the tassel (Fig. 14) in a few plants in the field. The pathogen was transmitted by P. maidis from maize to maize in the glasshouse (Autrey, unpublished; Ricaud and Felix, 1976) and from maize to barley (Autrey, unpublished).

Extensive attempts by C. Ricaud (personal communication) to purify the virus particles, claimed by Kulkarni (1973) to be the causal agent of the disease, have been unsuccessful. The recent report of Gingery et al. (1981) on a new type of virus particle, a filamentous nucleoprotein of 3 nm diam, is the causal agent of the disease explains Ricaud’s failure to isolate isometric particles. Recently, however, Autrey and R. D. Woods (unpublished) found 28 and 40 nm diam particles in young plants showing typical symptoms of MStpV by immune serum electron microscopy (ISEM) with Kulkarni’s MStpV antiserum. Whether these particles are the causal agent of maize stripe is not known at present. The MStpV antiserum is quite unspecific (Autrey, 1980; C. Ricaud and S. Felix, personal communication) and it apparently has antibodies to at least three viruses of maize (R. D. Woods, personal communication).

The disease has no economic importance in Mauritius and it has not been reported in the other islands. It is believed that MStpV is of such rare occurrence that it must have some alternate hosts which allow it to survive in the absence of maize. It is suspected that one of
Hosts could be *Setaria barbata* (Lam.) Kunth, because symptoms are found on this weed in the field (Autrey, unpublished).

**MAIZE LINE VIRUS**

After the description by Kulkarni (1973) of a virus disease inducing coarse distinct striping in maize, Ricaud and Felix (1976), on the basis of symptomatology, transmission studies with *P. maidis*, and positive serological reactions with the MLV antiserum, concluded that MLV existed in Mauritius and induced the coarse striping observed on maize. Attempts at purifying the 28 and 34 nm isometric particles described by Kulkarni were, however, unsuccessful (Autrey, unpublished; C. Ricaud, personal communication). Crude sap of plants infected with MMV-F, MMV-C, and MMV-B were found to give strong positive reactions with Kulkarni's MLV antiserum, while purified preparations did not react (Fig. 15). Attempts to separate MMV and MLV by various methods over 2 yr were unsuccessful (Autrey, unpublished).

K. R. Bock (personal communication) reported the presence of 28 nm diam isometric particles in symptomless plants and his discovery of MMV in Kulkarni's MLV cultures allowed Autrey (1980) to prove that Kulkarni had in fact prepared an antiserum to subviral fragments of MMV. This explained the positive reaction observed with crude sap but not with purified preparations unless the latter are treated with butanol (Fig. 16). Autrey (1580) while working with Kulkarni's so-called MLV cultures in 1977 found that the syndrome corresponded to MMV-B. Consequently, MLV is considered a misnomer and it is proposed that it be referred to as MMV-B in the literature.

**MAIZE CHLOROTIC STRIPE VIRUS**

During a visit to Rodrigues in 1980, Autrey (unpublished) observed a hitherto undescribed striping syndrome consisting of fairly broad yellow bands in the interveinal tissue of the lamina (Fig. 17) of a few plants in two localities on the island. Electron microscopic examinations revealed the presence of isometric particles of 45 nm diam (Autrey and R. D. Woods, unpublished). Later the syndrome was discovered in the south of Mauritius and a pathogen associated with the disease was readily transmitted in the glasshouse by *P. maidis* (Autrey, unpublished). In an experimental plot in the

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![Fig. 15. Immunodiffusion test with Kulkarni's antiserum to maize line virus (Kas) and antigens to maize mosaic virus (MMV). MMV-F = MMV-Fine; MMV-C = MMV-Coarse; MMV-B = MMV-Broken; MSV = maize streak virus; H = healthy sap; C = control (saline water).](image1)

![Fig. 16. Serological test with crude sap, butanol-treated, and untreated purified preparations of maize mosaic virus-Fine (MMV-F) and maize mosaic virus-Broken (MMV-B) against antisera to MMV-F (Fas) and Kulkarni's maize line virus (Kas). B, Bb, and Bp = crude sap, butanol-treated, and untreated purified preparations of MMV-B. F, Fb, and Fp = crude sap, butanol-treated, and untreated purified preparations of MMV-F.](image2)

![Fig. 17. Symptoms of maize chlorotic stripe virus on maize leaf.](image3)
south of Reunion, large numbers of plants (about 70%) were found with the same syndrome in 1981 (Autrey, unpublished). The syndrome is different from MMV, and the disease, called maize chlorotic stripe, is at present under study. It is not believed to be of economic importance in Mauritius and Rodrigues but could cause losses in Reunion. No report has been found in the literature of a similar virus in maize transmitted by P. maidis.

MAIZE DWARF MOSAIC VIRUS

A mechanically transmissible virus disease causing very mild mosaic symptoms in maize and Stenotaphrum dimidiatum (L.) Brongn. (Fig. 18) has been designated as MDMV by Ricaud and Felix (1976). The virus has been transmitted from maize to maize and to S. dimidiatum and vice versa. The symptoms are transient and in the glasshouse are visible at temperatures below 20°C. The disease is rarely seen in the field. Virus particles 750 nm long, typical of the potyvirus group, have been found associated with the disease. The virus was serologically related to SCMV from Madagascar (Ricaud and Felix, 1976). Very often such particles can be seen in the electron microscope in field-collected plants infected with MMV (Autrey, 1980; C. Ricaud, personal communication). The disease is of no economic importance and has not been reported on the other islands.

SUGARCANE MOSAIC VIRUS

Mauritius is one of the three sugarcane growing countries where SCMV has not been reported. The virus is present on sugarcane in Reunion but infection in maize in the field is not common. In Madagascar, Baudin (1968) observed SCMV, which was believed to have been eliminated from the country, in maize and sugarcane. Later, Baudin (1969) reported that three out of 362 seedlings issuing from seeds of maize plants inoculated with SCMV showed symptoms of the disease, a factor which could be important in the epidemiology of the disease. The present status of SCMV in maize in Madagascar is not known to the author.

MYCOPLASMA AND SPIROPLASMA

No mycoplasma or spiroplasma have been reported in the four islands.

CONCLUSIONS

Studies carried out at the Mauritius Sugar Industry Research Institute during the 1970's have led to the identification of five viruses in maize, two of which have shown strain variations. The relative importance of the viruses in Mauritius has been determined and it is evident that MSV economically is the most important pathogen, especially if foreign cultivars are grown. Lately MMV has been found to be more prevalent than was previously believed and the cultivation of maize genotypes resistant to MSV could lead to a buildup of MMV in Mauritius. Such a situation exists in Reunion where it has been observed that MMV was the most important pathogen. The exact situation of the maize viruses in Madagascar at present is not known. It is thought that research should be orientated towards finding hybrids resistant to both MMV and MSV. Further work should determine the etiology of MStpV and the pathogenicity of the 45 nm diam isometric particles associated with MCSV.
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Immunosorbent Electron Microscopy of Maize Viruses from East Africa

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ABSTRACT


Immunosorbent electron microscopy was used routinely to screen samples of virus infected maize (Zea mays) sent from East Africa. The multivalency of two antiserum preparations for Kulkarni's maize stripe and maize line viruses is described. The relationship between two isometric virus particles and certain maize virus diseases is discussed.

The electron microscope has provided plant virologists with one of the most sensitive techniques for the visualization of antigen-antibody reactions of viruses in suspension. Derrick (1973) has described a method for the trapping of virus particles in suspension to electron microscope grids which had previously been coated with a specific plant virus antiserum. We have been using this technique to identify maize viruses from East Africa and to try to throw some light on the maize stripe virus (MStpV) of Kulkarni (1973).

The losses attributed to maize viruses in Kenya have been recorded by Bock (1980). In Botswana, Molefe (personal communication) has reported that up to 50% infection with virus is not unusual, with infected maize (Zea mays L.) plants producing very small or no ears at all.

MATERIALS AND METHODS

Plant material. Samples of infected maize leaves were collected from the field and glasshouse in Botswana and Kenya. Surface moisture was blotted off and they were cut into 5-10 cm lengths, sealed into polyethylene bags, and dispatched by air mail.

Leaf dips. Leaves were ground in distilled water, the extract filtered through muslin, and the rinsed EM grids floated, carbon side down, on 50 μl drops of sap for 18 hr at 4 C. After incubation, the grids were rinsed with 20-50 μl drops of 0.03 M phosphate buffer, stained with 10 drops of 1% phosphotungstic acid, pH 7.0, and viewed in an electron microscope.

Ultrathin sectioning. Material for ultrathin sectioning was cut into pieces approximately 3 x 0.5 mm, fixed for 5 hr in 2.5% buffered glutaraldehyde, rinsed, post-fixed in 1% aqueous osmium tetroxide, dehydrated in a graded acetone series, and embedded in either Araldite CY212 or Spurr's low viscosity resin (Spurr, 1969). Ultrathin sections were cut on a Reichert Ultracut microtome, using a diamond knife, and stained on the grid with uranyl acetate (saturated in 50% ethanol) and lead citrate (Reynolds, 1963). The sections were examined in an electron microscope at 80 kv.

RESULTS AND DISCUSSION

The many symptoms produced by virus infection of plants can often help in identifying the virus, especially where these are studied under controlled conditions. However, in countries where plant viruses have been little studied, symptoms can be a poor guide in attempting to identify a particular virus. Plants may be infected with more than one virus as in the case of maize line virus (MLV) investigated by Kulkarni (1973). He attributed the symptoms to an isometric virus particle, an association which lead to difficulties with the identification of MLV in Mauritius (Bock, 1980). Kulkarni did, however, produce antisera to his MLV and to MStpV; both antisera contained antibodies to at least four distinct viruses due to contamination of his original virus cultures (Jones, unpublished). We have used these antisera routinely to enable us to visualize the four viruses in both field and glasshouse grown maize plants (Table 1).

The choice of antiserum dilution is a matter for individual experiments. Thomas (1980) reported using a dilution of five times more than the maximum react-
Fig. 1. Leaf mesophyll cell of maize from Botswana with maize stripe symptoms. a) Endoplasmic reticulum becomes swollen (E), and may lead to the formation of large granular bodies (viroplasm, V). Aggregates of virus particles (P) are present. B = 0.50 μ. b) Laminar inclusion bodies (L). Bar = 0.30 μ.
ing dilution in microprecipitin or gel-diffusion tests. We have standardized with a dilution of 1/1000 irrespective of the titer of the antiserum. ISEM is quick, reliable, and can be adapted to detect several viruses at the same time (Harville and Derrick, 1978) by incubating individual electron microscope grids with several different antisera. The same effect can be achieved by using an antiserum which contains antibodies to more than one virus, as in the case of Kulkarni's MLV and MStpV antisera.

There are five morphologically different viruses which we can detect in East African maize samples using ISEM (Table 1). The identification of maize streak (MSV), maize dwarf mosaic (MDMV), and maize mosaic (MMV) viruses by ISEM has not presented a problem; however, identification of MStpV-infected material has been a problem. Kulkarni (1973) described the agent of maize stripe disease as an isometric virus particle 35-40 nm in diam and transmitted by the plant-hopper, *Peregrinus maidis* Ashmead. On many occasions we have examined leaf dip material from East Africa having symptoms of maize stripe but could see no particles. Partially purified preparations of MStpV sent from Kenya by E. J. Guthrie would contain a few isometric particles.

In ultrathin sections, mesophyll cells showed aggregations of viroplasm, often with virus particles, crystalline inclusions, and discrete virus particles in the cell cytoplasm (Fig. 1). When we used material with stripe symptoms in ISEM tests with either Kulkarni's MStpV or MLV antiserum, we would find large numbers of 40 nm isometric particles (Fig. 2) of similar appearance to those isolated by Kulkarni (Fig. 3). The preparation contained both empty and full particles. A 28 nm sphere present in very low concentration in some ISEM tests with MStpV-infected material was considered to be possibly maize chlorotic dwarf virus (MCDV) in view of the ultrastructural alterations to the mesophyll cells. This 28 nm sphere was similar to the virus incorrectly identified as the cause of MLV disease by Kulkarni (1973). We obtained antiserum to MCDV from D. T. Gordon which gave negative results in all of our maize ISEM tests.

Gingery et al. (1981) have characterized MStpV as a fine stranded nucleoprotein. Following a visit by O. E. Bradfute to Rothamsted and a meeting with R. E. Gingery in Oxford in August 1981, we agreed to exchange material, as and when it became available, to corroborate the results we had been getting from East African infected maize. In spite of the small number of samples so far examined, our colleagues (O. E. Bradfute, R. E. Gingery, and D. T. Gordon, personal communication) at Wooster have confirmed: a) the presence of MStpV in maize from Botswana plus non-capsid protein crystals associated with MStpV in leaf dips; b) that neither of the isometric particles is related to MCDV; and c) that

### Table 1. Virus particles detected by immunosorbent electron microscopy in East African maize.

<table>
<thead>
<tr>
<th>Particle morphology</th>
<th>Virus name</th>
<th>Antiserum*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geminivirus</td>
<td>maize streak</td>
<td>MSV-3, MStpV, MLV</td>
</tr>
<tr>
<td>Potyvirus</td>
<td>maize dwarf mosaic</td>
<td>MStpV, MLV, SCMV-H</td>
</tr>
<tr>
<td>Rhabdovirus</td>
<td>maize mosaic</td>
<td>MStpV, MLV</td>
</tr>
<tr>
<td>40 nm sphere</td>
<td>'Kulkarni stripe'</td>
<td>MStpV, MLV</td>
</tr>
<tr>
<td>28 nm sphere</td>
<td>'Kulkarni stripe'</td>
<td>MStpV, MLV</td>
</tr>
</tbody>
</table>


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Fig. 2. 40 nm isometric particles from infected maize leaf. Immunosorbent electron microscopy test using maize line virus antiserum of Kulkarni. Bar = 0.15 μ.

Fig. 3. 40 nm isometric virus particles purified by Kulkarni from maize stripe symptommed material. Bar = 0.15 μ. Note similarity to particles in Fig. 2.
neither of the isometric particles is related to MStpV as described by Gingery et al. (1981).

We have still to resolve the roles and significance of the two isometric virus particles in maize from East Africa. Either or both of Kulkarni's viruses can often be detected together with MSV, MDMV, or MStpV in field collected material. Maize mottle virus (MMotV) (Storey, 1937) has transient diffuse mottle symptoms which may be masked by other viruses. Rossell and Thottappilly (1983) have shown maize mottle to be associated with a 40 nm spherical virus particle. However, both of Kulkarni's viruses were transmitted by Peregrinus and not by Cicadulina, the vector of MMotV. The possibility that the 28 nm isometric particles might be a so-called cryptic-virus (Lisa et al., 1981) should not be overlooked.

These two isometric viruses were recently detected in samples sent from Mauritius by J. C. Autrey. He has indicated that our results show the possibility that these viruses may be seedborne. If this indeed is the case, then the exchange of maize seed between African countries should be subject to strict quarantine procedures which ought to include checks with ISEM for these viruses. Our present state of knowledge about these two viruses is incomplete and cooperation among the interested researchers is clearly needed if a more complete understanding is to be achieved.

**LITERATURE CITED**


An Overview of Virus and Viruslike Diseases of Maize in India

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ABSTRACT


As many as 61 diseases of both tropical and temperate nature occur on maize (Zea mays) in India. Ten viruses have been reported to affect maize but only three diseases [maize mosaic, maize stripe (maize mosaic virus 1), and maize vein enation] have been observed under natural conditions. The other seven viruses have been shown to be infective only under experimental conditions. Maize mosaic virus (a strain of sugarcane mosaic virus) was the first virus to be reported affecting maize in India. It is most widespread, having been recorded in the northern plains as well as peninsular India. It is both mechanically and insect transmissible but is not transmitted through seed. Six aphid vectors have been identified. Aspects of etiology, epidemiology, host resistance, and inheritance of resistance have been elucidated. Virus particles are rod-shaped. Susceptibility seems to be inherited monogenically with "incomplete action". Remission of symptoms around flowering time has been observed. Maize mosaic virus 1, a member of the rhabdovirus group, has been reported to cause a stripe disease. The disease is restricted to the state of Maharashtra. Peregrinus maidis has been determined as its vector. Maize vein enaction occurs in the district of Darjeeling in West Bengal. An incidence of up to 15% has been observed. The disease pathogen is transmitted by Cicadulina mbila and is not sap transmissible. Critical study to elucidate its etiology is needed.

Maize (Zea mays L.) ranks fifth after rice (Oryza sativa L.), wheat (Triticum aestivum L.), sorghum [Sorghum bicolor (L.) M. enchen], and Pennisetum americanum (L.) R. Br. with regard to area and production in India. It is grown on 5.9 million hectares from which the annual grain production amounted to 6.4 million tons for the 1980-81 season. Almost 90% of the production is consumed directly as a staple food in the states of Bihar, Gujarat, Madhya Pradesh, Rajasthan, Uttar Pradesh, and hilly tracts in northern and northeastern areas. The remainder is used for industrial purposes and poultry feed. In recent years its use as green fodder for cattle feed has become popular.

Maize is mainly grown as a summer crop. However, in the states of Andhra Pradesh, Bihar, Karnataka, and Tamil Nadu, it is gaining importance as a winter crop. In Bihar, particularly, the farmers' choice is for white-grained cultivars; hybrids Ganga Safed-2 (white flint) and Hi-starch (white dent) are widely cultivated.

The All India Co-ordinated Maize Improvement Project (AICMIP) was initiated in 1957 in collaboration with the Rockefeller Foundation. It was the first multidisciplinary project of the Indian Council of Agricultural Research. Later, this multidisciplinary pattern was introduced in the other coordinated projects. Since 1961, AICMIP has released 15 double and double top cross hybrids and 11 composites.

Sixty-one diseases both of temperate and tropical nature affect maize (Payak and Sharma, 1980; Renfro and Ullstrup, 1976). These are seed rots and seedling blights, stalk rots, foliar diseases, downy mildews, smut, false smut, the common rust, banded leaf and sheath blight, and brown spot. As many as 10 virus diseases have been reported, only three of which (maize mosaic, maize stripe, and vein enation) have been observed to naturally affect India's maize crop (Table 1). The other seven are experimental pathogens of maize (Table 2).

MAIZE MOSAIC

The first record of a virus disease of maize in India was of maize mosaic (Chona and Seth, 1960). Since then, various aspects such as etiology, epidemiology, host resistance, and inheritance of resistance have been elucidated. Maize mosaic occurs in the Union Territory of Delhi, Andhra Pradesh, Himachal Pradesh, Karnataka, Punjab, Rajasthan, and West Bengal where the incidence ranges from 2.2 to 10.6%. Local open pollinated maize cultivars are believed to be more susceptible than the improved ones.

Symptoms begin as chlorotic specks which elongate and coalesce, giving a mottling effect. Affected plants remain pale and stunted. In severe cases ear development may be adversely affected. One noteworthy feature is that symptom remission has been observed as the crop matures and flowers. This suggests some role of flowering hormones in inhibiting virus multiplication.

The causal virus is a strain of sugarcane mosaic virus...
TABLE 1. Viruses which occur on maize in nature in India.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Sap transmissible</th>
<th>Symptoms</th>
<th>Virus morphology</th>
<th>Vector</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize mosaic</td>
<td>+</td>
<td>Chlorotic specks coalescing; later forming chlorotic blotchy areas. Infected plants remain pale and stunted. Ear development poor.</td>
<td>Rod shaped, 335-964 nm (av. 544 nm) in length, 27-35 nm in diam.</td>
<td>Rhopalosiphum maidis, R. rufulabdominalis, Aphis gossypii, Macrosiphum avenae, Schizaphis graminum, Myzus persicae</td>
</tr>
<tr>
<td>Maize stripe (maize mosaic virus 1)</td>
<td>-</td>
<td>Minute chlorotic spots develop on leaves; later they form small chlorotic stripes which become long gradually.</td>
<td>Bacilliform (300 nm) and bullet-shaped (280 nm); width 55 to 58 nm</td>
<td>Peregrinus maidis</td>
</tr>
<tr>
<td>Vein enation</td>
<td>-</td>
<td>Vein swelling and vein enation or galls on leaves; infected plants remain stunted and show leaf chlorosis.</td>
<td>Not determined</td>
<td>Cicadulina mbila</td>
</tr>
</tbody>
</table>

TABLE 2. Viruses which have been experimentally transmitted to maize in India.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Sap transmissible</th>
<th>Vector</th>
<th>Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ragi mosaic</td>
<td>+</td>
<td>Rhopalosiphum maidis, Schizaphis graminum, Schizaphis cyperi, Tetaneura nigriabdominalis, Aphis gossypii, Myzus persicae</td>
<td>Batra et al. (1970), Rao et al. (1965), Subbayya and Ravchoudhuri (1970)</td>
</tr>
<tr>
<td>Sugarcane mosaic</td>
<td>+</td>
<td>Aphid spp.</td>
<td>Seth (1967)</td>
</tr>
<tr>
<td>Bajra mosaic</td>
<td>+</td>
<td>Rhopalosiphum maidis, Myzus persicae</td>
<td>Seth et al. (1972a), Singh (1976)</td>
</tr>
<tr>
<td>Canna mottle</td>
<td>+</td>
<td>M. persicae, R. maidis, A. gossypii, A. fabae</td>
<td>Datta Gupta and Raychoudhuri (1975)</td>
</tr>
<tr>
<td>Chlorosis of sorghum</td>
<td>-</td>
<td>Peregrinus maidis</td>
<td>Capoor et al. (1968)</td>
</tr>
<tr>
<td>Eastern wheat striate</td>
<td>-</td>
<td>Cicadulina mbila</td>
<td>Nagaich and Sinha (1974)</td>
</tr>
<tr>
<td>Bajra (pearl millet), streak virus</td>
<td>-</td>
<td>C. mbila, C. bipunctella -zeae, C. storeyi, C. latens, C. parazae</td>
<td>Seth et al. (1971, 1972b)</td>
</tr>
</tbody>
</table>
(SCMV) and is sap transmissible. The incubation period is 5-22 days and appears to be temperature dependent. It is transmitted by aphid vectors. Six vector species have been identified: *Rhopalosiphum maidis* (Fitch), *Aphis gossypii* Glover, *Macrosiphum avenae* (F.) (Chona and Seth, 1960), *Schizaphis graminum* (Rondani) (Seth and Chona, 1961), *Myzus persicae* (Sulzer) (Bhargava and Shukla, 1966), and *R. rufidominalis* (Sasaki) (Singh, 1977).

The virus has a thermal inactivation point of 50-55 C, a dilution end point of 1:50 to 1:100, and longevity in *vitro* at 28-30 C is 16 hr and at 7 C, 7 days (Chona and Seth, 1960). The virus is inactivated at pH 4.0 and below, and at 4.4 it remains infectious up to 24 hr at 7-10 C. The optimum pH is 5.6-7.2.

The virus was found to be sensitive to many protein precipitants, denaturing agents, and organic solvents. These include commercial nicotine sulphate, copper sulphate, formalin, potassium permanganate, acetone, chloroform, ethyl alcohol, glycerine, and Lysol (Seth and Raychaudhuri, 1967).

The virus has rod-shaped particles of 339-964 nm length (average length = 544 nm) and 27-35 nm width (Paliwal and Raychaudhuri, 1966). The virus differed in particle size from that of SCMV.

Twenty-seven graminaceous hosts belonging to 16 genera including *Zea* were susceptible. Cross protection tests showed that the virus was related to SCMV. It may be noted, however, that sugarcane (*Saccharum officinarum* L.) remains unaffected following inoculation (Seth *et al.*, 1970). Paliwal *et al.* (1968) have differentiated three strains on the basis of host range and immunological relationship. The thermal inactivation points for these strains were 52-60 C, 46-48 C, and 44-46 C, while dilution end points were 1:60-1:70, 1:44-1:48, and 1:30-1:35, respectively. The *in vitro* longevity at 32-36 C for the three strains was 16-20, 6-8, and less than 4 hr, respectively. These three strains offered protection against each other, indicating that they were distinct forms. They differ also in geographical distribution. Strain one is prevalent in the plains of north India, strain two in the Himalayas, and strain three in peninsular India.

Maize mosaic is the most widespread virus disease in India and incidence is high when vector populations peak during February and March in Delhi.

A wide range of maize germplasm, indigenous and exotic, was tested for resistance to this disease. High levels of resistance were observed in single cross *Venz 1 × Venz 400*; hybrids *Ganga 101, Deccan,* and *Ganga 5,* and composites *Kisan* and *Jawahar.* Inbred lines CM (= Coordinated Maize) 104 and CM 103 were almost immune. Resistance of hybrids *Ganga 101* and *Deccan* may be attributed to these two lines which serve as female parents for these two hybrids. Inbred line CM 104, extracted from the Colombian flint variety "Amarillo Theobromina", has been found to be an outstanding source of resistance to many diseases (Payak and Sharma, 1979). Brewbaker (1972) rated it as resistant to Turcicum leaf blight (*Exserohilum turcicum* (Pass.) Leonard and Suggs), the common rust (*Puccinia sorghi* Schw.), and maize mosaic virus (a rhabdovirus) in Hawaii.

Agarwal *et al.* (1969) worked out the resistance inheritance. They made all possible crosses among seven inbred lines (three resistant, one moderately resistant, and three highly susceptible). F1 progenies were advanced to F2 and back crosses with resistant and susceptible parents were also made. These were inoculated with the type strain when seedlings were 7 days old. F1 progenies of *R × S* crosses showed intermediate reaction tending towards susceptibility. Disease development in the F1 generation was somewhat delayed in comparison to susceptible parents, which suggested the occurrence of dominance of susceptibility over resistance. On the basis of data obtained on F1, F2, and back crosses, it was concluded that the susceptibility to this virus is governed by a single gene whose action is incomplete. The presence of minor and modifier genes was also postulated.

The virus probably overwinters in northern India on infected stubble of certain grasses such as *Elesine indica* (L.) Gaertn., *Setaria glauca* (L.) Beauv., and off-season sprouts of sorghum. Aphid vectors colonize these plants and carry the virus to the spring-sown crop. *Brachiaria ramosa* (L.) Stapf, *Dactyloctenium aegyptium* (L.) Beauv., and *Echinochloa colonum* (L.) Link grow during the same season as maize and may serve as collateral hosts.

**MAIZE STRIPE**

This disease was reported from the State of Maharashtra (Chatterjee, 1971; Chatterjee and Nimbalicar, 1977) with incidence from 5 to 15% in different crop seasons. Disease symptoms appear as minute chlorotic spots which gradually elongate and coalesce to form parallel stripes. Leaf tips turn brown, a symptom that spreads along the leaf margins. Occurrence of symptoms on leaf sheaths, ear husks, and stalks has not been observed. For conditions at Pune, the incubation period was 9 days as compared to 4-21 days for the virus reported in the review of Granados (1969).

*Pergrinus maidis* (Ashmead) is the experimental vector of this virus. *M. persicae*, *R. maidis*, and *A. gossypii* did not transmit the virus. No other vector has been found. Efforts to transmit the virus mechanically were unsuccessful.


Thirty-eight cultivars including local open-pollinated varieties, hybrids, and composites were screened under conditions of artificial inoculation. Doubble cross hybrid 7, Giant White (African Tall), experimental hybrid 4050, Jawahar, Sonar, and Vikram were found to possess resistance. Among the inbred lines, least incidence (26.3%) was noticed in CM 103, while maximum
(83.3%) was observed in CM 110, a line extracted from a local open-pollinated variety of Punjab.

Electron microscopy showed that the virus does not infect uniformly all the cells of a leaf. Virions of two shapes, bacilliform (300 nm long) and bullet-shaped (280 nm long), were found. The width of virions was 55 to 88 nm. The former ones were observed free in cytoplasm, the latter were mostly in microcrystals (Varma et al., 1975). In the beginning, the virions formed microcrystals surrounded by a 10-12 nm thick membrane. Orientation of particles in these crystals was always side-to-side and not end-to-end, thus forming monolayered hexagonally packed microcrystals. According to Granados (1969), the particles have a size of 224 × 54 nm.

Microcrystals were detected in mitochondria but not in the nucleus. Each virion had a dense inner core, 10-11 nm in diam, and a middle layer of 10 nm. Between the layers, electron opaque regions 14 nm wide were present. In the middle layer 12 subunits per turn were resolved (Varma et al., 1975). Lastra (1977) showed that the virions possessed single stranded RNA. These observations suggest that the virus belongs to the rhabdovirus group and is considered related to maize mosaic virus 1.

MAIZE VEIN ENATION

This disease is prevalent in the district of Darjeeling and surrounding areas in West Bengal. An incidence of 15% has been observed (Ahlawat and Raychaudhuri, 1976). Affected plants are stunted with chlorosis of leaves. Such plants develop partially sterile tassels. Sometimes tassel emergence is prevented because of curling and twisting of the leaves. Premature death of affected seedlings has also been observed under experimental conditions; white spots on leaf veins were prominent. Swelling and white spindle-shaped galls or enations were observed on veins of the lower surface of leaves.

The virus is transmitted by a cicadellid vector, Cica dulina mbila Naude, and not by mechanical means or by the aphids M. persicae and R. maidis.

Both nymphs and adults of C. mbila were capable of transmitting the virus. Feeding for a minimum period of 15-20 min was necessary for the vector to become viruliferous. Incubation period in the vector was 20 hr. It takes 3-5 days for symptoms to develop. Viruliferous insects usually continued to transmit virus up to the time of their death (Ahlawat and Raychaudhuri, 1976).

The following hosts besides maize were infected: Coix lacryma-jobi L., Eleusine coracana (L.) Gaertn., E. indica, Paspalum sanguinale, rice, Setaria glauca, sorghum, sugarcane, and wheat.

The disease reported under the name of vein enation in India resembles what has been designated and described as maize wallaby ear by Grylls (1975) in Australia.

CONCLUSIONS

None of the three viruses known to occur naturally on maize has attained epidemic proportions in India. Maize mosaic is most widespread in the plains of northern India in the spring (February-March sown) crop. Maize, however, in these areas is cultivated mainly as a summer crop (June-October).

Currently, research on maize viruses is not being carried out at any of the seven maize pathological centers in India. Symptoms suggesting virus etiology are periodically observed on plants in many maize growing areas of the country. For example, during the 1980-81 season, one of the parental lines of hybrids Gauga Safed 2 and Hi-starch, CM 400 (Tenn 20), showed mosaic and mottling symptoms in as many as 40% of the plants. However, since the area is far away from the main centers of virus research, it was difficult to follow up the problem observed in the field. Work is contemplated on a systematic basis in the near future.

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A New Virus Disease of Maize in Thailand

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ABSTRACT


Maize (Zea mays) in Thailand was affected by a new virus disease with symptoms characterized by systemic mosaic and severe stunting. Host range of the virus was confined to the Gramineae. The virus had a thermal inactivation point between 70 and 80°C, a dilution end point between 10^-6 and 10^-8, and was still infectious after 11 days aging in vitro. A purification method yielded 3.5 mg of an icosahedral particle (averaging 27 nm in diam) per 100 g of infected tissue. Purified virus had a 260/280 absorbance ratio of 1.89 and showed serological relationship with antisera to rose mosaic, prunus necrotic ringspot, and maize chlorotic mottle viruses when tested by immunosorbent electron microscopy. Infected maize cells revealed aggregates consisting mainly of crystalline arrangements of virus particles.

Additional key words: Bromovirus, Illarivirus.

Materials and Methods

Virus source and transmission. A virus isolate was maintained on sweet corn. Mechanical transmission was made by rubbing sap prepared by grinding one part of leaf tissue in two parts of 0.01 M phosphate buffer, pH 7.0. The extract was filtered through cheesecloth and rubbed on carborundum-dusted leaves of test plants in a host range study.

Purification. A minor modification of the method of Niblett and Paulsen (1975) for the purification of pani­cum mosaic virus was used. Frozen tissue of systemically infected sweet corn was homogenized in 0.1 M phosphate buffer, pH 7.0, containing 0.01 M sodium sulfite and 0.01 M EDTA to give a 1:3 (w/v) dilution. The extract was squeezed through cheesecloth and clarified with an equal volume of chloroform:butanol mix­ture (1:1, v/v). The emulsion was kept at 4°C for 30 min, then centrifuged 10 min at 10,000 g. The aqueous phase was filtered through glass wool and centrifuged 2 hr at 65,000 g at 4°C. The pellets were suspended in 0.01 M phosphate buffer, pH 7.0, overnight at 4°C, after which two additional cycles of differential centrifugation were performed. The suspension was subjected to the 10-40% (w/v) sucrose density columns, and centrifuged at 25,000 rpm for 2.5 hr. The virus was collected and centrifuged 2 hr at 25,000 rpm. Purified virus was re­suspended in 0.01 M phosphate buffer, pH 7.0, and stored at 4°C.

Electron microscopy. Negatively stained prepara­tions of virus-infected sweet corn tissue were used for studying particle morphology. Fresh cut tissue was placed in contact with a drop of 2% uranyl acetate for 2-3 sec on formvar coated grids. For electron microscopy of tissue, small portions of systemically infected leaves of sweet corn were fixed for 2 hr in 2.5% glutaraldehyde in 0.05 M phosphate buffer, pH 7.0. Tissues were washed and postfixed in a 1.0% osmium tetroxide (w/v) for 3 hr, dehydrated through graded acetone, and embedded in Spurr’s medium (Spurr, 1969). Ultrathin sections were cut with a dia­mond knife on an LKB-ultramicrotome and stained with 2.0% aqueous uranyl acetate for 15-20 min and 0.02% lead citrate (w/v) for 2 min. All observations were made with a JEOL-100S electron microscope.

Immunosorbent electron microscopy (ISEM) was performed as described by Derrick (1973). All antisera tested were obtained from the Virus Research Labora­tory at Braunschweig, Federal Republic of Germany.
Figs. 1-5. 1) Symptoms of virus infected leaves of maize, *Zea mays*, showing leaf malformation and systemic mosaic. 2) Chlorotic streaks on infected sugarcane (*Saccharum officinarum*) leaf. 3) Electron micrograph of purified virus preparation stained with 2% uranyl acetate. 4) Virus-infected leaf cells of maize showing virus aggregates. 5) Portion of virus aggregate in virus-infected leaf cell of maize showing crystalline arrangement of virus particles.
RESULTS

Host range and symptomatology. The host range of the virus was mostly confined to the Gramineae (Table 1). Three maize cultivars including the most recommended Suwan I were susceptible to the virus. Symptoms on infected maize seedlings consisted of systemic mosaic, severe stunting, and chlorotic streak of the lower leaves (Fig. 1). Infected sugarcane exhibited large chlorotic streaks (Fig. 2).

Properties of the virus. Extracts prepared from infected sweet corn remained infectious after 11 days aging at 24-26°C. The virus tolerated heating at 70°C but not at 80°C for 10 min. Sap diluted in 0.05 M phosphate buffer, pH 7.0, was infectious at 10^{-4} but not at 10^{-8} dilution.

Purification. Purified virus was infectious and formed a single light scattering band in the sucrose density gradient column. The virus preparation had a 260/280 absorbance ratio of 1.89. The yield of purified virus was 3.5 mg/100 g tissue based on ultraviolet absorption and an extinction coefficient of E = 5.

Electron microscopy and immunosorbent electron microscopy (ISEM). Negatively stained preparations of purified virus revealed icosahedral particles averaging approximately 8 nm in diameter, of virus particles were observed in preparations stained with uranyl acetate but not with phosphotungstic acid.

Numerous particles were observed when antisera to prunus necrotic ringspot virus (PNRSV) and rose mosaic virus (RMV) were used in ISEM, indicating close serological relationships. Few particles were observed when antiserum to maize chlorotic mottle virus (MCMV) was used. Antiserum to tobacco necrosis virus, cocksfoot mild mosaic virus, sowbane mosaic virus, strawberry latent ringspot virus, and radish mosaic virus gave negative results, suggesting no serological relationship.

Virus aggregates were observed in virus-infected leaf cells of maize (Fig. 4). Aggregates were composed mainly of the crystalline arrangement of virus particles (Fig. 5). No other inclusion bodies were detected within infected cells or healthy controls.

DISCUSSION

General characteristics of the virus described in this paper resemble those of brome mosaic virus (BMV) (Bancroft, 1970), a member of the bromovirus group (Harrison et al., 1971), yet a serological relationship between BMV and this virus was not demonstrated in this study. MCMV, although serologically related to this virus, differed in host range (Uyemoto and Clafflin, 1981). Whether this virus and/or MCMV belong to the bromovirus group is subject to further study. The serological relationship of this virus to PNRSV and RMV in the illarvirus group invites further investigation.

<table>
<thead>
<tr>
<th>Family, genus, and species</th>
<th>Symptom*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amaranthaceae</td>
<td></td>
</tr>
<tr>
<td><em>Gomphrena globosa</em> L.</td>
<td>NL, SN</td>
</tr>
<tr>
<td>Chenopodiaceae</td>
<td></td>
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<tr>
<td><em>Chenopodium amaranticolor</em></td>
<td></td>
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<tr>
<td>Coste &amp; Reyn.</td>
<td>NL</td>
</tr>
<tr>
<td>C. <em>murale</em> L.</td>
<td>NL, SN</td>
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<tr>
<td>C. <em>quinoa</em> Wild.</td>
<td>CLR</td>
</tr>
<tr>
<td>Cucurbitaceae</td>
<td></td>
</tr>
<tr>
<td><em>Cucumis sativus</em> L.</td>
<td>N</td>
</tr>
<tr>
<td>Gramineae</td>
<td></td>
</tr>
<tr>
<td><em>Avena sativa</em> L.</td>
<td>SM</td>
</tr>
<tr>
<td>Hordeum vulgare L.</td>
<td>SM</td>
</tr>
<tr>
<td>Saccharum officinarum L.</td>
<td>SM</td>
</tr>
<tr>
<td><em>Sorghum bicolor</em> (L.) Moench (Cultivar 'Rio')</td>
<td>SM, SN</td>
</tr>
<tr>
<td>(Cultivar 'KU 257')</td>
<td>SM</td>
</tr>
<tr>
<td>S. <em>sudanense</em> (Piper) Stapf</td>
<td>SM</td>
</tr>
<tr>
<td>Triticum aestivum L.</td>
<td>SM</td>
</tr>
<tr>
<td>Zea mays L.</td>
<td></td>
</tr>
<tr>
<td>(Flint corn, cultivar ‘Suwan I’)</td>
<td>SM</td>
</tr>
<tr>
<td>(Glutinous corn)</td>
<td>SM</td>
</tr>
<tr>
<td>(Sweet corn, cultivar ‘Supersweet DMR’)</td>
<td>SM</td>
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<tr>
<td>Solanaceae</td>
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<tr>
<td><em>Nicotiana glutinosa</em> L.</td>
<td>N</td>
</tr>
<tr>
<td>N. <em>tabacum</em> L.</td>
<td>N</td>
</tr>
</tbody>
</table>

* Abbreviations: CLR = chlorotic local ring; N = noninfected; NL = necrotic local lesion; SM = systemic mosaic; SN = systemic necrosis.

The disease has a limited distribution in Thailand which may be due to the lack of an efficient vector. Whether the virus is transmitted by chrysomelid beetles has yet to be demonstrated.

LITERATURE CITED


Identification, Transmission, Host Range, and Epidemiology of Maize Dwarf Mosaic Virus in Northwestern China


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ABSTRACT


A sap and aphid transmissible virus isolated from field-infected corn (Zea mays), sorghum (Sorghum bicolor), and sugarcane (Saccharum officinarum) from northwestern China was identified as maize dwarf mosaic virus strain B (MDMV-B). The virus was transmitted by Schizaphis graminum, Rhopalosiphum maidis, R. padi, and Myzus persicae. Both S. graminum and R. padi acquired MDMV-B during probes as short as 0.5 and 1.0 min, respectively. S. graminum inoculated MDMV-B in 10 min; longer inoculation feeding times did not increase the transmission rate. Flexuous rod particles of MDMV-B were 749.6 nm long. The thermal inactivation point of the virus was 55-60°C; the dilution-end-point, 10^-5 to 10^-4; and the longevity in vitro, 1-2 days at 18-20°C and >6 days at 0-4°C. A low percentage (0.08%) of seed transmission was found in corn line Tien-yu 1. A total of 1292 Sorghum halepense seedlings were tested by sap and aphid inoculations of MDMV-B from different sources, including four from sugarcane, with negative results for infection. Of the 55 species and three cultivars of gramineous plants tested, 15 species and three cultivars were susceptible to MDMV-B. Virus isolated from three sugarcane lines (Zhen-tan 75-72, Nei-jian 907, and Ou-tan 73-39) produced characteristic systemic-mosaic symptoms on Sorghum sudanense cv. 1148 and S. bicolor cv. Broom, whereas the virus isolates from nine corn lines, one sorghum line, and two cereal lines produced only local and necrotic streaks. Therefore, these two Sorghum spp. may be useful hosts for identifying and separating the closely related virus strains.

Maize dwarf mosaic was first reported by Williams and Alexander (1965). Subsequently this disease was found in 37 states of the USA (Gorón et al., 1981). Maize dwarf mosaic is now considered to be the most important virus disease of corn (Zea mays L.) and sorghum [Sorghum bicolor (L.) Moench] in the world. Following the reports of Williams and Alexander (1965), maize dwarf mosaic was found in damaging proportions in many areas of China including Shaanxi, Gansu, Henan, Hebei, Shandong, Shansi, and Liaoning Provinces and Nei Monggol Autonomous Regions. Crop losses due to this disease have been estimated at 20-80% depending upon susceptibility of the genotype, time of infection, and other factors such as efficiency and quantity of the vector, availability of alternate hosts, and meteorological factors.

Because of the relatively high incidence of maize dwarf mosaic in the last 5 yr in northwestern China, we initiated a study of vector specificity, transmission characteristics by aphid vectors, properties-in-sap, host range, seed transmission rate, and epidemiology of maize dwarf mosaic virus (MDMV).
Five corn seedlings were planted in 10-cm diam clay pots containing standard potting soil mixture. Seedlings at the one-leaf stage were used throughout transmission tests. Test insects were starved for ca. 2 hr prior to the acquisition feeding tests. For testing acquisition efficiency of MDMV by the aphid, the feedings of test insects were timed and observed under a dissecting microscope. At the end of each acquisition feeding period (AFP), three insects were transferred to each test plant by means of a camel-hair brush. During the inoculation-access period (IAP), the test plants were caged with a glass lantern jar covered with a fine-mesh screen on the top. At the end of the 2-3 day IAP, test plants were fumigated and kept in an insect-free screenhouse.

For preparation of partially purified virus, infected tissue from the source plants was ground and mixed 1:1 with 0.01M phosphate buffer, pH 7.2. The crude-sap extract was filtered through two layers of cheesecloth. The filtrate was clarified with an equal volume of chloroform and precipitated by addition of 6% polyethylene glycol (PEG 6000), followed by a low speed centrifugation (3000 rpm) for 15 min. The sedimented material was suspended in the same buffer and given another low speed centrifugation (7000 rpm) for 15 min. To test the infectivity of the purified virus, the first leaf of seedlings at the two-leaf stage was dusted with 600-mesh carborundum and then rubbed with the partially purified virus preparation.

For properties-in-sap studies of the virus, the inoculum was prepared by triturating symptomatic young leaves with a mortar and pestle, diluting extracts 1:1 with 0.01M phosphate buffer, and filtering the extract through two layers of cheesecloth, followed by a low speed centrifugation (3000 rpm) for 15 min. For in vitro inactivation tests, the inoculum was held at 0-4 and 18-20 C and inoculated to the seedlings at 24 hr intervals. The dilution end point was determined by diluting the inoculum with distilled water. Heat inactivation of the virus was determined after exposing the inoculum to 45, 50, 55, and 60 C for 10 min.

RESULTS

Symptomatology. Symptoms on the inoculated corn plants first showed as a mosaic or chlorotic pattern of light and dark green areas at the leaf base. As the symptoms developed, the chlorotic areas often merged into continuous streaks along the veins to form an "A" shape or one or two chlorotic bands. In some instances a mottling or mosaic pattern developed on the entire leaf. Severe stunting was often observed in plants infected at the two- or four-leaf stage. In late infections, chlorotic symptoms only appeared on the upper leaves of mature plants and reddish streaks sometimes developed on mature leaves.

Symptoms on infected sorghum often first appeared as a mosaic or chlorotic streaks; later reddish or purple streaks developed on the leaves. In some cases, the reddish streaks became necrotic, followed by death of whorl leaves.

Aphid transmission. Six species of apterous aphids were tested for their ability to transmit MDMV from infected corn and sugarcane to healthy corn seedlings. *S. graminum* was the most efficient vector, followed by *R. maidis*, *R. padi*, and *M. persicae* in transmission of MDMV from corn to corn. However, the virus isolated from sugarcane was transmitted with different efficiencies. *M. avenae* and *A. dirhodum* did not transmit MDMV (Table 1). No transmission occurred with control aphids not fed on virus-source plants. Experiments with single aphids of *S. graminum* and *R. padi* showed an acquisition threshold of 0.5 and 1 min, respectively. The rate of MDMV transmission by *S. graminum* did not increase with an increase in probing duration from 1 to 5 min (Table 2).

<table>
<thead>
<tr>
<th>Aphid species</th>
<th>Infected maize</th>
<th>Infected sugarcane</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acyrthosiphon dirhodum</em></td>
<td>0/73 (0.0)</td>
<td>0/150 (0.0)</td>
</tr>
<tr>
<td><em>M. avenae</em></td>
<td>0/72 (0.0)</td>
<td>0/160 (0.0)</td>
</tr>
<tr>
<td><em>M. persicae</em></td>
<td>7/25 (28.0)</td>
<td></td>
</tr>
<tr>
<td><em>R. padi</em></td>
<td>27/82 (32.9)</td>
<td>6/137 (4.4)</td>
</tr>
<tr>
<td><em>R. maidis</em></td>
<td>49/85* (57.6)</td>
<td>19/19 (100.0)</td>
</tr>
<tr>
<td><em>S. graminum</em></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Numerator = no. of infected plants; denominator = no. of plants exposed to aphids post-acquisition feed on source plant. Each test plant was exposed to three aphids. Each trial consisted of at least three tests. *Zea mays* L. cv. Wei-er 156 was used as the test plant.
An inoculation threshold of 10 min was recorded for *S. graminum*; longer inoculation times (4-5 hr) resulted in decreased transmission rates (Table 3).

Properties-in-sap. The properties-in-sap of MDMV were as follows: thermal inactivation — infection after heating 10 min at 55 C but not after heating 10 min at 60 C; longevity *in vitro* — infection after 1 day but none after 2 days at 18-20 C and more than 6 days at 0-4 C; dilution end point — infection at 10-3 but none at 10-4 (Table 4).

Electron microscopy. In the electron microscope the virus particles appeared as flexuous rods with a 13-15 nm diam, but had a length that varied over a wide range (231.7-1115 nm), with 25 of 88 particles falling close to the average length of 749.6 nm.

Seed transmission. A total of 22,925 seedlings from five maize lines harvested from MDMV-infected plants

### TABLE 2. Transmission of maize dwarf mosaic virus strain B by *Schizaphis graminum* and *Rhopalosiphum padi* after various inoculation feeding periods.

<table>
<thead>
<tr>
<th>Test aphid</th>
<th>Transmission after specified inoculation feeding periods (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test</td>
</tr>
<tr>
<td><em>S. graminum</em></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td><em>R. padi</em></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2</td>
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</tbody>
</table>

* Numerator = no. of infected plants; denominator = no. of plants exposed to aphids post acquisition feed on source plant. Each test plant was exposed to three aphids. *Zea mays* L. cv. Wei-er 156 was used as the test plant.

### TABLE 3. Transmission of maize dwarf mosaic virus strain B by *Schizaphis graminum* and *Rhopalosiphum padi* after various inoculation feeding periods.

<table>
<thead>
<tr>
<th>Test aphid</th>
<th>Transmission (no. and %) after specified inoculation feeding periods (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
</tr>
<tr>
<td><em>S. graminum</em></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>3</td>
</tr>
<tr>
<td><em>R. padi</em></td>
<td>1</td>
</tr>
</tbody>
</table>

* Numerator = no. of infections; denominator = no. of trials. Each test plant was exposed to three aphids. *Zea mays* L. cv. Wei-er 156 was used as the test plant.

### TABLE 4. Properties-in-sap of maize dwarf mosaic virus strain B.

<table>
<thead>
<tr>
<th>Replicate</th>
<th>Thermal inactivation point (C)</th>
<th>Longevity <em>in vitro</em> (day) at</th>
<th>Dilution end point</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>45</td>
<td>50</td>
<td>55</td>
</tr>
<tr>
<td>1</td>
<td>69</td>
<td>44</td>
<td>19</td>
</tr>
<tr>
<td>2</td>
<td>75</td>
<td>55</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>65</td>
<td>45</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>35</td>
<td>30</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>38</td>
<td>0</td>
<td>81</td>
</tr>
<tr>
<td>6</td>
<td>19</td>
<td>0</td>
<td>69</td>
</tr>
<tr>
<td>7</td>
<td>15</td>
<td>5</td>
<td>70</td>
</tr>
<tr>
<td>Summary</td>
<td>55</td>
<td>60</td>
<td>1</td>
</tr>
</tbody>
</table>

* Numbers represent percent transmission by mechanical transmissions, with at least 15-20 test plants for each percent. *Zea mays* L. cv. Wei-er 156 was used as the test plant.
were tested for seed transmission. Seed transmission was detected only in the line Tien-yu 1. The rate of transmission varied from 0.0 to 0.27% in nine trials, with an average rate of 0.08% (Table 5). Virus was recovered from Tien-yu 1 maize infected through the seed by inoculation of maize line Wei-er 156. Typical symptoms subsequently developed on this assay plant. In a field study, corn lines Tien-dan 1 and Jin-bei 7 had seed transmission rates of 2.6 and 0.2%, respectively.

**Johsongrass insusceptibility.** A total of 1292 S. halepense seedlings were tested by sap and aphid inoculations with MDMV isolates from 11 maize lines, two sorghum lines, one cereal line, and four sugarcane lines. None of the tested plants developed maize dwarf mosaic symptoms after 6 wk (Table 6).

**Differential corn and sorghum responses.** During the course of this study, it was noted that MDMV isolated from infected corn was transmitted with higher efficiency by S. graminum and R. padi than the isolate from sugarcane (Saccharum officinarum L.), suggesting the existence of virus strains. In an experiment using nine hosts to test for differential reactions to inoculation with MDMV, symptom responses and rates of infection of six hosts (Z. mays cvs. Tien-yu 1 and Men 14; S. bicolor cvs. Bei-ping, WY786-E-1-2, Ni-oua, and Hsiung-yu 191) to the virus isolates from different sources were about the same (Table 7). However, two marked differences were noted on S. suda-

**TABLE 5.** Seed transmission of maize dwarf mosaic virus strain B in maize.

<table>
<thead>
<tr>
<th>Maize line</th>
<th>Replicate</th>
<th>No. seedlings in test</th>
<th>Seedlings showing symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tien-yu</td>
<td>1</td>
<td>605</td>
<td>1 (0.17)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>747</td>
<td>2 (0.27)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>572</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1060</td>
<td>1 (0.09)</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1228</td>
<td>1 (0.08)</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>2190</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>1880</td>
<td>1 (0.05)</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>3400</td>
<td>3 (0.09)</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>4407</td>
<td>5 (0.11)</td>
</tr>
<tr>
<td>Wei-er 156</td>
<td>1</td>
<td>148</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>450</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>483</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>744</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>557</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>375</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Wei-men single cross</td>
<td>1</td>
<td>599</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Ju-suan-1</td>
<td>1</td>
<td>516</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Tsang-dan-7</td>
<td>1</td>
<td>2964</td>
<td>0 (0.0)</td>
</tr>
</tbody>
</table>

*Efficiency and quantity of aphid vectors.* Five aphid species were commonly found to infest corn and sorghum. S. graminum, R. maidis, and R. padi were the predominant species. Although *A. dirhodum* and *M. avenae* were relatively abundant, they were not vectors of MDMV. Early in the season from May to June, *S. graminum* played a major role in the spread of MDMV due to low temperature and low humidity which resulted in extensive buildup of populations of this aphid. On the contrary, *R. padi* and *R. maidis* preferred...
TABLE 6. Inoculation of *Sorghum halepense* with maize dwarf mosaic virus from different sources.

<table>
<thead>
<tr>
<th>Virus source</th>
<th>Sap inoculation</th>
<th>Aphid inoculation*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of trials</td>
<td>SG</td>
</tr>
<tr>
<td>Maize line</td>
<td>No. of test plants</td>
<td>No. of trials</td>
</tr>
<tr>
<td>Huan-chao 4</td>
<td>1</td>
<td>22</td>
</tr>
<tr>
<td>Tung-da-li</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Liao-lung 8624</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>Lu 28</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Tien-yu 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AN₁₁₁₁H₁</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>RB₁₄H₁</td>
<td>1</td>
<td>22</td>
</tr>
<tr>
<td>Chun 714</td>
<td>1</td>
<td>14</td>
</tr>
<tr>
<td>H/Da-Chiu 36</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>Tien-dan 1</td>
<td>2</td>
<td>26</td>
</tr>
<tr>
<td>Mixture</td>
<td>11</td>
<td>342</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>470</td>
</tr>
<tr>
<td>Sorghum line</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hsiun-yu 253</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Bei-ping</td>
<td>2</td>
<td>19</td>
</tr>
<tr>
<td>Total</td>
<td>2</td>
<td>19</td>
</tr>
<tr>
<td>Setaria italica</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Sugarcane line</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zhen-tan 75-27</td>
<td>3</td>
<td>38</td>
</tr>
<tr>
<td>Nei-ji-an 907</td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>Ou-tan 73-39</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>Mixture</td>
<td>3</td>
<td>102</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>171</td>
</tr>
</tbody>
</table>

* SG = *Schizaphis graminum*; RP = *Rhopalosiphum padi*; RM = *Rhopalosiphum maidis*
high temperature and low humidity and they served as important vectors from July to August.

**Meteorological factors.** It was found that meteorological conditions not only determined the time of peak vector populations but also affected the number of population peaks. The most important factor was temperature. Early warm weather (monthly average 16°C) in the spring resulted in an early population build-up of *S. graminum* which in addition was responsible for a high incidence of barley yellow dwarf on wheat (*Triticum aestivum* L.) (Zhang et al., 1983). These early season large populations were followed by low aphid populations on corn and sorghum in the fall. When warm weather arrived late in the spring, heavy infestation of aphids on fall crops always followed.

**Conditions of cultivation.** A 2-yr field survey of maize dwarf mosaic under different cropping systems was made. Fields where corn and wheat were intercropped had about 90% fewer winged aphids than non-intercropped corn fields; subsequently, the incidences of maize dwarf mosaic in intercropped fields were 50% less than in non-intercropped corn fields. This was due to the wheat being a preferred host for *S. graminum*; therefore, very few winged aphids were produced. Consequently, the total number of apterous aphids in the intercropped fields was actually several times more than that of the non-intercropped fields. Another important factor affecting the maize dwarf mosaic epemics was planting date. Generally, an earlier planting date (April 9) resulted in only 25.0% of plants infected with MDMV at mid-season as compared to 79.3% infection for late plantings (April 29).

**TABLE 7. Differential host response to maize dwarf mosaic virus strain B from different sources.**

<table>
<thead>
<tr>
<th>Virus source</th>
<th>Zea mays</th>
<th>Sorghum bicolor</th>
<th>Sorghum sudanense</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tien-yu 1</td>
<td>Men 14</td>
<td>Wy 736-2-1-2</td>
</tr>
<tr>
<td></td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
</tr>
<tr>
<td>Maize line</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chun-lian-bei-ma-ya</td>
<td>18/19 (95)</td>
<td></td>
<td>1/10 (10)</td>
</tr>
<tr>
<td>Huan-chao 4</td>
<td>34/49 (69)</td>
<td>6/13 (46)</td>
<td>15/19 (79)</td>
</tr>
<tr>
<td>Tien-dan 1</td>
<td>19/38 (50)</td>
<td>28/49 (57)</td>
<td>17/26 (66)</td>
</tr>
<tr>
<td>Liao-tung 8624</td>
<td>14/16 (88)</td>
<td>4/9 (44)</td>
<td>8/15 (53)</td>
</tr>
<tr>
<td>Tung-da-li</td>
<td>18/19 (95)</td>
<td>5/18 (28)</td>
<td>12/19 (63)</td>
</tr>
<tr>
<td>Chun 714</td>
<td>12/19 (63)</td>
<td>5/11 (46)</td>
<td>4/11 (36)</td>
</tr>
<tr>
<td>H/Da-chiu 36</td>
<td>11/19 (58)</td>
<td>2/13 (15)</td>
<td>4/17 (24)</td>
</tr>
<tr>
<td>ANH1/H1</td>
<td>13/19 (68)</td>
<td>12/19 (63)</td>
<td>5/19 (26)</td>
</tr>
<tr>
<td>RB1/H1</td>
<td>18/20 (90)</td>
<td>6/9 (67)</td>
<td>9/16 (56)</td>
</tr>
<tr>
<td>Sorghum line</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bei-ping</td>
<td>27/50 (54)</td>
<td>4/9 (44)</td>
<td>10/32 (31)</td>
</tr>
<tr>
<td>Sugarcane line</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zhe-tan 75-72</td>
<td>17/56 (30)</td>
<td>15/46 (33)</td>
<td>14/31 (45)</td>
</tr>
<tr>
<td>Nei-Jian 907</td>
<td>10/19 (53)</td>
<td>4/14 (29)</td>
<td>13/17 (77)</td>
</tr>
<tr>
<td>Cereal line</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Setaria italica</td>
<td>18/29 (62)</td>
<td></td>
<td>3/10 (30)</td>
</tr>
<tr>
<td>Panicum miliaceum</td>
<td>12/17 (71)</td>
<td></td>
<td>1/10 (10)</td>
</tr>
</tbody>
</table>

*No. infections/No. trials, with 3-11 replicates.*
<table>
<thead>
<tr>
<th>Test species</th>
<th>Inoculation Sap</th>
<th>Aphid</th>
<th>Reisolated from corn</th>
<th>Result</th>
<th>Test species</th>
<th>Inoculation Sap</th>
<th>Aphid</th>
<th>Reisolated from corn</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aegilops squarrosa L.</td>
<td>NT</td>
<td>T</td>
<td>T</td>
<td></td>
<td>H. vulgare L.</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td></td>
</tr>
<tr>
<td>Agropyron sibiricum (Wild.) Beauv.</td>
<td>NT</td>
<td>T</td>
<td>T</td>
<td></td>
<td>H. vulgare var. nudum</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td></td>
</tr>
<tr>
<td>Agrostis alba L.</td>
<td>NT</td>
<td>T</td>
<td>T</td>
<td></td>
<td>Lolium perenne L.</td>
<td>NT</td>
<td>T</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td>A. tenuis Sibth.</td>
<td>NT</td>
<td>T</td>
<td>T</td>
<td></td>
<td>Opismenus undulatissolius (Arduino)</td>
<td>NT</td>
<td>T</td>
<td>T</td>
<td></td>
</tr>
<tr>
<td>Alopecurus aequalis Sobol.</td>
<td>NT</td>
<td>T</td>
<td>T</td>
<td></td>
<td>Roem. &amp; Schult.</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td></td>
</tr>
<tr>
<td>Aneurolepidium dasystachys (Trin.) Nevski</td>
<td>NT</td>
<td>T</td>
<td>NT</td>
<td></td>
<td>Oryza sativa L.</td>
<td>T</td>
<td>T</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td>Arrhenatherum elatius (L.) Persl.</td>
<td>NT</td>
<td>T</td>
<td>NT</td>
<td></td>
<td>Panicum miliaceum L.</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td></td>
</tr>
<tr>
<td>Arthraxon hispidus (Thunb.) Makino</td>
<td>T</td>
<td>NT</td>
<td>T</td>
<td>+</td>
<td>Pennisetum flaccidum Griseb.</td>
<td>NT</td>
<td>T</td>
<td>T</td>
<td></td>
</tr>
<tr>
<td>Avena nuda L.</td>
<td>NT</td>
<td>T</td>
<td>T</td>
<td></td>
<td>Phleum pratense L.</td>
<td>NT</td>
<td>T</td>
<td>T</td>
<td></td>
</tr>
<tr>
<td>Bothriochloa ischaemum (L.) Keng</td>
<td>NT</td>
<td>T</td>
<td>T</td>
<td></td>
<td>Phragmites communis Trin.</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td></td>
</tr>
<tr>
<td>Briza maxima L.</td>
<td>NT</td>
<td>T</td>
<td>T</td>
<td></td>
<td>Poa annua L.</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td></td>
</tr>
<tr>
<td>Bromus catharticus</td>
<td>NT</td>
<td>T</td>
<td>NT</td>
<td></td>
<td>R. psiloepis Peng</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td></td>
</tr>
<tr>
<td>B. inermis Leyss.</td>
<td>NT</td>
<td>T</td>
<td>NT</td>
<td></td>
<td>P. psiloepis Trin.</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td></td>
</tr>
<tr>
<td>B. plurinodis Keng.</td>
<td>NT</td>
<td>T</td>
<td>NT</td>
<td></td>
<td>Polypogon higegaweri Steud.</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td></td>
</tr>
<tr>
<td>B. tectorum L.</td>
<td>NT</td>
<td>T</td>
<td>T</td>
<td>+</td>
<td>Roegneria purpurascens Keng, sp. nov</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td></td>
</tr>
<tr>
<td>Buchloe dactyloides (Nutt.) Engelm.</td>
<td>NT</td>
<td>T</td>
<td>T</td>
<td></td>
<td>R. nutans (Keng) Keng</td>
<td>NT</td>
<td>T</td>
<td>T</td>
<td></td>
</tr>
<tr>
<td>Chloris virginia Swartz</td>
<td>T</td>
<td>NT</td>
<td>NT</td>
<td>+</td>
<td>R. ciliaris (Trin.) Nevski</td>
<td>NT</td>
<td>T</td>
<td>T</td>
<td></td>
</tr>
<tr>
<td>Clinelymus excelsus (Turecz.) Nevski</td>
<td>NT</td>
<td>T</td>
<td>T</td>
<td></td>
<td>Saccharum sincere Roxb. amend. Jeswiet</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td></td>
</tr>
<tr>
<td>C. nutans (Griseb.) Nevski</td>
<td>NT</td>
<td>T</td>
<td>T</td>
<td></td>
<td>Setaria glauca (L.) Beauv.</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td></td>
</tr>
<tr>
<td>Dactylis glomerata L.</td>
<td>NT</td>
<td>T</td>
<td>NT</td>
<td></td>
<td>S. italica (L.) Beauv.</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td></td>
</tr>
<tr>
<td>Deyeuxia xylatica (Schrad.) Knuth</td>
<td>NT</td>
<td>T</td>
<td>T</td>
<td></td>
<td>S. viridis (L.) Beauv.</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td></td>
</tr>
<tr>
<td>Digitaria adscendens (H.B.K.) Henrard.</td>
<td>NT</td>
<td>T</td>
<td>T</td>
<td></td>
<td>Sorghum nitidum (Vahl) Pers.</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td></td>
</tr>
<tr>
<td>Echinochloa crusgalli var. mitis (Pursh) Peterm.</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>+</td>
<td>S. propinquum (Knutz) Hitchc.</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td></td>
</tr>
<tr>
<td>Eleusine indica (L.) Gaertn.</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td></td>
<td>S. sudanense (Piper) Stapf cv 1146</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td></td>
</tr>
<tr>
<td>Elvtrigia trichophora (Link) Nevski</td>
<td>NT</td>
<td>T</td>
<td>NT</td>
<td></td>
<td>S. sudanense cv 1147</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td></td>
</tr>
<tr>
<td>Eragrostis ciliaris (All.) Lutati</td>
<td>NT</td>
<td>T</td>
<td>NT</td>
<td>+</td>
<td>S. sudanense cv 1149</td>
<td>T</td>
<td>T</td>
<td>T</td>
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</tr>
<tr>
<td>E. pilosa var. imberbis Franch.</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td></td>
<td>Stipa bungeana Trin.</td>
<td>NT</td>
<td>T</td>
<td>T</td>
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<tr>
<td>E. poaeoides Beauv. ex Roem.</td>
<td>NT</td>
<td>T</td>
<td>NT</td>
<td>+</td>
<td>Triticum aestivum L.</td>
<td>T</td>
<td>T</td>
<td>T</td>
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</tr>
<tr>
<td>Hordeum brevissulatum (Trin.) Link</td>
<td>NT</td>
<td>T</td>
<td>NT</td>
<td></td>
<td>Zea mays L.</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td></td>
</tr>
</tbody>
</table>

* NT = not tested; T = tested; + = susceptible; − = not susceptible. Schizaphis graminum was used for aphid inoculation tests throughout the experiment, except for a few occasions when Rhopalosipum padi was also used.
DISCUSSION

Currently 23 species of aphids have been reported as vectors of MDMV in the USA (Knoke and Louie, 1981), including the six species tested in this study. However, in our study only four species, S. graminum, R. maidis, R. padi, and 2M. persicae, transmitted the MDMV-B isolate from northwestern China. S. graminum was the most efficient vector. This is in agreement with reports in the USA and elsewhere (Daniels and Toler, 1969; Nault and Bradley, 1969; Onazi and Wilde, 1974; Shaunak and Pitre, 1971, 1973). Although R. maidis, R. padi, and M. persicae were less efficient vectors than S. graminum, their transmission efficiency was still higher than that of the same species reported by other authors (Bancroft et al., 1966; Knoke et al., 1977; Louie and Knoke, 1975; Messieha, 1967; Shaunak and Pitre, 1971, 1975). A. dirhodum and M. avenae failed to transmit MDMV in our trials. They have been reported as MDMV vectors by other authors (Knoke et al., 1977; Louie and Knoke, 1975). Both S. graminum and R. padi transmitted MDMV in a stylet-borne manner; they were able to acquire MDMV in 0.5-1 min. Nault and Bradley (1969) reported that S. graminum acquired MDMV in as short a time as 15 sec. The inoculation of MDMV by S. graminum also occurred in 15 sec (Nault et al., 1971). We did not test inoculation thresholds shorter than 10 min, but it is reasonable to assume that most inoculations in our tests occurred in less than 10 min. Thus, increasing inoculation duration to 5 hr did not increase transmission efficiency (Table 3).

Our data on properties-in-sap of MDMV are consistent with the values reported by many researchers. Most published values for MDMV properties are: thermal inactivation point of 54-58 C, dilution end point of 10^{-2} to 10^{-6}, and longevity in vitro of 1-2 days at room temperature and 3-5 days at 0-4 C (Gingery, 1981; Shih and Hsu, 1979). The particle of MDMV is a flexuous rod of the potyvirus type and measured 700-755 nm x 12-16 nm (Anonymous, 1976; Gingery, 1981; Shih and Hsu, 1979). An isolate of MDMV collected from Shaanxi province was found to have similar particle morphology (O. E. Bradlute and J. H. Tsai, unpublished).

We have demonstrated MDMV transmission through seed of three commonly grown corn lines (Tien-yu 1, Tien-dan 1, and Jin-bei 7). Although the average transmission rate in our controlled experiments (Table 5) was only 0.08%, this nevertheless has great ecological and epidemiological significance. We feel that seed transmission is one of the most important factors in the introduction, long-distance dispersal, survival, and annual recurrence of maize dwarf mosaic. This could also be the reason for sudden outbreaks of this disease throughout northern and northwestern China in the last decade. After extensive inoculation of johnsongrass with different isolates of MDMV including sugarcane isolates, we concluded that MDMV in northwestern China is strain B or the non-johnsongrass strain. The isolate collected from Shaanxi province, PRC, was serologically related to MDMV-B (D. T. Gordon and J. H. Tsai, unpublished). While various isolates of MDMV in this study have been identified as MDMV-B, identification by differential host reactions has enabled us to distinguish between isolates of MDMV-B from sugarcane and other sources with otherwise very similar properties. Furthermore, S. sudanense cv. 1148 and S. bicolor cv. Broon could be used for identifying and separating the strains of MDMV in northwestern China into substrains of the non-johnsongrass strain of MDMV (viz MDMV-B). The use of differential hosts for separating MDMV-A and -B and sugarcane mosaic virus strain B has been reported by Rosenkranz (1978).

We have identified 15 species of gramineous plants as hosts of MDMV-B. Of these, A. hispidus, B. tectorum, C. virgata, E. crusgalli, E. ciliaris, E. poaeoides, P. miliaceum, S. glauca, S. italica, S. viridis, S. sudanense, and Z. mays have also been reported as hosts of MDMV-B in the USA (Rosenkranz, 1981). The remaining three susceptible species, D. ascendens, O. undulatifolius, and S. nitidum have not been reported as hosts, but species of the same genera are hosts (Rosenkranz, 1981). Among these 15 susceptible hosts tested, one particular host, B. tectorum, plays an important role in the epidemiology of MDMV as a potential overwintering host under severe conditions in northwestern China. Among those non-host species in our test, E. indica, E. pilosa, and O. sativa were recorded to be susceptible to MDMV elsewhere (Rosenkranz, 1981).

Several commonly grown corn lines in northwestern China were highly susceptible to MDMV. Those corn lines were bred solely for resistance to Helminthosporium leaf spots and head smut [Sphacelotheca reiliana (Kuehn) Clint.]. Since maize dwarf mosaic was a relatively new disease appearing only within the last decade, tests for resistance to it had not been included in previous breeding programs.

Both temperature and humidity play an important role in the epidemiology of maize dwarf mosaic. These factors greatly affected the efficiency and quantity of aphid vectors in our study. S. graminum developed high populations under low temperature and low humidity early in the season, resulting in massive dispersals of viruliferous adults during the corn growing season. However, this insect was not considered an important vector in the spread of MDMV in the USA because of low populations during the corn growing season (Knoke et al., 1977). According to our study, early planting dates were desirable in control of maize dwarf mosaic. This cultural practice has also been tried successfully in several parts of the USA (Keaster et al., 1969; Pitre, 1970; Zubet, 1967).

We have pointed out important factors which contribute to the development of maize dwarf mosaic epidemics and which have been previously presented by other authors (Knoke and Louie, 1981). Other interrelated biological factors such as moisture stress, light, wind, plant age, host plant conditions, and alternate hosts for virus and vectors affect the development of the disease. Further study will be directed to these factors and interactions involved in the dynamics of the complex system.
LITERATURE CITED


Viruses Diseases of Maize in the Philippines

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ABSTRACT


Mosaic, stripe, and leaf gall are known viral diseases of maize (Zea mays) in the Philippines. Leaf gall, after more than 5 decades since first reported, is now endemic in most of the corn-growing areas of the country. Symptoms of leaf gall generally involve stunting and dark green coloration of the leaves. Narrow, elongated galls appear on the lower surface of leaves. Galls start as small, white, hardly discernible specks on the lower surface of leaves. These galls gradually elongate following the veins and form a string of separate spindle-shaped swellings. With age, galls coalesce and form a continuous rough corky surface; the greener tissues between these galls become wrinkled. The virus has been transmitted by adult Cicadulina bipunctella after a 2-hi acquisition feeding and 1-20 hr inoculation feeding. Symptoms appeared after 15 to 20 days of incubation. No transmission has been obtained by sap injection, needle pricking, or bruising, or through insect exuviae and excreta or seeds.

The virus occurs naturally on Coix lachryma-jobi, Dactyloctenium aegyptium, Eleusine indica, Ischae­num rugosum, Rothboellia exaltata, and Sorghum halepense. Experimentally, it has been transmitted to sugarcane (Saccharum officinarum), sorghum (Sorghum bicolor), and wild rice (Zizania aquatica).

Compared to the corn downy mildew caused by Peronosclerospora philippinensis (Weston) Shaw and the bacterial stalk rot caused by Erwinia carotovora var. chrysanthemi Dye, viral diseases of maize (Zea mays L.) in the Philippines are of minor importance. No comprehensive record of their occurrence, distribution, and effects on yields is available. However, the economic significance of these diseases, particularly corn mosaic, is reflected in the ability of the corn mosaic virus to infect other economically important crops such as sugarcane (Saccharum officinarum), abaca (Musa textilis Nee), and even cultivated sorghum [Sorghum bicolor (L.) Moench] (Benigno and Karganilla, 1973; Celino and Ocfemia, 1941; Lawas and Fernandez, 1949; Ocfemia, 1949; Paulsen and Karganilla, 1973). In the case of the corn leaf gall, its causal virus also has the capacity to infect important crops such as sugarcane, sorghum, and a variety of wild rice (Zizania aquatica L.) (Agati and Calica, 1949).

MAIZE VIRUS DISEASES

Among the virus diseases of maize in the Philippines, mosaic, corn stripe, and leaf gall have been reported (Agati and Calica, 1950; M. B. Capito, personal communication; Lawas and Fernandez, 1949; Reyes, 1950). In the case of mosaic and stripe, the symptomatologies, transmission characteristics, viral properties, and host ranges have already been published (Exconde, 1977). Since no additional information is available, no attempt will be made to discuss these diseases. Suffice to say that mosaic is still endemic in some of the corn growing areas of the Philippines.

MAIZE LEAF GALL

This disease was first reported in September 1929 in a field planted to yellow flint of which 90% was affected (Ocfemia, 1931). After more than 5 decades, the disease has been consistently noted in varying proportions on some commercial hybrids and varieties in the corn-growing areas of central and southern Mindanao. In one field, the disease affected nearly 95% of the crop. If this trend continues, and evidence indicates that the infection is increasing every year, maize leaf gall could become the principal maize virus disease in the Philippines.

Symptoms. Diseased plants are generally stunted and produce leaves that are darker green than normal (Fig. 1). Narrow, elongated galls appear on the lower surface of leaves (Fig. 2). On early infected plants, these galls start as small, white, hardly discernible specks on the lower surface. These specks gradually elongate following the veins and form a string of separate spindle-shaped swellings. With age, galls along such a string may coalesce end-to-end and form a continuous rough corky surface (Fig. 3). As the galls develop on the veins, the greener tissues between the veins become wrinkled (Fig. 4). In many ways the production of the galls and the effect on the growth of corn are similar to that of Fiji disease of sugarcane. Leaves of badly affected corn plants show a tendency to twist in various directions or roll inward (Fig. 5). Diseased plants produce small abnormal ears with poorly developed kernels or no ears at all.

The diagnostic features of the symptoms on Coix
Fig. 1. Severe stunting of a maize plant due to maize leaf gall (left) and a healthy maize plant (right).

Fig. 2. Narrow elongated galls on the under surface of maize leaves.

Fig. 3. Galls on the lower surface of maize leaf which have coalesced to form a continuous rough corky surface.

Fig. 4. Galls on the lower surface of maize leaf showing wrinkling of the greener tissues between galls.
lachryma-jobi L., Dactyloctenium aegyptium (L.) Beauv., Eleusine indica (L.) Gaertn., Ischaemum rugosum (L.) Salisb., Rottboellia exaltata L., and Sorghum halepense (L.) Pers. are stunted growth followed by the development of dark-green coloration of the foliage. These are the persistent symptoms, whereas the degree of dwarfing and the extent of gall formation vary with different hosts.

Transmission. The maize leaf gall pathogen has been transmitted to corn by the leafhopper, Cicadulina bipunctella Matsumura, following feeding on diseased corn plants for 48 to 72 hr. Attempts to transmit the pathogen mechanically by sap infection, needle prickling, bruising, and through insect exuviae and excreta yielded negative results. Likewise, the pathogen was not transmitted through seeds obtained from either naturally or artificially inoculated plants. The pathogen from both the naturally and experimentally infected C. lachryma-jobi, D. aegyptium, E. indica, I. rugosum, R. exaltata, and S. halepense was recovered and subsequently transferred to corn using C. bipunctella (Agati and Calica, 1950).

Host range. In addition to maize, the virus also has been reported to occur naturally on C. lachryma-jobi, D. aegyptium, E. indica, I. rugosum, R. exaltata, and S. halepense (Agati and Calica, 1950). Experimentally, it has been transmitted to sugarcane (cv. POJ 2882, Alunan and M-1900), sorghum (Fig. 6), and wild rice (Zizania aquatica L.).

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Characteristics of Viruses Affecting Maize in Australia

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ABSTRACT


Five viruses have been recorded causing diseases of maize (Zea mays) in Australia. The most important is an Australian johnsongrass (Sorghum halepense) strain of sugarcane mosaic virus (SCMV-Jg) which causes the maize dwarf mosaic disease. However, recent use of resistant hybrids is decreasing the importance of this disease. SCMV-Jg is a typical potyvirus; it is aphid-transmitted, has flexuous rod particles, and produces pinwheel and laminated aggregate inclusions in infected cells. The coat protein is distinct from that of three other SCMV strains from Australia.

Cereal chlorotic mottle virus (CCMV) is a subgroup II plant rhabdovirus, transmitted by the cicadellids, Neocicadula pallida and Cicadulina bimaculata. It causes fine chlorotic striations on leaves, stunting, and tassel sterility of susceptible maize. The particles have a distinctive morphology and are not serologically related to other rhabdoviruses which infect maize. It produces two and sometimes three lines in agar gel-diffusion serology and five polypeptides are resolved by polyacrylamide gel electrophoresis.

Maize sterile stunt virus (MSSV) is a subgroup I plant rhabdovirus, transmitted by delphacid planthoppers, chiefly Sogatella longifurcifera. It has a largely festucoid host range and infects only a few maize genotypes, in which it causes severe stunting, top necrosis, and sterility.

Maize stripe virus (MStpV), transmitted by Peregrinus maidis, affects maize and sweet corn in coastal and tropical districts. It has similar characteristics to MStpV in the USA and Africa. The host range includes Zea spp., Sorghum spp., barley (Hordeum vulgare), rye (Secale cereale), and triticale.

Chloris striate mosaic virus (CSMV) is transmitted by N. pallida and produces fine, pale striations on leaves of affected maize. Only a few lines and hybrids are susceptible. CSMV is a geminivirus but is not serologically related to maize streak virus.

Wallaby ear disease is caused by feeding of C. bimaculata, and recent work has shown that the reovirus, commonly present in this leafhopper, is not involved in the stunt and vein-enation disease produced in maize.

Maize (Zea mays L.) is grown in Australia from about 12 to 37° S latitude. Some of the virus diseases appear to be endemic, although the vectors range outside the continent. Maize was introduced only with European settlement and it seems likely that the endemic viruses have spread to maize from reservoirs in native grasses. Sugarcane mosaic virus (SCMV) is the only virus recorded which has been shown to be seed-borne and may have been introduced. However, strains of SCMV present in Australia are different from those found elsewhere (Teakle and Grylls, 1973; Taylor and Pares, 1968). This paper deals with ecological and epiphytological characteristics of maize viruses as disease agents and briefly describes their physico-chemical, morphological, and serological properties as well as host relationships.

Maize is an important summer cereal, but is second to sorghum (Sorghum bicolor (L.) Moench) in both total area and cash value. Many commercial maize hybrids grown in Australia are derived from public and private inbreds originating from the USA. However, there are also two active public breeding programs, one centered at Grafton in New South Wales and one at Kairi in north Queensland. These programs use some public U.S. inbreds and others developed within the programs from old Australian open pollinated cultivars and exotic lines of more recent derivation from America (especially Caribbean countries) and Africa (Colless, 1979; Persley et al., 1981). Many of our most virus-susceptible lines are of U.S. origin and several excellent sources of virus resistance derive from old Australian material.

MAIZE VIRUS DISEASES

Historical aspects. The earliest record of transmission of a maize virus in Australia appears to be that of maize stripe virus (MStpV) by Blackford in 1948 using the vector Peregrinus maidis (Ashmead) (Simmonds, 1966). SCMV received little attention until the 1960's and work was stimulated by investigation of SCMV infection of the Sorghum spp. breeding lines of A. Pritchard (N. Grylls and Greber, unpublished) in
1961. The first record of transmission of SCMV from naturally infected maize was by Greber in 1965, but symptoms suggesting SCMV infection had been recorded by McKnight in 1948 (Simmonds, 1966). Teakle (Teakle and Pritchard, 1971; Grogan and Teakle, 1969; Teakle and Grylls, 1973) subsequently examined strains of SCMV and the reactions of maize and sorghum to SCMV infection. Host resistance, yield loss, and epidemiological studies have been made periodically (Persley and Greber, 1977; Persley et al., 1976-1981).

Viruses other than SCMV have been investigated recently by Greber (1977a, b, c, 1979b, 1981a, b, c, 1982a, b), whereas maize wallaby ear disease was examined by Grylls (1975, 1979) and Reddy et al. (1976). Wallaby ear disease was first described as a disease of maize by Tryon (1910) and insect transmission by Cicadulina bimaculata (Evans) was demonstrated by Schindler (1942) and Grylls (1975), but recent work (Boccardo et al., 1980; F. Ofori and R. Francki, personal communication; Greber and D. Gowanlock, unpublished) has failed to demonstrate any viral etiology for this disease in maize.

Chloris striate mosaic virus (CSMV) was first described by Grylls (1963) and first shown to produce natural infection of maize by Greber (1977a). Characteristics of the virus were described by Francki et al. (1979, 1980).

Importance of virus diseases and changes resulting from the use of resistance and cultural practices. Maize dwarf mosaic is the most important virus disease of maize in Australia. It is the only aphid-transmitted virus disease recorded on Australian maize and is caused by a johnsongrass [Sorghum halepense (L.) Pers.] strain of SCMV (SCMV-Jg). The incidence varies from high (>50%) on susceptible hybrids in southeast Queensland during mid and late summer, to low (0-20%) in very early crops, and is also low in the most southerly and in parts of the far northerly range of maize culture. Some districts in Victoria and northern Queensland are virtually free of the disease. Ratings have been published each year by the Queensland Department of Primary Industries indicating the relative susceptibility to maize dwarf mosaic of the hybrids currently being offered for sale. This has resulted in commercial pressure to offer hybrids with adequate resistance for sale in those districts prone to the disease. Consequently, the importance of the disease is diminishing and there are very few highly susceptible hybrids now being marketed. Some locally bred hybrids from the Grafton and Kaiiri programs are highly resistant to the virus. These high resistances are based on lines derived from old Australian open-pollinated cultivars and lines extracted from material of Central American and Caribbean origin (Grogan and Teakle, 1969; Persley et al., 1981). In view of the stability and efficacy of these resistances, it is surprising they have not been incorporated into SCMV-resistance breeding programs in other countries. The location for selection of some of the old Australian lines (Gatton, Queensland) is also coincidentally the district with the most consistently high incidence of SCMV-Jg in Australia.

Cereal chlorotic mottle virus (CCMV) has been recognized only recently (Greber, 1977b). Deletion of hybrids highly susceptible to SCMV has made minor virus diseases more conspicuous. Publication of ratings for CCMV-susceptibility of some maize hybrids (Greber, 1981c) was followed quickly by replacement of two of the most susceptible by CCMV-resistant equivalent types. One of these replacements, unfortunately, proved to be highly susceptible to SCMV. The incidence of CCMV, like all leafhopper-borne viruses, is very dependent on weather conditions. Dry seasons following good early planting rains and heavy weed-grass contamination of crops can induce incidences up to 60%, but most districts frequently show only trace infection. There are highly resistant hybrids with good agronomic performance and application of this knowledge should soon reduce the disease incidence.

Maize sterile stunt is another recently described virus disease (Greber, 1982c). It affects only a few maize genotypes. The seriousness of this disease arose from its high incidence in parental lines in seed production plantings. Affected plants were almost invariably sterile as either male and female parents. Early attempts were made by a plant breeder to select out the “stunting gene” from lines B37 and H84 and failure to do so precipitated the examination of affected plants for virus. Districts prone to maize sterile stunt can be avoided for production of those hybrids made from susceptible parents. A resistance back-crossing program may soon provide resistant forms of the most susceptible lines.

CSMV has been a problem only in one commercial hybrid and its parental lines. In retrospect, Grylls (1963) was fortunate to select the line used to establish maize as a host of this virus, as almost all other lines are immune. Some sweet corn lines and hybrids are slightly susceptible to CSMV.

Wallaby ear disease can seriously affect many hybrids in coastal areas and even tolerant hybrids can be affected under extreme pressure from high populations of C. bimaculata. Dry seasons, growing crops late in the summer, and weed grass contamination are associated with increased severity of the disease.

Maize stripe disease follows the distribution of the vector, P. maidis, and is most severe close to the coast and in north Queensland, where incidences can reach 40%. Similarly high incidences may occasionally occur in sweet corn, which appears to be more susceptible than maize. There is little resistance available (Greber, 1981a), but the low frequency of severe outbreaks probably does not warrant substantial effort on control.

**PROPERTIES AND RELATIONSHIPS OF THE VIRUSES AND THE DISEASES THEY PRODUCE**

Data presented are largely derived from the literature cited, but where new hosts or other data are reported the methods used were similar to those reported for work on the individual viruses (Greber, 1977c; Persley and Greber, 1977; Greber, 1979a; Greber, 1981a, b; Greber and Gowanlock, 1979; Persley et al., 1981).

**Relationship of viruses and vectors.** The classification groupings and vectors of viruses infecting maize and other Gramineae in sub-tropical Australia are...
shown in Table 1. Digitaria striate virus (DSV) has not been shown to infect Sorghum or Zea spp. but has not been extensively tested on a wide range of genotypes. An unnamed isometric virus of the Gramineae (not shown in Table 1) has not been transmitted in glasshouse tests and it is not known whether it can infect maize. CCMV and MSSV are both rhabdoviruses but have cicadellid and delphacid vectors, respectively, and belong to different subgroups using the criteria of Peters (1981). SCMV-Jg is a potyvirus and is capable of transmission by high populations of *Rhopalosiphum maidis* (Fitch) within maize crops throughout most of the geographic range in Australia make it likely that this species is the major vector. Populations of *R. padi* (L.) in Queensland are exceptionally low and this species would contribute little to the transmission of SCVM in that area. Sugarcane Fiji disease virus is sometimes listed with maize viruses since maize is a host, but there is no record of this plant reovirus naturally infecting maize in Australia. The delphacid vectors of Fiji disease virus have a narrow host range and this fact inherently restricts the virus host range.

**Taxonomic patterns in host ranges of the viruses.**

Most maize viruses are restricted in host range to the family Gramineae, and especially those with leafhopper vectors. Table 2 compares the taxonomic range within the Gramineae of some maize and grass viruses from Australia. Barley yellow dwarf virus (BYDV) and barley stripe mosaic virus (BSMV) have been omitted. The data are compiled from hosts demonstrated for Australian isolates only and are listed for a selected range of genera which gives a satisfactory balance within the taxonomic groups chosen (Watson and Gibbs, 1974). Characteristically, the host range of SCVM is non-festucoid, while CCMV, CSMV, and MSSV favor this taxonomic group. SCVM has more hosts in the Panicoïdes and Andropogonïdes, while the hosts of MStpV are spread mainly in the Festucoid and Andropogonïdes. Known hosts for the unnamed isometric virus are still too few to draw any conclusions.

Apparent virus host range is probably a composite of real plant host range and vector host range, particularly with viruses requiring transmission feed times of moderate or long duration. Forced feeding under insectary conditions can extend the range, but many grass and cereal leafhoppers refuse to feed adequately on many species even when the alternative is to die of starvation. Reported host range, especially the negative aspect, has little value without specification of the genotype, especially in a species like maize which has been extensively genetically manipulated by the development of inbred lines. The same narrow genetic constraints makes these same species especially valuable for virus strain differentiation (Persley and Greber, 1982; Persley et al., 1981).

Sugarcane mosaic virus strains causing maize dwarf mosaic disease. This is the most serious virus disease of maize and sweet corn in Australia and regularly causes losses in late crops of susceptible hybrids in southeast Queensland. The Australian SCVM-Jg can be distinguished from maize dwarf mosaic virus strain A (MDMV-A) which occurs in the USA by the reaction of several sorghum lines (Teakle et al., 1970; Persley and Greber, 1982). The two viruses are distantly related serologically (Taylor and Pares, 1968). Symptoms produced by SCVM-Jg vary with time of infection and the host genotype and range from chlorosis and mosaic with a blotchy or streaked pattern between the veins (Fig. 1a) to ringspotting. Stunting is more severe in susceptible hybrids infected early. Yield loss in the worst cases can be up to 50% (Persley et al., 1976). Incidence is usually highest in good seasons with adequate rainfall — conditions which generally depress the leafhopper-borne viruses.

Four strains of SCVM (Table 3) have been recorded in Australia, each based on an exclusive perennial host

<table>
<thead>
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<th>Virus group</th>
<th>Vectors</th>
<th>Vector group</th>
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<td>Plant rhabdovirus (Subgroup II)</td>
<td>Nesoclthia pallida</td>
<td>Cicadellid leafhoppers</td>
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<tr>
<td>Maize sterile stunt virus</td>
<td>Plant rhabdovirus (Subgroup I)</td>
<td>Sogatella longisignifera</td>
<td>Delphacid planthoppers</td>
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<tr>
<td>Digitaria striate virus</td>
<td>Plant rhabdovirus (Subgroup I)</td>
<td>Sogatella kolophon</td>
<td>Delphacid planthoppers</td>
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<tr>
<td>Maize stripe virus</td>
<td>New group</td>
<td>Peregrinus maidis</td>
<td>Delphacid planthoppers</td>
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<td>Sugarcane mosaic virus</td>
<td>Potyvirus</td>
<td>Rhopalosiphum maidis and other species</td>
<td>Aphids</td>
</tr>
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<td>Geminivirus</td>
<td>Nesoclthia pallida</td>
<td>Cicadellid leafhoppers</td>
</tr>
<tr>
<td>Sugarcane Fiji disease virus</td>
<td>Plant reovirus (Subgroup II)</td>
<td>Perkinsiella saccharicida</td>
<td>Delphacid planthoppers</td>
</tr>
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</table>

**TABLE 1. Some viruses of Gramineae in the Australian sub-tropics.**
TABLE 2. Comparison of host range of some grass and cereal viruses.

<table>
<thead>
<tr>
<th>Taxonomic group</th>
<th>Genus</th>
<th>SCMV</th>
<th>CCMV</th>
<th>MSSV</th>
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<th>DSV</th>
<th>MStpV</th>
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<td>Festucoids</td>
<td>Aegilops</td>
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<td>+</td>
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<td>Avena</td>
<td>+</td>
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<td>Bromus</td>
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b SCMV = sugarcane mosaic virus, CCMV = cereal chlorotic mottle virus, MSSV = maize sterile stunt virus, CSMV = chloris striate mosaic virus, DSV = digitaria striate virus, MStpV = maize stripe virus, and IV = unnamed isometric virus.
Fig. 1. a) Severe mosaic symptoms produced by the johnsongrass strain of sugarcane mosaic virus in sweet corn. b) Fine chlorotic striations caused by cereal chlorotic mottle virus infection of maize. c) Stunted maize plants (line B37) and *Echinochloa calona* affected by the maize sterile stunt virus. d) Broad chlorotic striations and apical bending caused by the maize stripe virus in sweet corn.
Only SCMV-Jg causes significant infection of maize. The Queensland blue couch grass (*Digitaria didactyla* Willd.) strain occasionally infects maize and sweet corn, but these infections are only readily detected in areas where SCMV-Jg is absent. The other two strains have not been recorded from maize. They are readily aphid transmitted (Teakle and Grylls, 1973), but even when they comprise the major source of inoculum near maize plantings, it is found that infections in the maize crop are by the SCMV-Jg strain, often apparently derived from a much more obscure inoculum source. The Sabi grass [*Urochloa mosambicensis* (Hack.) Dandy] and sugarcane (*Saccharum officinarum* L.) strains infect maize readily when mechanically inoculated (Persley et al., 1981).

Johnsongrass has a wide distribution in Queensland and New South Wales (Monaghan, 1978; Penrose, 1974). It frequently forms a continuous fringe along roadways and SCMV-Jg infection is almost invariably present, although symptoms are often mild in the grass host. However, at least one district, the Atherton Tableland in north Queensland, has ample johnsongrass but SCMV-Jg has not become established despite a long history of maize culture.

SCMV-Jg is a typical potyvirus. It has flexuous rod particles and both pinwheel and laminated aggregate inclusions are found in infected cells (Figs. 2a, b). The coat protein subunit has a molecular weight of 34,200 daltons. On the basis of amino acid composition and tryptic peptide maps, the SCMV-Jg strain is quite distinct from the other three Australian strains (Gough and Shukla, 1981). The Sabi grass and blue couch grass strains have very similar coat proteins with molecular weights of 40,500 and 39,100 daltons, respectively. Examination of the strains by the infection spectrum on maize differentials which have resistance to one or more strains (Persley et al., 1981) does not correlate well with the coat protein comparisons. Although the blue couch grass strain produces milder mosaic symptoms on most hosts, it appears to have the widest infection spectrum within resistant maize lines when mechanically inoculated.

### TABLE 3. Ratings for susceptibility of maize lines and related species to Australian viruses and viruslike diseases.

<table>
<thead>
<tr>
<th>Maize line or species</th>
<th>MSSV*</th>
<th>CCMV</th>
<th>WED</th>
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* MSSV = maize sterile stunt virus; CCMV = cereal chlorotic mottle virus; WED = wallaby ear disease; SCMV = sugarcane mosaic virus.
* Teakle and Grylls, 1973; USA inbreds.
* MSSV and CCMV: 0 = not susceptible; + = slightly susceptible; ++ = susceptible.
* WED: + = moderately affected; ++ = severely affected; T = tolerant.
* SCMV: HR = highly resistant even to sap inoculation; R = moderately resistant to sap inoculation; + = susceptible; PR = not fully resistant to sap inoculation with all isolates; FR = field resistant, but susceptible to sap inoculation.

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* = no data.

**Kaari inbreds.**

* Gatton inbreds.

* Grafton inbreds.

* Kairi sweet corn lines.
These four SCMV strains were each thought to be relatively homogeneous, but recent examination of isolates from resistant maize lines at Gatton, Queensland, has shown that some johnsongrass isolates are more aggressive than others and can be differentiated by their ability to infect lines such as KL308 which are highly resistant even to mechanical inoculation with other johnsongrass isolates (D. Persley and Greber, unpublished; Persley et al., 1981) (Table 3). Although SCMV-Jg is readily differentiated from MDMV-A by the N-gene reaction in sorghum lines, the resistances to SCMV available in both sorghum and maize show good correlations between the Australian and American viruses (Henzell et al., 1982; Persley et al., 1981; Grogan and Teakle, 1969).

Cereal chlorotic mottle virus. CCMV belongs to subgroup II (Peters, 1981; Greber, 1982b) of the plant rhabdovirus group. It causes fine chlorotic striations on maize leaves (Fig. 1b) as well as variable stunting and tassel sterility (Greber, 1979a; 1982b). This disease was probably confused with maize dwarf mosaic when many SCMV-susceptible hybrids were grown, but became obvious when maize dwarf mosaic-resistant hybrids became more widely used. It is readily diagnosed by examination of negatively stained sap with an electron microscope.
microscope. The bullet-shaped particles in unfixed preparations containing potassium phosphotungstate show conspicuous surface ornamentation patterns (Greber, 1979a; 1982b) and a wide axial core (Fig. 3a). CCMV particles in sap almost invariably reach a high concentration. Dual and triple infections of CCMV, MSSV, and SCMV (Figs. 3a, b, c) also occur and are readily distinguished by electron microscopy. Thin section electron microscopy shows that individual cells can become infected by both SCMV and MSSV or by SCMV and CCMV (Figs. 2a, b), but we have not found the two rhabdoviruses together in a single cell (D. H. Gowanlock and Greber, unpublished). Symptoms produced by the virus complexes are extremely variable and are expressed as combinations of chlorosis, mosaic, purple coloration, streaks, and stunting. Symptom diagnosis is difficult and unreliable for mixed infections.

Host species demonstrated for CCMV are *Avena sativa* L., *Bromus unioloides* Kunth, *Dactyloctenium aegyptium* (L.) Beauv., *Digitaria ciliaris* (Retz.) Koeler, *Dinebra retroflexa* (Vahl) Pantz., *Echinochloa colonia* (L.) Link, *Eleusine indica* (L.) Gaertn., *E. coracana* (L.) Gaertn., *Eragrostis ciliaris* (All.) E. Mosher, *Hordeum vulgare* L., *Leptochloa filiformis* (Lam.) Beauv., *Rottboellia exaltata* L., *Secale cereale* L., *Setaria italic*a (L.), Beauv., *Setaria verticillata* (L.) Beauv., x *Triticosecale* Wittmack, *Triticum aestivum* L., *T. monococcum* L., *T. tauschii* (Coss.) Schmal., *Urochloa panicoides* Beauv., and *Z. mays*. The virus occurs naturally in most of these species and can reach high incidences (ca. 80%) in temperate cereals such as wheat (*T. aestivum*) and triticale when these are grown at times of high vector activity (Greber, 1981b). However, serious disease in these crops does not normally occur because they are grown in cooler times of the year. The virus infects maize over a geographical range of more than 1000 km, which correlates better with the distribution of *C. bimaculata* than with that of the other cicadellid vector, *Nesochlata pallida* (Evans).

CCMV is more efficiently spread by *C. bimaculata* than by *N. pallida*, although the latter provides better overwintering and early-season vectoring (Greber, 1981c). Some host species such as *E. colona*, *D. ciliaris*, and *E. ciliaris* are exclusive to the vector *C. bimaculata*. *N. pallida* could not acquire CCMV from or transmit it to these hosts, although it transmitted to other hosts after injection of infective sap from these species (Greber, 1982b). *C. bimaculata* breeds freely on lower surfaces of mature maize leaves during mid- and late summer, and nymphs collected from field plants infected by CCMV transmitted efficiently as adults in glasshouse tests (Greber, 1981b). Because it reaches much higher population numbers on maize than *N. pallida*, *C. bimaculata* is considered to be the major vector during epiphytotics.

Infected grass hosts are present in the field throughout the year and provide inoculum sources for maize crops. *L. filiformis* produces a conspicuous reaction, with a rusty red necrosis on the leaves and deformed inflorescences (Greber, 1982b). *N. pallida* can also act as a persistent inoculum source as it survives well in cool conditions and can live for several months. *C. bimaculata*, however, thrives only under warmer conditions and has a shorter life span.

CCMV particles are readily purified by a method involving extraction in tris-HCl buffer, centrifugation, and collection from the interface of layered sucrose solutions, followed by celfite filtration and centrifugation on sucrose gradients (Greber, 1982b). Purified preparations are only moderately good immunogens. Antisera produce two and sometimes three precipitin lines in agar gel diffusion tests and the reaction is usually enhanced by adding 1% Triton X-100. Two antigens can be separated by centrifugation (Greber and Gowanlock, 1979; Greber, 1982b) from CCMV particles after treatment with detergent or storage degradation, but in freshly extracted sap all antigens are pelleted with the particles during centrifugation. Polycrylamide gel electrophoresis separates five polypeptides from CCMV particles (J. Dale and Greber, unpublished). One of these is glycosylated (G-protein) and two of the others are presumably M1 and M2 proteins, in the manner of most subgroup II rhabdoviruses (Peters, 1981). Gel-diffusion reactions are useful diagnostically with sap preparations, but would normally be used only to confirm the electron microscope tests.

In electron microscopy of thin sections of infected plants, CCMV particles are grouped mainly within the perinuclear space and appear to bud from the inner nuclear membrane. Brain and salivary gland cells of both vectors also contain virus particles in the perinuclear space, but the particles are sometimes embedded in a granular matrix (Greber and Gowanlock, 1979; Greber, 1982b).

**Maize sterile stunt virus.** This virus produces severe stunting, sterility, purple coloration, and often top necrosis of a few maize genotypes (Fig. 1c). The causal agent is a rhabdovirus belonging to subgroup I (Peters, 1981). Because the disease results in stunting and occasionally causes some striations in maize and could be transmitted by *P. maidis*, there was some early difficulty providing evidence that this disease was not caused by a form of maize mosaic virus (MMV). This problem was not alleviated by the proliferation in the literature of erroneous references to the occurrence of MMV in Australia. This confusion began before the rhabdovirus etiology of MMV was established (Herold, 1972) and the Australian disease referred to was probably caused by MSStV (Greber, 1981a, 1982c). When MSSV was first described (Greber, 1977a), transmission was reported by *S. longifurcifera* (Esaki & Ishihara). *P. maidis* is a very inefficient vector of MSSV after acquisition from plants (0-10%), but can transmit efficiently (70% of individual insects) after injection of infective sap. *S. longifurcifera* is an efficient vector of MSSV, but will not breed on maize. It is, nevertheless, commonly found on maize and in drier situations there are usually far higher populations of adults of *S. longifurcifera* than *P. maidis*. *S. longifurcifera* will breed well on *E. colona* and on wheat, both of which are hosts of MSSV. Festucoid hosts (Table 2) are better acquisition sources for
the virus than maize. Other hosts of MSSV are *Aegilops variabilis* Eig, *A. sativa*, *D. aegyptium*, *H. vulgare*, *S. italica*, × *Triticosecale*, *T. aethiopicum* Jakubz., *T. monococcum*, and *T. turgidum* L. Symptoms in festucaoid hosts usually are broad chlorotic striations on leaves produced soon after infection, reducing to diffuse mottled chlorosis in later growth. Stunting, notching of leaf margins, and narrow-leaved tillering also occur. Other hosts first show mild chlorotic striations followed by stunting.

Both the virus and vector can originate from *E. colona*, but other grass species may also be involved. The temperate cereal hosts are highly susceptible when grown during summer, when leafhopper populations are high, but only a trace infection has been recorded during their normal cool-weather growing season. Because the vector does not breed on maize and this species is a poor virus acquisition host except in the first stages of infection, maize probably occupies a largely terminal position in the epidemiology of MSSV (Greber, 1981b).

Susceptible maize lines include H84, B37, N7B, KE31, and close relatives of these. Most of these lines are also susceptible to SCMV and CCMV. MSSV symptoms are dominant, especially when the infection precedes that of the other viruses. Thus some triple infections can show obvious symptoms only of MSSV, while others may show extensive mosaic or chlorotic striations. The F1 hybrids of B37 with the resistant lines Oh07, Oh7B, Pa405, and 38-11 are all fully resistant to MSSV.

MSSV particles resemble those of DSV (Greber, 1979a; 1982c) and show no prominent surface pattern in unfixed negative stain preparations (Fig. 3a). They have narrower axial core penetration than CCMV, frequently disrupt to several fragments, and are often penetrated by the stain to show the nucleoprotein helix. In thin sections they are seen to accumulate in cytoplasmic vesicles, often in parallel orientation and always enclosed by a vesicle membrane (Fig. 2b). In particle cross-sections, a darkly stained annulus surrounds an unstained core with a central dot (Fig. 3b). In infective vector planthoppers, bundles of tubular structures of the size and configuration of unenveloped particles are found in brain and salivary gland tissue (Greber, 1982c).

MSSV was not satisfactorily purified by methods described for CCMV (Greber and Gowanlock, 1979) or maize mosaic virus (Lastra and Acosta, 1979) or wheat striate mosaic virus (Sinha and Behki, 1972), and did not react with either MMV or CCMV antisera.

Maize stripe virus. Maize stripe was sometimes confused with maize mosaic in early literature, but is readily differentiated by negative stain electron microscopy because it does not have the characteristic rhabdovirus particles of MMV. MStpV causes prominent chlorotic striations of varying width and intensity, often with a "brushed-out" appearance on the first affected leaves (Fig. 4a). Bending of the apical growth is also a characteristic symptom (Fig. 4b). Maize stripe is also typically a disease of coastal areas and those with frequent rainfall. It parallels the distribution of the vector *P. maidis* which it is transovarially propagated (Gingery et al., 1981). *P. maidis* colonizes the apical region of maize, sorghum, and related genera. Unfurled leaves of the apical region produce an enclosure which establishes a protected environment for emerging nymphs against drowning; the unfolding of these leaves is partially prevented by the sticky planthopper exudate. In contrast, planthopper colonies hatched on exposed leaf surfaces are decimated when rainfall is frequent. Emergence of the tassel largely evicts the *P. maidis* colony and provides an effective dispersal mechanism for the virus.

Maize stripe is more severe on sweet corn than on maize, although the incidence in maize is sometimes high in north Queensland. Sorghum rarely shows more
than a trace infection. This may partly reflect the fact that this crop is grown farther from the coastal locations preferred by the vector. The disease is more common in sorghum x sudangrass hybrids grown as rotational green manure crops in higher rainfall districts.

The host range of MStpV includes several Sorghum spp. as well as Zea spp. The virus can infect barley, rye, and triticale under experimental conditions (Greber, 1981a). *P. maida* feeds adequately on barley, but under Queensland conditions it could not be induced to breed on this host as stated by Kulkarni (1973). No complete resistance to MStpV was found in any maize line tested, although some were much less efficiently infected than others, when the same group of insects fed sequentially on each. Only a low infection efficiency was found with the festucoid hosts.

Establishment of single insect transmission series showed that a range of isolates with different symptom severity could be propagated from a single field infection source.

Gingery et al. (1981) demonstrated that MStpV was consistently associated with a thin (3 nm) filamentous particle and they proposed MStpV as a member of a new virus group. No particles typical of previously described virus groups had been found in infected plants, extracts from them, or infective vectors in extensive tests with Australian isolates. Although Kulkarni (1973) reported isometric particles in his preparations of MStpV, his antisera nevertheless reacts well with isolates of MStpV from North and South America and Australia and presumably was prepared from mixed immunogens. An antisera with a low titer but specific reaction to MStpV was prepared from a Queensland isolate and produced lines confluent with those using Kulkarni’s antisera. These antisera react with sap extracts from all the MStpV hosts. Infective sap contained a highly diffusive antigen which produced a straight line reaction in agar gel-diffusion serology tests, but this reaction was supplanted that of a less diffusive antigen in tests with purified preparations (Greber, 1981a). Initial high speed pellets produced in buffers containing 0.5% 2-mercaptoethanol or thiolglycollate were infective when injected into *P. maida*, but no final purification products were infective. Sap from infected plants was not infective without use of 2-mercaptoethanol in the extraction medium (Greber, unpublished). This corresponds to infectivity assay data for MStpV published by Gingery et al. (1981), but contrasts with results with MSSV and CCMV (Greber, 1981b, 1982c) in which infectivity was well maintained in extracts diluted with distilled water and injected into the vectors. The protein molecular weight of 33,000 daltons determined for an Australian isolate (Greber, 1981a) was similar to that found for the Florida ‘isolate by Gingery et al. (1981).

**Chloris striate mosaic virus.** Infection of maize by this virus is rare because most maize is highly resistant. A few maize lines and hybrids are susceptible, as well as some sweet corn hybrids. Symptoms include fine, pale, chlorotic striations on the leaves (Fig. 4b). There may be slight stunting, but male and female inflorescence func-

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maize rough dwarf (Milne and Lovisolo, 1977) or sugarcane Fiji disease (Hatta and Francki, 1977). In common with other investigators (Grylls, 1975; Boccardo et al., 1980; Reddy et al., 1976), we have found reoviruslike particles in C. bimaculata. Sap from gall tissue of severely affected maize, when injected into N. pallida, did not cause the insects to produce wallaby ear when subsequently fed on wallaby ear disease-susceptible maize plants (Greber, unpublished). Further examination of wallaby ear disease at the Waite Agricultural Research Institute by F. Ofori and R. Francki (personal communication) has shown that C. bimaculata free of the reovirus infection can still induce wallaby ear disease symptoms. Further critical work on the etiology of wallaby ear disease is required on both the toxin and viral aspects, but present data indicate that the symptoms most likely result from the effects of a toxin secreted by C. bimaculata.

Most maize is susceptible to wallaby ear disease when subjected to feeding by large populations of C. bimaculata. However, there is a wide range of tolerance to the disease in maize (Table 3). Good ratings for tolerance can be obtained by allowing early contamination of plots by grass such as E. colona which will induce higher populations of C. bimaculata than will breed on maize itself and also cause higher disease pressure at an earlier stage of growth. Feeding by C. bimaculata also causes enations on several cereal species and a severe disease results in triticale.

Viruses not recorded as natural infections in maize. Several other viruses affect Gramineae in Australia. These include BYDV (Smith and Sward, 1982), BSMV (Greber, 1971), DSV (Greber, 1979b), and a newly described isometric virus (Greber, 1982a). None of these have been shown to cause field diseases of maize in Australia. BYDV and BSMV can infect maize experimentally; DSV has not been tried on a wide range of maize genotypes and no vector has been found for the isometric virus, which is not sap-transmissible.

Relative distribution and climatic effects. The importance of each maize virus disease varies between districts within the wide range of latitudes where maize is grown in Australia and also with the distance from the coast, which influences rainfall patterns. Where uniform moderate rainfall is experienced and especially within the mid range of latitude, maize dwarf mosaic is by far the most important virus disease. In the far north, where maize is subject to frequent rainfall during the growing season, maize stripe is more important. Leafhopper-borne viruses tend to occur in sporadic epidemics and are generally worse when a dry season follows good planting rain. Furrow irrigation does not inhibit leafhopper populations because it does not wet leaf surfaces where nymphs are hatching.

Control by resistance breeding. A good range of resistant hybrids based on resistant lines (Table 3) is available in Australia for all except MStpV. The resistance varies from immunity through field resistance to tolerance. Field resistance, as shown by U.S. lines such as Oh07, Oh7B, and 88-II to maize dwarf mosaic, tends to break down under high disease pressure and infection levels of >50% can occur. Several of these lines, however, show quite mild symptoms when infected by SCMV-Jg. Resistance sources based on Australian-derived lines such as L1, L23, HB, and KL57 confer very high resistance to SCMV when used in one of a pair of single cross hybrid parents. Similarly KPI, KP190, and KL308 of Central American origin and Pa405 of North American origin produce resistant hybrids when used with a susceptible partner (Grogan and Teakle, 1969; Persley et al., 1981; D. Persley and Greber, unpublished). Lines which showed a less complete spectrum of resistance to four Australian SCMV strains (Persley et al., 1981) (Table 3) probably have poorer durability prospects in the long term. Recent assessment of KL308 and KP190 reactions to a range of SCMV-Jg isolates has shown that some aggressive isolates can infect a percentage of plants from these lines when mechanically inoculated or when subjected to high infection pressure in some field locations (D. Persley and Greber, unpublished). Since this indicates that the virus has a potential...
tial to produce variants capable of overcoming these resistances, their use in hybrids intended for districts with high SCMV-Jg incidence may be inadvisable. However, we have found no evidence that any isolates of SCMV in Australia can infect Pa405, HB, or L12.

Resistance to MSSV is common in maize lines (Greber, 1982c) (Table 3). Current work at the Queensland Department of Primary Industries is attempting to incorporate SCMV and MSSV resistance from Pa405 into the lines B37 and H84, and MSSV resistance from another line into KE31. A combination of emerging seedling challenged with MSSV by caged feeding of infective S. longifurcata and then sap inoculation of SCMV-Jg is being used. CCMV resistance in most commercial hybrids is adequate (Greber, 1981c) and some highly susceptible ones have been replaced by equivalent CCMV-resistant hybrids. Experimental rating of hybrids should ensure that those which are highly susceptible are not grown in areas likely to be affected by the disease.

Several hybrids of sweet corn are slightly susceptible to both CCMV and MSSV (Greber, 1982b, c). The incidences, however, would not cause severe losses. Sweet corn in general is highly susceptible to maize stripe and losses can be severe in near-coastal districts. Use of insecticides may give effective control, if needed. Almost all sweet corn hybrids are highly susceptible to SCMV-Jg. Some lines, cultivars, and synthetics released by Brewbaker in Hawaii have considerable resistance (J. L. Brewbaker, personal communication) and some of this material has been included in sweet corn breeding populations at Kairi. Screening of lines from these populations following cycles of recurrent selection (D. Persley, I. Martin, and Greber, unpublished) has identified two highly resistant lines (Table 3) which have apparently evolved by recombinations from the original field-resistant material. When used on one side of a single cross, these produce highly resistant hybrids, one of which has now been released by I. Martin and T. McCarthy from the Kairi program.

**LITERATURE CITED**


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Some Serological Techniques for Detecting Plant Viruses

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ABSTRACT


Methods used in our laboratory for detection of plant viruses fall into two major categories: a) precipitin and b) amplified antibody reactions. Brief descriptions of the more important methods are given. Some specific applications are indicated with emphasis on advantages and disadvantages of each. Various applications of the enzyme-linked immunosorbert assay and electroblotting as new techniques for detection of plant virus protein are discussed in more detail.

It is not within the limits of this contribution to list and discuss every serological method used for plant virus detection. This subject has been well reviewed (Ball, 1974; Torrance and Jones, 1981; van Regenmortel, 1978, 1981). Rather, this paper will cover methods and their applications as used in our laboratory over the past 5 yr in studying a variety of viruses.

Antiserum preparation. Rabbits are used most commonly for antiserum production, although goats have been used when large quantities were required. A wide range of antiviral antibodies has also been obtained from egg yolks of immunized hens (Bar-Joseph and Malkinson, 1980; Polson et al., 1980a,b). Immunization procedures vary from one laboratory to another and the relative merits of different approaches may be argued. However, little substantiating evidence is available to prove any particular procedure superior to another. Since the response of individual rabbits subjected to the same immunization regime may vary considerably (van Regenmortel and von Wechmar, 1970; von Wechmar, unpublished), it is advisable to immunize two or three animals with the same viral antigen. This will enable the researcher to select the animal giving the most desirable immune response. For instance, when two animals were immunized with the same preparation of barley stripe mosaic virus (BSMV), one animal yielded antibodies which reacted with dissociated viral protein as well as the undisassociat ed viral capsid and the other yielded antibodies reacting only with undissociated viral capsid. Both species of antibody are useful, but for different purposes (von Wechmar, unpublished). The latter antiserum will find application in immunosorbent electron microscopy (ISEM) and agar double diffusion tests, whereas the former antiserum, rich in antibodies directed against dissociated viral protein, is useful in enzyme-linked immunosorbent assay (ELISA) and immuno-electroblotting techniques (IEB).

The procedure generally followed in our laboratory produces antiserum with high titers (du Plessis and von Wechmar, 1981; Rybicki and von Wechmar, 1981). It is common practice to collect several antiserum bleedings and to select those with desired characteristics.

Several procedures for the preparation of dissociated anti-viral capsid proteins used for immunization have been reported for both elongated and isometric viruses (Moghul and Francki, 1976; Purcifull and Batchelor, 1977; Purcifull et al., 1981).

Antigen preparation. No general recipe can be given for the preparation of viral antigens. Procedures depend mainly on the morphology and biochemical characteristics of a particular virus which will determine the purification program. Specific details can be found in descriptions of a particular virus. Generally, plant viruses are excellent antigens and the majority elicit relatively high titers ranging from 1:250 to 1:2000 in gel precipitin tests. The antigenicity of unstable viruses can be improved by crosslinking amino groups on the capsid surface using formaldehyde or glutaraldehyde treatment (Francki and Habil, 1972; Rybicki and von Wechmar, 1981; von Wechmar and van Regenmortel, 1968).

Serological techniques in common use for plant virus detection can be divided into two major categories: precipitin techniques and amplified antibody binding techniques.

PRECIPITIN TECHNIQUES

Tube precipitin test. This is a classic, simple, and quick test that will detect viral antigens to a lower limit of 0.01 mg/ml in purified preparations or in clarified crude plant sap. It is useful as a diagnostic test and to determine relative concentrations of virus in clarified crude plant extracts. Its reliability for relationship studies, however, is surpassed by ELISA, ISEM, IEB, and radioimmunoassay (RIA).

The precipitin reaction is based on the interaction of antibody and antigen molecules forming a complex
when suspended in a liquid. Depending on the relative concentration of the two reactants, precipitation may occur. For elongated viruses the precipitate is flocculant and for small isosahedral viruses it is granular. Since precipitation will only occur when reactants are present in optimal proportions, it is advisable to test a series of ten-fold dilutions of clarified sap against a series of two-fold dilutions of antiserum prepared in saline.

Clarified crude plant sap may be prepared as follows. Leaves are homogenized in a suitable buffer containing 10-20% chloroform, followed by low speed centrifugation and incubation of the supernatant fluid at 37°C for 30 min. Precipitated host protein is sedimented by low speed centrifugation. For details of the test procedure and interpretation, see Matthews (1957).

Disadvantages of this test are that large quantities of antigen are required, mixtures of antigens will go undetected, and non-specific precipitations of host components may interfere.

**Gel diffusion. Double diffusion in two dimensions (Ouchterlony method).** Details for setting up gel-diffusion tests can be found in van Regenmortel (1966) and Bercks et al. (1972). It is ideal as a diagnostic test for smaller, particularly isometric viruses and viral proteins, for relationship studies, and to detect different antigenic components. Limits of resolution are in the region of 0.01 mg/ml for isometric viruses and 25μg/ml for viral proteins. Accurate titrations of rod and elongated viruses are possible due to difficulties in diffusing through the agar medium. These difficulties may be partially overcome by degrading virions and/or incorporating detergents in the agar (Hamilton, 1965; Purcifull and Batchelor, 1977; Shepard et al., 1974; Slack and Shepherd, 1975). BSMV will diffuse readily into 0.7% physiologically buffered saline, pH 7.0 (PBS)-buffered agar containing 0.05% Leonil-SA without prior treatment of the virus sample (Fig. 1). This application was successfully in detecting mixtures of seed transmitted brome mosaic virus (BMV) and BSMV in gramineous plants (Rybicki and von Wechmar and von Regenmortel, 1981).

This test is ideally suited for antigen relationship studies between virus strains and groups of viruses. Partial fusion or crossing of precipitin lines indicates the lack of identical antigenic determinants, whereas complete fusion will indicate identity (van Regenmortel, 1966). For relationship studies the reagents should be tested in optimal proportions to avoid artifact reactions.

The buffer incorporated in the agar medium also plays an important role in the stability of the viral antigen. Although most antibody-antigen precipitation reactions are performed in physiologically buffered saline, pH 7, these conditions do not favor all viral or viral-protein antigens. For BMV and BVM-protein, for instance, it can be shown that the choice of suitable ionic conditions inside the agar gel is as critical as the selection of the buffer used for initially suspending the different preparations contained in the wells. Greater stability was obtained with cacodylate-saline-agar than with phosphate agar (von Wechmar and von Regenmortel, 1968). Changes of pH are also known to affect capsid swelling of bromoviruses which in turn may alter serological relationships. In a relationship study of the bromoviruses (Rybicki and von Wechmar, 1981), it was shown that capsid swelling due to changes of pH altered the antigenicity of both BMV and cowpea chlorotic mottle virus; coat proteins were shown to be related at both pH 6 and pH 7, but the intact viruses appeared to be related only when swollen at pH 7.

Advantages of this test are the simple equipment it requires, the visual presentation of reactions and relatively easy interpretation of results, the possibility of adjusting ionic and pH conditions for different antigens, detection of more than one antigen in a preparation, and the use of crude plant sap or extracts. Disadvantages of the Ouchterlony test are limited sensitivity and the relatively large quantities of reagents required.

**“AMPLIFIED” IMMUNE TECHNIQUES**

Over the years a large number of techniques have evolved which employ some means of amplification of the antibody-antigen reaction to make it more easily detectable. Many of these have been applied, at one time or another, for the detection and characterization of plant viruses (van Regenmortel, 1981). The most popular and applicable of these techniques are briefly described as follows.

**Immunosorbent Electron Microscopy (ISEM).** The simplest serological technique involving the electron microscope is visualization of “clumped” antibody-virus aggregates after treatment of sap with specific antibody. The method has been applied in a variety of situations (van Regenmortel, 1981); its specific applications include virus detection, establishing strain relationships, and identifying antigenic determinants on characteristic virion capsid features. One obvious drawback of clumping is that it is similar to standard precipitin assays in that an optimal proportions point needs to be established for each virus-antiserum combination.

A solid-phase assay that is perhaps more applicable is the “trapping” technique originally described by Derrick (1973), termed serologically specific electron microscopy (SSEM), and since improved by several...
Fig. 2. Immunosorbent electron microscopy. (a) Brome mosaic virus (BMV) particles isolated from plants infected with viruliferous uredospores. Carbon-coated grids were coated with a 1:500 dilution of anti-BMV serum to "trap" the virus particles. (b) Barley stripe mosaic virus (BSMV) trapped and decorated with anti-BSMV-serum at a dilution of 1:500. The virus-antibody complexes were stained with phosphotungstate. Scale bar represents 200 nm.
other researchers. The technique, referred to herein as ISEM, relies on the specific attachment of virions in suspension to antibodies adsorbed non-specifically onto a grid and subsequent assay by counting “trapped” negatively stained virions (Fig. 2a). The technique can be extremely sensitive since as little as 10 ng virus/ml can be detected (Derrick, 1973), and may be made even more sensitive by pre-coating grids with staphylococcal protein A to increase the number of antibodies for trapping (Shukla and Gough, 1979). Trapping techniques are useful both for detection of specific viruses (spherical or elongated) and for determining strain relationships (Nicolaef and van Regenmortel, 1980). Again, a problem with this technique is that optimal antibody concentrations must be established for each different antibody-antigen pair to be tested (van Regenmortel, 1981).

A useful follow-up to the trapping technique is “decoration” of trapped virions with a second antibody (Fig. 2b). This serves both to enhance the visibility of the virions and as a check on the specificity of trapping (Milne and Lusioii, 1977). The method may also be used to check antigenic relatedness among strains of viruses by decorating with antibodies to the strains. Another important use is in the localization of specific antigenic sites on capsid surfaces (van Regenmortel, 1981).

There are two major disadvantages inherent in any ISEM technique. The first is that relatively few laboratories outside of the main centers either possess electron

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**Fig. 3. Flow diagram of different applications of enzyme-linked immunosorbent assay (ELISA) for plant viruses.**
microscopes or have technicians skilled in these quite complicated techniques. The second is that serological EM techniques are fine for detecting virions, but clumping and trapping may both be severely inhibited by an excess of free coat protein, which may be present in infected sap. These antigens are effectively too small to visualize, so false negative results may be obtained in the presence of virus (R. G. Milne, personal communication).

Enzyme-linked immunosorbent assays (ELISA). Solid-phase microplate ELISA is now well established in the field of plant virus serology) Torrance and Jones, 1981; van Regenmortel, 1981). All such assays depend upon a colorimetric assay of the amount of enzyme-labeled "detecting protein" bound either directly or indirectly to an immobilized antigen. The three techniques most applicable to plant virology are described.

Double antibody "sandwich" ELISA. The technique described by Clark and Adams (1977) and by Koenig (1978) has found wide application as a means of plant virus detection. The method allows specific detection of virus at concentrations as low as 1 ng/ml by "trapping" of virions with specific antibodies adsorbed to a surface and detecting their presence by means of binding enzyme-conjugated antibody specific to the virions and testing for the presence of these bound enzyme-conjugated antibodies with an appropriate substrate (Figs. 3, 4a, and 4b). The main drawbacks of the technique are that it is extremely strain-specific (Koenig, 1978; Rochow and Carmichael, 1979; Rybicki and von Wechmar, 1981) and that a different virus-specific conjugate is needed for each virus to be assayed. Advantages are that it can be used for either spherical or elongated viruses (van Regenmortel, 1981) and that free protein as well as virus is easily detectable (Rybicki and Coyne, 1983).

Indirect ELISA. This simple technique consists of immuno-enzymatic detection of antibodies bound to immobilized antigen (Voller and Bidwell, 1977) (Fig. 3). It has been applied with great success to the detection of distant serological relationships among purified bromoviruses and their coat proteins, after initial attempts by immunoprecipitin techniques had proved unsuccessful (Rybicki and von Wechmar, 1981). An advantage of this method is that only one enzyme conjugate is needed for any assay using antibodies from a particular animal species. For example, we have used the same goat-anti-rabbit-alkaline phosphatase conjugate in assays for tobamoviruses, bromoviruses, tymoviruses, haemocyanins, and bacterial proteases (Rybicki, unpublished). Two disadvantages of the technique are: a) it is not useful as a means of virus detection, because of the inhibition of virus adsorption to the solid phase by competing contaminants in plant sap (Rybicki, 1979), and b) not all pure viruses adsorb equally well to microtiter tray surfaces (Koenig, 1981; Rybicki, 1979).

"Sandwich indirect" ELISA. This procedure has become popular only recently (van Regenmortel and Burckard, 1980). It entails trapping viruses onto a solid phase with specific antibodies as with sandwich ELISA, "probing" for antigen with virus-specific antibodies, and detecting the bound second antibody by antiglobulin-enzyme conjugate as in indirect ELISA (Fig. 3). An important feature of the technique is that virus-specific antibodies from two different animal species

![Image](image_url)

**Fig. 4.** Sandwich enzyme-linked immunosorbent assay (ELISA) for the sensitive differentiation of viral antigens. (a) Differentiation of natural strains or mutants of bromo mosaic virus (BMV). Test conducted at pH 6.0 to maintain virions in compact configuration for stability. BMV-G9: South African isolate; BMV-type: ATCC P47; BMV-#2 and #4: electrophoretic variants of BMV-type (Lane and Kaesberg, 1971); WCuMV: Wild cucumber mosaic virus, an unrelated tymovirus. (b) Differentiation of different morphological and chemically altered forms of BMV. Test conducted at pH 6. BMV-F pH 7.4: BMV formalinized with 2% HCHO (von Wechmar and van Regenmortel, 1968) at pH 7.4, i.e., stabilized in swollen configuration; BMV-F pH 6 as above but stabilized in the compact configuration. Starting antigen concentrations were 5 mg/ml. The test illustrates: a) the effect of swelling on the antigenicity of the virus (compare BMV-F pH 6 and pH 7.4), and b) the effect of chemical modification on BMV antigenicity (compare native BMV and BMV-F pH 6).
must be used: one for trapping and one for probing. For instance, if chicken IgY antibodies are used to coat a microtiter tray, then the second or probing antibody could be rabbit-derived and the detecting antibody conjugate could be goat-derived (e.g., goat-anti-rabbit IgG (GAR)). We have also used rabbit coating antibody, chicken IgY probing antibody, and rabbit-anti-chicken IgY (RAC) detecting conjugate (Rybicki, unpublished). The technique is valuable as a general means of virus detection, given the (unlikely) availability of virus-specific antibodies from two animal species for each virus to be detected. Another, possibly more useful, variant of the technique uses F(ab')2 IgG fragments to coat the solid phase, the same whole antibody to probe for antigen, and Fc-specific anti-globulin conjugate to detect whole antibody (Barbara and Clark, 1982). F(ab')2 IgG fragments are IgG antibodies from which the entire Fc portion, or heavy-chain dimer "handle" of the antibody, has been removed by pepsin cleavage. "Fc-specific anti-globulin conjugate" (which may be either an anti-IgG Fc antisera or the naturally anti-Fc Staphylococcus aureus protein A) will then only bind to the probing antibody and not the coating F(ab')2. Both variants are very useful for the detection of viruses, as they combine the advantages of sandwich ELISA (i.e., specific "trapping" of antigen) with those of simple indirect ELISA (i.e., only one enzyme-labeled conjugate is needed for all assays). The various authors claim that the technique is far less strain-specific than the double antibody-sandwich ELISA, and consequently can be used to detect heterologous related strains with antisera to one virus. However, personal experience has shown that indirect ELISA is more useful for detecting strain relationships among purified tobamoviruses, probably because only one antigen-specific selection occurs in the simpler assay (Rybicki, unpublished).

Radioimmunoassay (RIA). RIA is mainly a feature of animal, human, and insect virology and has seldom been applied to the detection of plant viruses (van Regenmortel, 1981). A relatively simple procedure, very similar to microplate ELISA techniques, has been described for plant virus detection (Ball, 1973). Exactly the same protocols as used in ELISA can be applied to RIA, as the radioisotope label serves exactly the same function as the enzyme label in ELISA. However, the relatively slight increase (if any) in detection efficiency (Schetters et al., 1981) offset in terms of day-to-day use by the need for far more rigorous safety precautions in laboratories that may not be equipped to handle radiochemicals.

Immunofluorescence (IF). Antibodies labeled with fluorescein isothiocyanate (FITC) have long been used for both direct and indirect localization of cell-associated antigens, because of the possibility of combining IF staining of cells or sectioned tissue with ultra-violet illumination in a light microscope. The method is largely used in routine clinical virology with human and animal cell cultures. However, with the advent of plant cell tissue and protoplast culture techniques, the possibility exists for using it to detect plant virus in individual cells (van Regenmortel, 1981). The method is not really suited for detection of viruses in sap; however, it has a valuable place in the localization of viruses in sectioned or cultured plant tissue. We have recently used FITC-labeled antibodies for the successful direct and indirect detection of in vivo-acquired BMV on the surface of wheat stemrust (Puccinia graminis Pers. f. sp. tritici) uresdospores (Erasmus, 1982). This was performed as part of an investigation into rust spore transmission of BMV in small grains, a subject that should interest maize virus researchers (Erasmus, 1982; von Wechmar, 1980) (Figs. 5a, 5b, and 5c).

"Western Blotting" techniques. An important recent development in plant virology has been the application of immuno-Western blotting to the detection of plant virus coat proteins. O'Donnell et al. (1982) have described a protocol for the electrophoretic transfer of SDS-PAGE-fractionated plant sap onto activated paper, and the subsequent detection of specific plant virus proteins at high efficiency by indirect RIA using rabbit antibodies and 125I-labeled protein A. The technique enables detection and characterization of viral protein on the basis of both molecular weight and serological specificity, thereby making it a valuable analytical tool. O'Donnell et al. (1982) have applied the technique to the detection of tobacco mosaic virus, four different sugarcane mosaic virus (SCMV) strains, and five different isolates of potato leafroll virus, a luteovirus akin to barley yellow dwarf virus.

We have applied a similar immuno-enzymatic technique (Towbin et al., 1979) for the same purpose for detecting tobamovirus, bromovirus, and SCMV coat proteins electroblotted onto nitrocellulose paper by means of indirect enzyme-immunoassay (EIA) using virus-specific rabbit antibodies and horseradish peroxidase-labeled goat-anti-rabbit globulins (Rybicki and von Wechmar, 1982). The latter technique can detect as little as 1 ng of protein per gel track, and because it is an indirect EIA, related virus strains may be detected with a single antisera. The EIA technique is probably more easily applied than RIA for routine use. However, the technique is both less sensitive and less quantifiable and thus is not a true "assay". Both techniques may be used in routine virus detection, although their value probably lies more in analysis of antigenic mixtures, the identification of antibody specificities, and serving as checks on routine ISEM or ELISA detection of viruses.

Our investigation of IEB methodology has been of great value to recent work on: a) the investigation of serological relationships of virus strains and groups, b) the detection of unstable viruses in plant extracts, and c) the analysis of antiserum specificity (purity) after long immunization schedules with supposedly pure viruses. The latter point is especially important, as it has afforded indirect proof of seed contamination in commercial small grains. This proof is based on rabbits acting as "biological amplifiers" in the instance where repeated injection of small amounts of contaminant virus in the presence of large amounts of "pure" virus leads to production of anti-contaminant antibodies,
Fig. 5. Stem rust (Puccinia graminis tritici) uredospore transmission of brome mosaic virus (BMV) (Erasmus, 1982; von Wechmar, 1980). (a) Procedural steps for indirect fluorescent staining technique used to indicate the presence of virus on the surface of the infected tissue: (1) Virus on tissue surface. (2) Binding of anti-virus antibodies to virus particles. (3) Coupling of fluorescein isothiocyanate (FITC)-labeled goat-anti-rabbit bodies to bound anti-virus rabbit antibodies. (b) Using the indirect fluorescent staining procedure, strong fluorescence was obtained with BMV-antibodies + FITC-labeled goat-anti-rabbit bodies on viruliferous uredospores (taken from pustules of BMV-infected wheat). (c) No fluorescence was obtained with spores taken from pustules on non-virus infected plants. Test conditions the same as in (a, above.
Fig. 6. An electroblot illustrating the multiplicity of antibodies present in an antiserum prepared to "pure" barley stripe mosaic virus (BSMV). Tracks: 1. 'Uninfected' Goudveld maize with dead growth-points, processed with phosphate buffer to extract seed-transmitted virus; 2 and 3. Field-collected diseased maize leaf processed in phosphate buffer, pH 7, and sodium acetate buffer, pH 5, respectively; 4. Purified preparation of sugarcane mosaic virus (SCMV) (Winburg-isolate); 5. Purified brome mosaic virus (BMV); 6. Purified BSMV. This electroblot illustrates that the 'uninfected' Goudveld maize plants contained proteins with a molecular weight (MW) similar to the purified isolate of SCMV-Winburg, and samples 2 and 3 contained proteins with a MW similar to BMV. The BSMV used to immunize the rabbit was multiplied in barley and wheat infected with low levels of BMV and SCMV (unknown at the time and extremely difficult to detect). The sensitive rabbit immune system acts as a "biological amplifier".

even though the heterologous contaminant may be effectively undetectable in the inoculum. By analysis of antiserum to "pure" BMV, BSMV, and SCMV, we have demonstrated seed transmission of all three viruses in commercially grown small grain cultivars (Rybicki and von Wechmar, unpublished) (Fig. 6). One very useful feature of IEB is that several viruses with different known coat protein molecular weights may be probed for simultaneously by mixing several virus antisera together for use on the same blot.

A possible disadvantage with IEB techniques is that viruses are detected in the form of their dissociated SDS-denatured coat proteins and that virus-specific antisera may not recognize the subunits. However, this could be circumvented by use of antisera to native or SDS-unfolded subunits (Purcifull et al., 1981). An inherent advantage of IEB lies in the observation that viral coat proteins are often serologically more closely related than the intact virions (Shepard et al., 1974), meaning that the range of viruses detectable with a single antiserum may be wider in this technique than in most others.

GENERAL OBSERVATIONS

The choice and the successful application of any serological method to a particular virus problem requires a thorough knowledge of: a) the biophysical and biochemical nature of the virus, b) the properties of antibodies and antisera, and c) the shortcomings and advantages of the technique. In addition, the choice of any of the more sophisticated "amplified" serological techniques for a particular purpose should depend upon whether the method is to be used for detection of virus and/or virus protein, for studying antigenic rela-
tionships among strains, or for basic immunochemical research.

Perhaps the best technique for routine detection of specific viruses is sandwich ELISA because of its sensitivity. Its specificity is also useful for the differentiation of very closely related viral isolates (Rybicki, 1979; Rybicki and von Wechmar, 1981). For general purpose virus detection, the F(ab')2 sandwich-indirect procedure appears very attractive. ISEM techniques have also been widely applied in routine diagnostic work, subject to the limitation of having an EM on hand. IEB techniques are very well suited to the detection and identification of "difficult" viruses which may be hard to purify or extract from plant tissues. In this technique, plant tissue may be efficiently solubilized in SDS-mercaptoethanol buffer for SDS-PAGE, and antisera to crude extracts may be efficiently absorbed with healthy plant material by dilution of whole antiserum in clarified sap just before use (Rybicki and von Wechmar, 1982). All of the above techniques are as effective for elongated as for spherical viruses, and ELISA and IEB are also efficient at detecting free coat protein subunits, unlike ISEM. Serological studies of group-specificity for elongated as for spherical viruses, and ELISA and IEB are perhaps best performed at university or strain-relationships are perhaps best performed using indirect ELISA if pure virus is available, or ISEM decoration and IEB if viruses are impure or in plant tissue extracts. Immunochemical studies on plant viruses and their proteins may be effectively performed using ELISA and RIA techniques (van Regenmortel, 1978; 1981).

The choice of technique and approach will obviously differ according to both individual preference and the facilities on hand. The simple precipitin-based assays are applicable almost anywhere, whereas the various "amplified" techniques require more sophisticated equipment and support staff. However, ELISA tests are extremely cost-effective as well as being relatively easy to use on a routine basis, and IEB with peroxidase-antibody conjugates may be performed in any laboratory equipped for analytical electrophoresis. The dissemination of such techniques to workers in even isolated field laboratories is vital for a broad-based understanding of virus epidemiology.

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Sorghum Cultivars Differentiating Sugarcane Mosaic and Maize Dwarf Mosaic Virus Strains

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ABSTRACT


In this investigation with Atlas and Rio sorghum (Sorghum bicolor) as standards, sorghum cvs. OKY8, SA8735, Tx3197, NM31, Martin, SC0097, Q7589, and QL11 developed distinctive symptoms which qualify them as differential hosts to distinguish sugarcane mosaic virus (SCMV) and maize dwarf mosaic virus (MDMV) strains. MDMV strain A, SCMV johnsongrass strain (Jg), and one isolate of a virus causing mosaic on maize (Zea mays) in Yugoslavia (MDMV-YU) were identical and should be considered one strain. MDMV strain B and SCMV strain E behaved identically; henceforth these two strains should be considered as one strain. The other SCMV strains (SCMV-A, -B, -D, -H, and -I) differed enough to warrant their separate strain designations. A set of sorghum cultivars is proposed for differentiation of MDMV and SCMV strains.

MATERIALS AND METHODS

During these investigations the following sorghum cultivars were studied: OKY8, SA8735, Tx3197, Atlas, NM31, Rio, Martin, SC0097, Q7539, and QL11. Seed samples of these sorghum cultivars were kindly supplied by D. M. Persley (Plant Pathology Branch, Department of Primary Industries, Meires Road, Indooroopilly, Q. 4068, Australia). Sorghum seeds were planted in 15 x 10 cm pots filled with soil, and these pots were maintained in the greenhouse. Seedlings were inoculated mechanically at the three to four leaf stage.

The following strains of SCMV and MDMV were studied: SCMV-A, -B, -D, -E, -H, -I, and -Jg; and MDMV-A (Iowa 65-74), -B (Iowa 66-188), and -YU (an isolate causing mosaic on maize in Yugoslavia); all strains had been used in previous experiments (Tosic, 1974; Tosic and Ford, 1972; Tosic and Malak, 1973). Virus isolates were maintained in sweet corn seedlings under greenhouse conditions and transferred by mechanical inoculations periodically. Inocula were prepared by homogenizing young corn leaves showing mosaic symptoms 2 wk after inoculation. Virus inocula with added carborundum powder were used to rub-inoculate sorghum seedlings. Control plants were rubbed with water only. Each virus isolate and the control treatment were applied to seedlings of each tested sorghum cultivar growing in separate pots. After inocu-
TABLE 1. Reactions of some sorghum cultivars to maize dwarf mosaic virus (MDMV) and sugarcane mosaic virus (SCMV) strains.

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<tr>
<th>Sorghum cv.</th>
<th>MDMV and SCMV strains</th>
<th>Symptoms</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atlas</td>
<td>MDMV-A, -Yu; SCMV -Jg</td>
<td>L:rLN a</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>S:M, rN</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MDMV-B; SCMV-E</td>
<td>L:LN ty</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SCMV-A, -B, -D</td>
<td>L:LN Nst</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SCMV-H, -I</td>
<td>L:LN</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>S:N</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>N appears sooner with SCMV-I</td>
</tr>
<tr>
<td>Tx3197</td>
<td>MDMV-A, -Yu; SCMV-Jg</td>
<td>L:rLN N</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>S:M, N</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MDMV-B; SCMV-E</td>
<td>L:LN</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SCMV-A, -B, -D</td>
<td>S:rM, N</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SCMV-H, -I</td>
<td>L:LN</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>S:N</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>N usually starts terminally on shoots</td>
</tr>
<tr>
<td>Rio</td>
<td>MDMV-A, -Yu; SCMV</td>
<td>L:rLN N</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-A, -B, -D, -H, -I, Jg</td>
<td>S:M, N</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MDMV-B; SCMV-E</td>
<td>L:LN</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SCMV-A, -B, -D, -H, -I</td>
<td>S:LN</td>
<td></td>
</tr>
<tr>
<td>SA8735</td>
<td>MDMV-A</td>
<td>S:M, rNSp</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MDMV-Yu; SCMV-Jg</td>
<td>L:LN</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MDMV-B; SCMV-E</td>
<td>L:LN</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SCMV-A, -B, -D, -H, -I</td>
<td>S:LN</td>
<td></td>
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<tr>
<td>OKY8</td>
<td>MDMV-A, -Yu; SCMV</td>
<td>S:M</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-H, -I, -Jg</td>
<td>S:CSk</td>
<td></td>
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<tr>
<td></td>
<td>SCMV-B</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>MDMV-B; SCMV-A, -D, -E</td>
<td></td>
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<tr>
<td>NM31</td>
<td>MDMV-A, -Yu; SCMV</td>
<td>L:LN</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-H, -Jg</td>
<td>S:M, N</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MDMV-B; SCMV-A, -B, -D, -E</td>
<td>L:LN</td>
<td></td>
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<tr>
<td></td>
<td>SCMV-I</td>
<td></td>
<td></td>
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<tr>
<td>Martin</td>
<td>MDMV-A; SCMV-B, -I</td>
<td>S:M</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MDMV-Yu; SCMV-Jg</td>
<td>S:M, rN</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MDMV-B; SCMV-E</td>
<td>S:rM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SCMV-H, -I</td>
<td>L:rLN</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SCMV-A</td>
<td>S:M</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M present on up to 10% of inoculated plants</td>
</tr>
<tr>
<td>Q7539</td>
<td>MDMV-A, -Yu; SCMV-Jg</td>
<td>S:M</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SCMV-I</td>
<td>S:rM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MDMV-B; SCMV-A, -B, -D, -E, -H</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SC0097</td>
<td>S:M</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MDMV-A, -Yu; SCMV-Jg</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>MDMV-B; SCMV-A, -B, -D, -E, -H, -I</td>
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<tr>
<td></td>
<td>QL11</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MDMV-A, -B, -Yu; SCMV-A, -B, -D, -E, -H, -I, -Jg</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\*Strain designations are explained in text.
\*Abbreviations: L = local symptoms on inoculated leaves; S = systemic symptoms on new leaves; r = rare; a = atypical, usually tiny; ty = typical; LN = local necrosis; M = mosaic; N = necrosis; NS = necrotic stripes; NSp = necrotic spots; CSk = chlorotic streaks; CSp = chlorotic spots; CSt = chlorotic stripes; and - = no symptoms.
ulation, sorghum seedlings were kept in a greenhouse and observed for symptoms during the following 1 mo.

All experiments were repeated three times, one each in the spring and fall of 1981 and the third in the spring of 1982.

RESULTS AND DISCUSSION

Primary symptoms observed, recognizing that some minor differences occurred probably due to different environmental conditions, are presented in Table 1. Sorghum cv. Atlas reacted primarily as reported (Dean, 1970; Gordon and Williams, 1970; Tosic and Ford, 1972; Tosic and Malak, 1973), although some minor differences exist between those results and our work. Some researchers reported mosaic on Atlas caused by MDMV-B, SCMV-H or -I (Tosic and Ford, 1972; Tosic and Malak, 1973), whereas others did not report observing necrotic local lesions on the leaves of Atlas inoculated with SCMV-A, -B, and -D (Dean, 1970), nor with MDMV-A and SCMV-A and -B (Gordon and Williams, 1970). Local lesions on Atlas leaves caused by MDMV-A and -YU and SCMV-A, -B, -D, -H, -I, and -Jg are tiny, elliptical, and usually with a pale center (Tosic and Ford, 1972), which differ from those produced by MDMV-B or SCMV-E and for that reason they were not always recognized. On the basis of reactions on sorghum cvs. Atlas (Fig. 1) and Tx3197, which react similarly to MDMV and SCMV strains, there are four groups of virus strains. Group one includes MDMV-B and SCMV-E which cause identical local lesions. Group two includes SCMV-H and -I which caused extensive necrosis of infected plants; although not easy to differentiate, SCMV-I caused somewhat faster necrosis than SCMV-H. Group three contains MDMV-A and -YU and SCMV-Jg which caused atypical local lesions and mosaic and rare necrosis on new leaves. Group four includes SCMV-A, -B, and -D which caused mosaic and pronounced necrosis on new leaves.

The reaction of sorghum cv. Rio to SCMV and MDMV strains has not been reported. We found no obvious differences in symptoms on Rio caused by MDMV-A and -YU and SCMV-A, -B, -D, -H, -I, and -Jg, all of which rarely induced local lesions on inoculated leaves and necrosis on new leaves but rather all induced a systemic mosaic. Reportedly Rio reacts to SCMV-A, -B, -D, and -H with mosaic and to SCMV-I with mosaic, yellowing, and necrosis (Tippett and Abbott, 1968). This disagreement with our findings may be due to the different environmental conditions for experiments or to differences in the origin of Rio seed. On the basis of virus reactions on Rio, the strains form two groups; one group includes MDMV-B and SCMV-E and the other all other strains.

Sorghum cv. SA8735 (Fig. 2) differentiated these viruses into three groups: a) MDMV-A and -YU and SCMV-Jg; b) MDMV-B and SCMV-E; and c) SCMV-A, -B, -D, -H, and -I. Likewise, sorghum cv. OKY8 (Fig. 3) differentiated these strains into three groups: a) MDMV-A and -YU and SCMV-H, -I, and -Jg which caused only mosaic; b) SCMV-B which caused chlorotic streaks on new leaves; and c) MDMV-B and SCMV-A, -D, and -E which were not infective.
Fig. 3. The reaction of sorghum cv. OKY8 to systemic infection by maize dwarf mosaic virus strain A (MDMV-A) and sugarcane mosaic virus strains E (SCMV-E), H (SCMV-H), and I (SCMV-I).

Fig. 4. The reaction of sorghum cv. Q7539 to systemic infection by maize dwarf mosaic virus strain A (MDMV-A) and sugarcane mosaic virus strains I (SCMV-I) and johnsongrass (SCMV-Jg).

TABLE 2. Sorghum cultivars for differentiating maize dwarf mosaic virus (MDMV) and sugarcane mosaic virus (SCMV) strains.

<table>
<thead>
<tr>
<th>Sorghum cv.</th>
<th>MDMV-A, -Yu; SCMV-Jg</th>
<th>MDMV-B; SCMV-E</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>H</th>
<th>I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atlas</td>
<td>M;N</td>
<td>LN</td>
<td>M;N</td>
<td>M;N</td>
<td>M;N</td>
<td>N</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>Rio</td>
<td>M;rN</td>
<td>CSk, rNSp</td>
<td>M;rN</td>
<td>M;rN</td>
<td>M;rN</td>
<td>M;rN</td>
<td>M;rN</td>
<td>M;rN</td>
</tr>
<tr>
<td>SA3735</td>
<td>M;rN</td>
<td>LN</td>
<td>CST</td>
<td>CST;N</td>
<td>CST;N</td>
<td>CST;N</td>
<td>CST;N</td>
<td>CST;N</td>
</tr>
<tr>
<td>OKY8</td>
<td>M</td>
<td>-</td>
<td>-</td>
<td>CST</td>
<td>-</td>
<td>M</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td>NM31</td>
<td>M;rN</td>
<td>LN</td>
<td>LN</td>
<td>LN</td>
<td>LN</td>
<td>M;rN</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>Martin</td>
<td>M</td>
<td>rM</td>
<td>-</td>
<td>M</td>
<td>M</td>
<td>M;rN</td>
<td>M;rN</td>
<td>M;rN</td>
</tr>
<tr>
<td>Q7539</td>
<td>M</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>rM</td>
</tr>
<tr>
<td>SC0097</td>
<td>M</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*a Strain designations are explained in text.
*b Abbreviations are: M = mosaic (new leaves); r = rare; N = necrosis (new leaves); LN = local necrosis (inoculated leaves); CSk = chlorotic streaks (new leaves); NSp = necrotic spots (new leaves); CST = chlorotic stripes; CSP = chlorotic spots; and - = no symptoms.
Sorghum cv. NM31 also differentiated strains into three groups: a) MDMV-A and -YU and SCMV-H and -Jg which caused mosaic on new leaves followed by necrosis; b) MDMV-B and SCMV-A, -B, -D, and -E which caused only local infection; and c) SCMV-I which caused only necrosis. Likewise, sorghum cv. Q7539 (Fig. 4) differentiated these strains into three groups: a) MDMV-A and -YU and SCMV-Jg which caused mosaic; b) SCMV-I which caused rare mosaic; and c) all other strains which were not infective. Similar results were obtained with sorghum cv. SC0097, except that SCMV-I did not infect this cultivar.

Sorghum cv. Martin showed more diverse reactions to virus infection. MDMV-A and SCMV-B and -D caused only mosaic on new leaves, whereas MDMV-YU and SCMV-Jg caused mosaic and rare necrosis. MDMV-B and SCMV-E caused only rare mosaic on new leaves, and SCMV-H and -I caused rare local lesions followed by mosaic and rare necrosis on new leaves. Finally, SCMV-A was not infective.

We believe that MDMV-A and -YU and SCMV-Jg belong to the same subgroup and should be considered as the same strain as suggested before (Bond and Pirone, 1971; Tosic, 1974; Tosic and Ford, 1974; Tosic and Malak, 1973). Our work showed that MDMV-B is closer to other SCMV strains than to SCMV-Jg or MDMV-A as suggested before (Tosic and Ford, 1974). In the present study MDMV-B and SCMV-E behaved identically on all sorghum cultivars tested and should be considered as one strain. The other strains, SCMV-A, -B, -D, -H, and -I, differed markedly on the sorghum cultivars tested and accordingly they are considered as different strains.

Similarities and differences among sorghum cultivars reported here allow us to propose a set of sorghum cultivars to differentiate MDMV and SCMV strains (Table 2). This set may be combined with the host set for separating SCMV and MDMV strains proposed earlier (Tosic, 1974).

**LITERATURE CITED**


Potential for International Spread of Viruses

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ABSTRACT


Plant virus dispersal mechanisms are many and varied. Dispersal systems utilizing arthropod vectors are difficult to confine, although the insect carriers rarely move over great distances. Systems utilizing seed transmission or vegetatively propagated host tissue are more easily controlled by exclusion practices but are also very efficient mechanisms of dispersal. Any virus-host-vector system in which man plays some transport role removes geographical and geophysical barriers normally in operation in stable ecosystems. Factors affecting plant virus dispersal and establishment and general recommendations for controlling spread are discussed.

Organisms have limited intrinsic abilities to move within and between ecosystems. For a species to spread successfully into a new area, it must meet three major conditions: a) it must possess the physiological potential to survive and reproduce in the new area, b) it must have the ecological opportunity to become established in the new habitat, and c) it must have physical access to the new range (Keeton, 1972).

A species (virus) must be minimally preadapted for survival in a new area, i.e., there must exist a susceptible host in which it can multiply and survive and a means to spread within the new environment, viz., a vector. Furthermore, the incoming virus must be able to compete if other established viruses already occupy its intended niche. Finally, the virus must have access to the new area. Unfortunately, in many cases new viruses are more virulent and cause more severe disease outbreaks than do established viruses.

The above limitations to spread apply in the absence of man's intervention. When man becomes involved, intentionally or not, the potential for movement changes drastically.

Since ancient times, organisms have spread passively into new areas on water and wind currents and animal carriers. The recolonization of Krakatoa in the East Indies between 1883 and 1906 is an example of such passive dispersal. Nine months after complete desolation of all land life forms within 25 nautical miles, one spider was found on the island. Three years later the shore was populated by plants with seeds resistant to salt water and ferns with aerial spores. After 13 yr the island was covered with vegetation, and within 3 yr the island was densely covered with plants and populated by 283 species of animals (Keeton, 1972).

Beginning with the 15th century-worldwide explorations, most geographical barriers to the spread of plants, animals, and pathogens were removed. Columbuses brought the smallpox and measles viruses to the New World and took the syphilis spirochaete, corn (Zea mays L.), and potatoes (Solanum tuberosum L.) back to Europe. Forced migration of black slaves from Africa to West Indian sugar plantations introduced malarial protozoans and yellow fever virus to the Western Hemisphere. Movement of sugarcane (Saccharum officinarum L.) cuttings from country to country has played a significant role in the spread of sugarcane mosaic and other viruses around the world. Americans have lost the mighty American chestnut (Castanea dentata [Marsh.] Borkh.) and may soon lose the American elm (Ulmus americana L.) to diseases introduced inadvertently in the last century (Kimball, 1974).

Some species have become important pests after intentional introduction for other purposes. Johnsongrass [Sorghum halepense (L.) Pers.] was introduced as a forage crop but has become a major nuisance in most southeastern states. The gypsy moth, Lymantria dispers (L.), introduced into Massachusetts in 1869 in the hope of starting a silk industry, ravaged 12.5 million acres of timber in 1981, mostly in the northeast U.S. The European rabbit (Oryctolagus cuniculus L.) was introduced into Australia for sport in 1859 but, without natural enemies, became too prolific. In 1950, a myxoma virus was introduced into Australia and the rabbit population was reduced to less than 0.5% of its peak (Keeton, 1972). Although not as graphic as the above example, we have evidence for international dispersal of a few plant viruses, i.e., plum pox virus in Europe and citrus tristeza virus in North and South America (Thresh, 1980).

Plant viruses are spread by arthropods (aphids, leafhoppers, planthoppers, whiteflies, thrips, mites, mealybugs, and mandibulates), nematodes, grafting, seed, soil (probably fungi), dodder, and mechanical methods. Viruses traditionally have been classified as "mosaics"
Plant viruses which require insects for spread must have either an introduced insect-carrier, which itself must become established, or become associated with an endemic insect vector. Viruses transmitted non-persistently must rely on some mechanism other than the insect vector for long-distance spread. However, the apparent recent spread of maize dwarf mosaic virus (non-persistently transmitted) over several hundred miles without intermediate virus sources might suggest that other mechanisms for long-distance transmission may be operative in some alate aphid vectors.

Plant viruses are obligate parasites that require some extrinsic mechanism for transfer between living host tissue. Seed-borne viruses have a distinct advantage for extrinsic mechanism for transfer between living host tissue. Seed-borne viruses have a distinct advantage for spread. However, in the 10 yr since the report, none of these plant viruses have become established, although one virus (maize stripe virus) has been identified in Florida.

A strong potential exists for pests, including plant viruses, to enter a country such as the U.S. It has a large land mass, recently developed, with most of the crops and pests introduced. Since Columbus discovered the Western Hemisphere, more than 1200 new insect species have become established in the U.S.; since 1920 an average of nine new insects per year have become established. There are more than 1300 important, exotic organisms which pose threats to the U.S., of which more than 550 are plant pathogens. While quarantine inspection of passenger baggage and agricultural cargoes catches some new arrivals, others inevitably get through. Increasing emphasis on Agricultural Source Inspection and Surveillance Technique (ASIST) is necessary to provide early detection at the site of new outbreaks (McGregor, 1973).

Past history shows that pests, including viruses, are continually introduced into new areas. However, it remains for that virus to become established in the new ecosystem. Several factors play significant roles in the early stages of establishment, the interruption of any of which may prevent final establishment of the virus or alter the course of its development (Table 2).

Continental land masses such as the U.S. or Canada and insular land masses such as Hawaii offer “inviting” food sources for insect vectors and susceptible germplasm for viruses. However, there are many barriers to establishment. The vector of a virus must land in the proper geographic location, find suitable food hosts, and be either parthenogenetic or a fertilized female. Establishment may occur when an endemic vector species is readily available. There also must be susceptible virus hosts on which the insects feed.

Prior to man’s colonization of the Americas, movement of insect species carrying new viruses over great distances was extremely uncommon. Not only were there few long-distance insect carriers, but the germplasm variability of indigenous wild flora and stability of predatory and parasitic fauna made it extremely difficult for new pests to become established.

As the Europeans populated the Americas, they brought with them a variety of new plant and animal

<table>
<thead>
<tr>
<th>TABLE 1. Factors associated with plant virus dispersal into new areas.</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Nature of transmission</td>
</tr>
<tr>
<td>A. Seed transmission</td>
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<tr>
<td>B. Propagative plant parts</td>
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<tr>
<td>C. Persistent vector-virus relationship</td>
</tr>
<tr>
<td>D. Non-persistent vector-virus relationship</td>
</tr>
<tr>
<td>II. Pathways of entrance</td>
</tr>
<tr>
<td>A. Abnormal weather patterns affecting jet streams</td>
</tr>
<tr>
<td>B. Air traffic passengers and cargo</td>
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<tr>
<td>C. Ships—passengers and cargo</td>
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<td>D. Postal service</td>
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<tr>
<td>E. Scientists (research purposes)</td>
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<tr>
<td>F. Military usage (special situations)</td>
</tr>
<tr>
<td>III. Control measures or constraints</td>
</tr>
<tr>
<td>A. Quarantine</td>
</tr>
<tr>
<td>B. Early detection</td>
</tr>
<tr>
<td>C. Retroactive responsibility</td>
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</tbody>
</table>
TABLE 2. Factors involved in the establishment of a plant virus in a new area.

<table>
<thead>
<tr>
<th>Location of entry port</th>
<th>Seasonal effects on entry</th>
<th>Natural biological barriers</th>
<th>Intrinsic factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Coastal vs. inland</td>
<td>A. Winter vs. summer and dry vs. wet season</td>
<td>A. Host resistance to vector</td>
<td>A. Environmental effects on vectors in the first few generations (30-180 days)</td>
</tr>
<tr>
<td>B. Northern vs. southern port.</td>
<td>1. Summer season with overwintering (alternate host available) (+)</td>
<td>B. Predators of vectors</td>
<td>B. Number of vectors introduced</td>
</tr>
<tr>
<td></td>
<td>2. Summer minus alternate hosts (+/−)</td>
<td>C. Parasites of vectors</td>
<td>C. Sex and fertility of vectors</td>
</tr>
<tr>
<td></td>
<td>3. Winter season with alternate hosts available (+/−)</td>
<td></td>
<td>D. Endemic vector available to transmit virus</td>
</tr>
<tr>
<td></td>
<td>4. Winter minus alternate hosts (−)</td>
<td></td>
<td>E. Number of infective propagules</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Seasonal effects on entry</th>
</tr>
</thead>
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<td>1. Summer season with overwintering (alternate host available) (+)</td>
</tr>
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<td>2. Summer minus alternate hosts (+/−)</td>
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<td>B. Predator of vectors</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Intrinsic factors</th>
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</thead>
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<td>A. Environmental effects on vectors in the first few generations (30-180 days)</td>
</tr>
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<tr>
<td>D. Endemic vector available to transmit virus</td>
</tr>
<tr>
<td>E. Number of infective propagules</td>
</tr>
</tbody>
</table>

* (+) = increased potential for establishment; (+/−) = less potential for establishment than (+); (+/−) = less potential than (+/−); and (−) = least potential for successful establishment.

Types, introduced cultural changes, and greatly expanded the production crops with reduced germplasm variability. These actions increased the potential for spread of existing viruses and for the introduction of new viruses.

The mechanism of transmission drastically affects the likelihood of introduction. Obligately insect-transmitted viruses require much different environmental barriers to prevent introduction than do seed-transmitted or host propagatively transmitted viruses. Of more immediate danger are those scientists and tourists who knowingly carry pests into the country through a lack of understanding of the basic dangers involved. Quarantine laws have been enacted by states and countries to exclude seed, nursery stock, improper shipment, etc. These are valid only if adhered to by every citizen.

Some plant virus-vector systems are well suited to introduction and establishment; others are restricted by one or more barriers. There is adequate host germplasm for maize streak virus and its vector, but the vector is not present in the U.S. and it is temperature restricted (Damsteegt, 1980; Damsteegt, 1981). Maize rayado fino virus has been found in the U.S. where it has access to an endemic vector species [Graminella nigrifrons (Forbes)] but lacks an overwintering host (Damsteegt, 1981; Gamez, 1980; Nault and Knoke, 1981). Maize chlorotic mottle virus has been introduced, has susceptible germplasm and endemic vectors, but is mostly limited to river bottom lands and controlled by trap cropping (Uyemoto and Claflin, 1981). Maize stripe virus has been introduced, has available susceptible germplasm, but the vector is restricted by winter temperature (Damsteegt, 1981; Nault and Knoke, 1981). Soybean dwarf virus has susceptible soybean [Glycine max (L.) Merr.] germplasm; susceptible perennial overwintering hosts and endemic vectors are available. However, American biotypes of the aphid vector, Acrithosiphon solani (Kaltenbach), differ in efficiency of transmission, and living, infected plant material or aphids would be required to introduce the virus (Damsteegt, 1983; Tamada, 1975). Rice hoja blanca virus has susceptible hosts available and vectors migrate sporadically into the U.S., but temperature requirements prevent insect establishment (Anonymous, 1960).

What is the potential for international spread of plant viruses? There are no data, no magic crystal balls, no mathematical formulae which can be used to predict answers. Many pests of minor importance in their centers of origin become extremely serious in new areas, while others are very important in endemic areas but fail to become established elsewhere. The combination of rapid worldwide travel, more restrictive germplasm bases in developing countries, and more travelers and joint scientific endeavors increase the opportunities for viruses and/or their vectors to move across geographical barriers into new areas. Perhaps the greatest hope for limiting major calamities is to increase the awareness of the problem in all sectors of society utilizing some graphic examples of past and present successes or failures of organisms to colonize new areas. The American public is aware of the threat of the Mediterranean fruit fly [Ceratitis capitata (Wiedemann)], Dutch elm disease, gypsy moth, Japanese beetle (Popillia japonica Newman), oak wilt, golden nematode [Globodera rostochiensis (Wt.) Muldey and Stone], johnsongrass, screw worm [Cochliomyia hominivorax (Coquerel)], etc. However, too often there is little sense of urgency by the general public because monetary loss is usually indirect and food shortages are non-existent. This, unfortunately, is not the situation in many other countries. New viruses and virus strains will continue to appear and opportunities for dispersal will increase as countries change their cultural practices toward increased yields on a narrow germplasm base. Laboratories such as the USDA Plant Disease Research Laboratory in Frederick, Md., provide some opportunity to compare viruses and viral strains on common germplasm in an attempt to provide solutions to problem infestations before they arise. Stricter quarantine inspection at either the export or import depot and early field detection offer some hope for preventing or controlling major pest problems.

There is a need for closer cooperation between plant pathologists, plant breeders, entomologists, and related scientific disciplines within and between countries to educate and emphasize the importance of exclusion as one avenue of control of plant viruses. Where exclusion is not feasible, early detection, restricted movement, eradication, or other measures can be used to limit movement of pests to new areas.
LITERATURE CITED


Classification and Prediction of Maize Dwarf Mosaic Intensity

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ABSTRACT


Early and total seasons in southern Ohio were classified as 'mild' or 'severe' for the relative intensity of maize dwarf mosaic in exposed maize (Zea mays) trapplants. Thirteen years were analyzed with stepwise discriminant analysis and 100% of the seasons were correctly classified using winter and spring climatic variables. For the early seasons, the following variables were significant predictors of disease classification: a) number of days in January, February, and March with an average temperature less than 0 C; b) average minimum temperature in December, January, and February; and c) number of days with rain in March and April. 'Severe' early seasons were preceded by warmer winters and drier springs than the 'mild' early seasons. For the total seasons, three variables also were significant: a) number of days with rain in March and April; b) total rain in April; and c) total rain in March and April. 'Severe' total seasons were preceded by drier springs than 'mild' seasons. For northern Ohio, where johnsongrass (Sorghum halepense), the overwintering host of most MDMV strains, is not present and disease intensity is always low, years were classified using the discriminant function derived from the southern Ohio data. For the northern Ohio site, three early seasons and six total seasons were classified as 'severe' for maize dwarf mosaic. These results indicate that environmental conditions are often favorable in northern Ohio for maize dwarf mosaic and this disease could become a problem if johnsongrass became established and widespread.

We have been attempting for the past 2 yr to model the components of epidemics of the most common virus disease of maize (Zea mays L.) in the U.S., maize dwarf mosaic (Gordon et al., 1981). The virus, maize dwarf mosaic virus (MDMV), is transmitted by at least 23 aphid species in a nonpersistent manner (Knoke and Louie, 1981). Fifteen of the 23 species have been trapped in Ohio; six of these are prevalent (Madden et al., 1983). Previously we used multiple regression analysis to relate infection of a susceptible maize hybrid to the natural logarithms of numbers of these six aphids (Madden et al., 1983). The significant species varied between locations in Ohio and also among years.

We also have related MDMV infections of maize to distance from a virus source using nonlinear and linear regression models (Knoke et al., 1983; Madden, Knoke, and Louie, unpublished). Since the pattern of virus-infected plants in fields can indicate the type of disease progression, we developed methodology for analyzing this pattern in naturally infected maize fields (Madden et al., 1983). Results indicated that the degree of aggregation of infected plants, i.e., clustering, varied among locations and increased over time (Madden, J. J. Abt, Louie, and Knoke, unpublished).

The objective of the study presented herein was to predict the relative intensity of maize dwarf mosaic by using winter and spring ambient environmental variables. We hypothesized that these environmental conditions influence the survival of johnsongrass [Sorghum halepense (L.) Pers.], the overwintering host of most MDMV strains, and the aphid vectors, as well as the virus. Thus, these conditions partly determine the relative intensity of maize dwarf mosaic during the following season. The multivariate statistical technique of discriminant analysis was used to perform the analyses.

The goal of this research was to develop pre-planting predictive systems for maize dwarf mosaic in Ohio. The means of analysis and their results from this study should be applicable to other virus disease systems.

MATERIALS AND METHODS

Field data. Disease intensity data were collected at experimental sites in southern and northern Ohio. Table 1 contains specifications of the two locations.
The proportion of individual maize seedling trap plants infected by MDMV was used as a measure of maize dwarf mosaic disease intensity. The merits and disadvantages of trap plants have been described previously (Knoke et al., 1977). Trap plants of the MDMV-susceptible dent hybrid W9xOh51A were grown in 10.2 cm diam plastic pots in a greenhouse and transported to the experimental plots 14 days after planting. The seedlings were left in the field for 7 days and then returned to a greenhouse where they were observed for symptoms for 3-5 wk. During most years, 50 trap plants/week/location were used. Partially buried plastic cups served as receptacles for the pots. A row of these cups was centrally located in a 3.05 X 32.98 m area from which weeds were removed each week. Trap plants were maintained in southern Ohio from 1968-81 and in northern Ohio from 1969-81.

Local ambient temperature and precipitation data were obtained from the National Oceanic and Atmospheric Administration (Asheville, NC 28801). Averages were calculated as necessary for the analyses. Forty year temperature and precipitation averages were calculated in order to characterize partially the two experimental plot locations (Table 1).

Discriminant analysis. For each year in southern Ohio from 1968 to 1980, 18 winter-spring environmental variables (E1-E18) (Table 2) were used in the calculations. These variables were based in part on winter-spring environmental variables used in other studies to predict disease severity (Shaner and Finney, 1976; Watson et al., 1975), but were limited by the availability of only temperature and precipitation data. The average percent of trap plants infected by MDMV for the month of June was calculated for each year in southern Ohio. Based on a histogram of these averages, each year was classified as 'mild' or 'severe' for the level of maize dwarf mosaic. In other words, the years were clearly separated into groups with low and high average levels of maize dwarf mosaic in the trap plants; severe years always had an average trap plant infection greater than 75%, whereas mild years had an average less than that percentage. The years were then reclassified using a weighted average of trap plant infections for the total season. Little maize dwarf mosaic was observed during any year in northern Ohio and therefore the years were not classified.

Discriminant analysis was used to predict the classification of each year in southern Ohio using the winter and spring environmental variables. The objective of a discriminant analysis is to assign an unknown observation to one of two or more groups on the basis of a multivariate observation, i.e., a set of discriminating variables, with a low error rate (Lachenbruch, 1975). The groups in this study correspond to the two levels of maize dwarf mosaic; the discriminating variables are the 18 variables listed in Table 2. The groups are separated by finding a linear combination of the discriminating variables that gives the greatest amount of squared difference between the groups relative to the variance within the two groups (Lachenbruch, 1975). A stepwise procedure was used to select only those variables that significantly (P = 0.05) discriminated between the two groups. The selection criterion minimized Wilk’s lambda, a measure of group discrimination (Morrison, 1976). The discriminant functions derived from the southern Ohio data were used to classify the years in northern Ohio using northern Ohio environmental data. This allowed us to evaluate the discriminant function for an independent location where little natural maize dwarf mosaic is present. The Statistical Package for the Social Sciences (SPSS) (Nie et al., 1975) was used for the analysis.

The discriminant functions were evaluated by calculating their error rates using two different methods. The error rate, a number between 0 and 1, is an indication of the proportion of misclassifications in the sample used for the analysis. It also indicates the probability of
misclassifying future observations. The "apparent" error rate was estimated by the number of misclassifications in the sample divided by the number of observations (i.e., years).

The "leave-one-out" or "jackknife" error rate is more complicated computationally but makes efficient use of the available data. The method classifies each year by removing that year from the total number of observations, calculating the discriminant function from the remaining data, and using this discriminant function to classify the omitted year. After the omitted year is classified, it is replaced and then the procedure is repeated until all years are classified. The total number of incorrect classifications divided by the number of years is the error rate of the "leave-one-out" method.

RESULTS

Site specifications. The southern Ohio site was characterized by a long-term temperature average ca. 4°C warmer as well as 15 cm/year more precipitation than the northern Ohio site (Table 1). Days of precipitation were different by only 1 day. Johnsongrass, the overwintering host of MDMV, was abundant in southern Ohio, whereas it was absent at the northern Ohio location.

The median maize dwarf mosaic disease intensity for southern and northern Ohio for each week during the growing season is presented in Fig. 1. The median curves represent the typical proportion of trap plants infected by MDMV during a season. Southern Ohio was characterized by a minor peak around day 170 followed by a very high maize dwarf mosaic intensity from day 200 to day 270. Northern Ohio was characterized by low disease intensity for the entire season, with only minor peaks starting at day 220.

 Discriminant analysis. The classifications of maize dwarf mosaic level for the early (June) and total seasons in southern Ohio are listed in Table 3. Three years were classified as 'severe' using both schemes, although the years were not always the same. Stepwise discriminant analysis of the early season data resulted in three environmental variables significantly separating the groups. The three variables were: number of days in January, February, and March with an average temperature less than 0°C (E1); average minimum temperature in December, January, and February (E11); and number of days with rain in March and April (E16). Thus, discrim-

<table>
<thead>
<tr>
<th>Year</th>
<th>Actual</th>
<th>Pred.</th>
<th>D</th>
<th>p</th>
<th>Actual</th>
<th>Pred.</th>
<th>D</th>
<th>p</th>
</tr>
</thead>
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<td>0.03</td>
<td>0.99</td>
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<td>1.00</td>
<td>m</td>
<td>m</td>
<td>2.33</td>
<td>1.00</td>
</tr>
</tbody>
</table>

* Classification based on average of trap plants with maize dwarf mosaic during June (early season) or total season.

"m" indicates a 'mild' year; 's' a 'severe' year.
The linear discriminant function (D) can be written as:

\[ D = 0.23(E1) + 0.97(E11) + 0.48(E16) - 14.69 \]  

(1)

The mean discriminant score for the ‘mild’ years was 0.93, and for the ‘severe’ years — 3.11. Each year was assigned a discriminant score and then classified into the appropriate group, i.e., assigned to the closest group. Using equation 1, all years were correctly classified (Table 3). The “apparent” and “leave-one-out” error rates both were equal to 0 for this discriminant analysis. The posterior probability (p) of an observation belonging to the classified group also is presented in Table 3. The posterior probability is a function of the discriminant score, and therefore of the environmental variables (Lachenbruch, 1975). The average values of the three significant environmental variables are presented in Table 4.

### Table 4: Significant variables from stepwise discriminant analysis and their average values for ‘mild’ and ‘severe’ years based on two classification periods.

<table>
<thead>
<tr>
<th>Classification period</th>
<th>Variable</th>
<th>Wilk’s lambda</th>
<th>p</th>
<th>Average value</th>
</tr>
</thead>
<tbody>
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<td>Early season</td>
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<td>0.35</td>
<td>0.004</td>
<td>35.90</td>
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<tr>
<td></td>
<td>E11</td>
<td>0.23</td>
<td>0.006</td>
<td>-4.05</td>
</tr>
<tr>
<td></td>
<td>E16</td>
<td>0.46</td>
<td>0.003</td>
<td>26.40</td>
</tr>
<tr>
<td>Total season</td>
<td>E16</td>
<td>0.68</td>
<td>0.046</td>
<td>26.00</td>
</tr>
<tr>
<td></td>
<td>E17</td>
<td>0.32</td>
<td>0.003</td>
<td>9.06</td>
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<tr>
<td></td>
<td>E18</td>
<td>0.21</td>
<td>0.002</td>
<td>18.99</td>
</tr>
</tbody>
</table>

* See Table 2 for description of variables.

a Measure of group discrimination (Morrison, 1976).

b Significance of Wilk’s lambda.

### Table 5: Predicted classification of years in northern Ohio for the relative intensity of maize dwarf mosaic together with the discriminant scores (D) and posterior probability of group membership (p) based on the discriminant functions derived from the southern Ohio data.

<table>
<thead>
<tr>
<th>Year</th>
<th>Pred.</th>
<th>D</th>
<th>p</th>
<th>Pred.</th>
<th>D</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td>68</td>
<td>m</td>
<td>1.12</td>
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<td>m</td>
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</tr>
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<td>m</td>
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<td>1.00</td>
</tr>
</tbody>
</table>

* Predicted classifications for early and total seasons based on the discriminant functions in equations 1 and 2.

b ‘m’ indicates a ‘mild’ year; ‘s’ a ‘severe’ year.

Table 4. ‘Severe’ years were preceded by warmer winters (i.e., lower E1, higher E11 and drier springs (lower E16) than the ‘mild’ years.

Stepwise discriminant analysis of the total season data resulted in three environmental variables significantly separating the groups. The three variables were: number of days with rain in March and April (E16); total rain in April (E17); and total rain in March and April (E18). Thus, discrimination was based on spring precipitation (Table 2). The linear discriminant function can be written as:

\[ D = 0.67(E16) - 0.70(E17) + 0.19(E18) - 13.68 \]  

(2)

The mean discriminant score for the ‘mild’ years was 0.98, and for the ‘severe’ years — 3.28. Using equation 2, all years were correctly classified (Table 3). The “apparent” error rate was equal to 0, whereas the “leave-one-out” method misclassified 1 yr which produced an error rate equal to 0.077. The average values of the three significant environmental variables are presented in Table 4. ‘Severe’ years had drier springs (lower E16, E17, E18) than the ‘mild’ years.

The classifications of maize dwarf mosaic at the northern Ohio site, where johnsongrass does not occur, using discriminant functions derived from the southern Ohio data are presented in Table 5. Three early seasons and six total seasons were predicted as ‘severe’. Based on trap plant infections, no years were considered ‘severe’.

### DISCUSSION

Several workers have been able to predict disease intensity using winter-spring variables. Stevens (1954) developed a simple system for predicting the occurrence and severity of Stewart’s bacterial wilt [Erwiniastewar­tti (Smith) Dye] of maize based on the previous winter’s average temperature. This system has since been modified and validated, and recently was implemented into a computer program to allow forecasting on a large scale (Castor et al., 1977). Watson et al. (1975) were able to predict the incidence of beet yellows and beet mild yellows in England using number of days in January, February, and March when temperatures fell below —0.3 C and the average temperature in April. Shaner and Finney (1976) predicted severe years for Septoria leaf blotch of wheat [Triticum aestivum L.] by using the frequency of rainfall and daily temperatures less than or equal to a threshold for the fungus from 1 April to 14 June. The variables tested in this study (Table 2) were derived from these and similar investigations (Coakley and Line, 1980; Kemp and Troup, 1978) under the constraints of available environmental data.

Using discriminant analysis, we correctly classified all years in southern Ohio with low or zero error rates (Table 3). For early season classification, two temperature and one precipitation variable (E1, E11, and E16) were significant. For the total seasons, however, three precipitation variables (E16, E17, and E18) and no temperature variables were significant. With both classifications, ‘severe’ years had relatively dry conditions in March and April (Table 4). The ‘severe’ early seasons were also significantly associated with relatively warm
winters, whereas the 'severe' total seasons were only associated with the frequency and amount of precipitation in March and April (Table 4). These results suggest that the early season infections originated from aphids and/or virus that survived the previous winter. Warmer winters resulted in higher early infection. Infections for total seasons could not be related to winter or spring temperatures. Dry springs may favor the development of aphids or increase their probability of acquiring MDMV from alternate hosts. Dry springs also indicate more cloud-free days and thus a higher level of solar radiation than wet springs. Kemp and Troup (1978) observed a significant correlation between sunshine hours in April and an index for pepper virus disease caused primarily by cucumber mosaic virus. We do not have the sunshine-hours data to test these possibilities.

Our ability to classify correctly maize dwarf mosaic intensity using local winter and spring environmental variables does not support the theory that viruliferous aphids migrating from southern states are responsible for MDMV infections (Zeyen et al., 1978). If the migration theory was correct, we would not expect to classify correctly 100% of the years in southern Ohio.

Several seasons in northern Ohio were predicted as 'severe' even though little disease was observed. Aphid species which are significantly related to MDMV infections are found in high numbers at the northern Ohio site (Madden, Knoke, and Louie, unpublished). These results suggest that the environment was favorable for MDMV in northern Ohio and that this virus may become a serious problem if johnsongrass became established and widespread in the North.

Most posterior probabilities of group membership were greater than 0.99, which suggested that all classifications were far from borderline (Table 3). An observation, i.e., year, is classified into the group with the highest posterior probability. A classification with a posterior probability of 0.90 is more likely correct than when the probability equals 0.60. Posterior probabilities are only approximate with a small number of observations (<30) as in this study. Nevertheless, the posterior probability allows the researcher to assign a future observation to a group and assess the approximate probability that the observation belongs in that group.

Our results indicate that it is possible to predict the relative intensity of maize dwarf mosaic in trap plants using winter and spring environmental data. Validation studies are now being planned. Our goal is to determine if the derived discriminant functions successfully classify years at other locations. If the discriminant function successfully classifies future observations and if the relationship between trap plant infections and incidence in field planted maize remains strong, then it will be possible to warn growers prior to planting of probable 'severe' maize dwarf mosaic years.

LITERATURE CITED


Integrating Techniques of Vector and Weed-Host Suppression into Control Programs for Maize Virus Diseases

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ABSTRACT


Many of the diseases produced by viruses in maize (Zea mays) offer a variety of options for control due to the complexity of interactions among the protagonists [i.e., virus x maize x vector(s) x alternate hosts(s)]. Since no single method is completely effective in controlling maize virus diseases, pest management should be an integrated control program. Our research over the past 8 yr with maize chlorotic dwarf disease indicates that the optimum management strategy is a combination of early planting, resistant hybrid, johnsongrass (Sorghum halepense (L.) Pers.) suppression with herbicides, control of the leafhopper vector (Graminellina nigrifrons) with carbofuran, and irrigation. However, it is apparent that this is not the most cost-efficient system. From the standpoint of cost, agronomic suitability, and effectiveness as a disease control system, it is concluded that use of disease resistant hybrids, if available, and early planting should be the heart of a pest management program for maize virus diseases. Other methods such as carbofuran, herbicide(s), or irrigation entail substantial costs and their use either individually or in combination must be weighed against the yield benefits achieved. Also, factors such as multiple pest suppression by a pesticide and crop enhancement, in addition to disease suppression, should be a consideration along with these techniques. It is apparent that the threshold for controlling johnsongrass to levels that markedly reduce disease (maize chlorotic dwarf and maize dwarf mosaic) is lower than its weed pest threshold. Eradication of the weed is often extremely difficult in fields continuously cropped with maize. Crop rotation with a non-graminaceous crop like soybeans (Glycine max) may be advisable in fields with a persistent johnsongrass problem and a history of maize virus diseases. This has special merit with the advent of new “over-the-top” graminicides that can be used throughout the season in soybean fields to eradicate johnsongrass with no residue carryover the following year.

A variety of management options are possible for reducing the impact of many of the viruses that produce disease in maize (Zea mays L.). This is due to the greater complexity of interactions among the protagonists of the disease syndrome [i.e., virus x maize x vector(s) x alternate host(s)] as compared to other pathogen x host associations (Fig. 1). Thus, although greater flexibility may be present for disrupting one or more of the protagonist linkages, it is apparent that no single method is completely effective in controlling maize virus diseases. Therefore, pest management should involve a multicomponent strategy and the integrated control concept is especially important with these diseases.

Control strategies for maize virus diseases have been reviewed recently (Gordon et al., 1981). This paper does not attempt to expand on these reviews, but will consider their potential utilization in developing a cost effective management system for maize virus diseases. Emphasis will be on maize chlorotic dwarf and maize dwarf mosaic diseases and discussion will center on our experience with the control methods listed in Table 1. Two high-cost techniques that we have studied extensively in recent years are vector suppression with systemic insecticides and control of johnsongrass (Sorghum halepense (L.) Pers.) with herbicides. These techniques are effective and have potential utilization in an integrated control program for maize chlorotic dwarf and maize dwarf mosaic, but their cost effectiveness has not been clearly established.

DISEASE RESISTANT HYBRIDS

The greatest success to date in the control of maize virus diseases has occurred with the development of hybrids with resistance to the pathogens (Findley et al., 1981). It is evident that use of resistant hybrids, if available, should be the central strategy in maize virus disease management. The value of using a resistant hybrid in an integrated control program is easily demonstrated by comparing it to a susceptible hybrid in experiments with one or more control procedures applied similarly to each hybrid. We have done this for several years using DeKalb 1214, which is susceptible to both maize chlorotic dwarf and maize dwarf mosaic, and Pioneer 3147, which is relatively resistant to both diseases. The hybrids have similar yields in non-disease situations (Kuhn and Jellum, 1975). General trends of maize chlorotic dwarf in the two hybrids are differentially influenced by planting date and use of the systemic insecti-
Many maize virus diseases (e.g., maize chlorotic dwarf) involve complex interactions of virus x vector(s) x maize x alternate host(s) (protagonists). These environmental mediated interactions are linked through vector activities among maize and alternate hosts.

Symbols refer to: pathogen \( P \), host (maize) \( H \), and alternate host \( A \)

**TABLE 1.** Control strategies available for virus diseases of maize and estimates of usefulness and cost effectiveness.

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Usefulness</th>
<th>Cost effectiveness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hybrids resistant or tolerant to the virus</td>
<td>Agronomically acceptable hybrids are available for certain major diseases of maize such as maize chlorotic dwarf (MCD).</td>
<td>Highly effective; additional cost minimal or none</td>
</tr>
<tr>
<td>Early planting</td>
<td>Occurrence and impact of disease is often reduced in early plantings.</td>
<td>Low to moderate effectiveness; often no additional cost of operation</td>
</tr>
<tr>
<td>Vector control (Insecticides)</td>
<td>Reduction of disease impact shown only for MCD and corn stunt. Broad spectrum pesticides such as carbofuran also control other insect pests and phytophagous nematodes.</td>
<td>Moderately effective; cost is moderately high</td>
</tr>
<tr>
<td>Vector control (Biological)</td>
<td>Vectors of maize virus diseases are vulnerable to a variety of parasitoids, predators, and diseases. Techniques for manipulating and enhancing control by these agents are not available.</td>
<td>Low effectiveness; high probable cost for introduction or inundation strategies; low or no cost for enhancement strategies</td>
</tr>
<tr>
<td>Johnsongrass eradication</td>
<td>Epiphytotics of MCD and maize dwarf mosaic (MDM) are associated with johnsongrass infestations. Additionally, johnsongrass is a serious weed pest of maize. Control of johnsongrass greatly reduces disease and weed impact on maize yield.</td>
<td>Probably highly effective; eradication in continuous maize difficult and costly</td>
</tr>
<tr>
<td>Crop rotation</td>
<td>Rotation of maize with a non-susceptible crop eliminates maize virus disease problems for the season it is used. It often provides a better opportunity to control johnsongrass and other alternate hosts of the pathogens.</td>
<td>Highly effective where feasible; additional cost of operation variable</td>
</tr>
<tr>
<td>Irrigation</td>
<td>Recent research (All, unpublished) indicates that yield impact of MCD and MDM is reduced in irrigated maize. Improved growth through irrigation during dry periods can produce substantial increase of yield.</td>
<td>Low to moderately effective; high cost</td>
</tr>
</tbody>
</table>
cide carbofuran (Fig. 2). Yield is generally associated with these trends and demonstrates the consistently greater performance of Pioneer 3147 as compared to DeKalb 1214 with either control methodology.

Fig. 2 also demonstrates that while use of a resistant hybrid is important, its performance can be improved by combining other control methodologies. Recent research (All, unpublished) indicates that johnsongrass control with glyphosate (in no-tillage maize) and irrigation can further improve hybrid performance in an integrated program involving carbofuran and early planting. It is apparent that optimum pest management for maize chlorotic dwarf occurs with a combination of early planting, resistant hybrid, johnsongrass control, suppression of *G. nigrifrons* (Forbes) with carbofuran, and irrigation. However, analysis of costs of the various control methods relative to their individual impact on maize chlorotic dwarf and yield indicates that this management system often may not be the most cost efficient program.

**SYSTEMIC INSECTICIDES**

Several researchers have demonstrated the efficacy of systemic insecticides for control of leafhopper vectors of maize chlorotic dwarf virus (MCDV) and corn stunt spiriplasma (All et al., 1981). *G. nigrifrons*, the major vector of MCDV in the eastern USA, seems particularly susceptible to carbofuran (Bhirud and Pitre, 1972; All et al., 1977). We have done considerable field research with this insecticide and results indicate that 2.2 kg active ingredient (AI)/1000 m row produces optimum cificity with a rapidly diminishing rate of control at higher dilutions. Carbofuran is toxic to the leafhopper up to 55 days after application and efficacy drops rapidly thereafter (All, unpublished).

Reduction of maize chlorotic dwarf and higher yield are associated with control of *G. nigrifrons* by carbofuran, especially with a maize chlorotic dwarf susceptible hybrid such as DeKalb 1214. Yield has been increased by as much as 124% for this hybrid with carbofuran application. In experiments using carbofuran with the resistant hybrid Pioneer 3147, yield increases have averaged 0-25%. For 1982, $43/hectare (ha) was estimated as the cost of carbofuran at a rate of 2.2 kg AI/1000 m row in a planting time application. This estimate was based on the manufacturer's recommended price and did not include application costs. Maize price trends since 1979 indicate that yield should be increased by approximately 500 kg/ha to justify the cost of carbofuran solely for maize chlorotic dwarf control. However, other pest management factors also must be weighed in decisions on the use of carbofuran. This insecticide is active on several major pests of corn and also is a potent nematicide (e.g., All and Jellum, 1977). Thus, additional benefits could be anticipated with using carbofuran where a multiple pest complex is present. Conversely, many entomologists are concerned about environmental contamination and insect resistance associated with heavy use of certain insecticides, including carbofuran, in maize cropping systems.

**JOHNSONGRASS ERADICATION**

The association of johnsongrass as a perennial host for several maize virus pathogens is well known (Gordon et al., 1981). Spread patterns of maize dwarf mosaic virus (MDMV) from johnsongrass have been examined (Damsteegt, 1976) and we have studied the movement of *G. nigrifrons* from johnsongrass sprayed with RbCl, a biological tagging agent (Alverson et al., 1980a, b). These studies demonstrate that vectors can move inoculum quickly from johnsongrass into maize within a field or from border areas.

Johnsongrass is considered the worst weed pest of maize in Georgia and control of this weed in continuous maize is one of the most difficult problems confronting growers, not only in Georgia but in many areas of the world (McWhorter, 1981). Information now available suggests that even low levels of rhizome johnsongrass in maize fields early in the season have substantial influence on movement of virus inoculum within seedling maize (All et al., 1977).

Seasonality of johnsongrass is especially important in no-tillage systems because rhizome johnsongrass is present earlier relative to germination of maize. In conventional tillage cropping, plowing operations disrupt growth of the weed for up to several weeks. Research over several years has demonstrated a consistent trend for higher maize chlorotic dwarf levels in no-tillage as compared to conventional tillage maize (All, unpublished; All et al., 1977). This is associated with higher populations of rhizome johnsongrass in no-tillage.
plots. *G. nigrifrons* levels are similar in either tillage system.

During the past 4 yr, we have evaluated preplanting, planting time, and post-planting time johnsongrass control as a suppressive strategy for maize chlorotic dwarf and maize dwarf mosaic. These tests included no-tillage and conventional tillage and cropping systems of continuous maize (one crop annually) and double cropping with the maize planting following the harvest of a winter grain in late spring. The johnsongrass control program in no-tillage plots used a systemic herbicide, glyphosate, at planting time. The herbicide was broadcast on fields containing johnsongrass seedlings ca. 20-30 cm tall. Also, glyphosate was used as a directed spray in a post-planting time application in no-tillage. In conventional tillage, the johnsongrass control program was to use several tillage operations to disrupt rhizome growth, followed by application and incorporation of the herbicide EPTC plus protelant (Eradicane) or non-incorporated application of glyphosate plus atrazine or parquat plus atrazine.

These tests have had mixed results and in several cases they have been discouraging. A major problem has been incomplete control of rhizome johnsongrass in any system: Disease is usually decreased in the weed control plots, but differences from control plots are usually low and often not statistically significant. A contributing factor in these tests has been drought that has contributed to poor herbicide effectiveness. However, eradication of johnsongrass is not typical with any of the herbicides in continuous maize (Miller, 1978).

A major problem in no-tillage maize for johnsongrass control with a systemic herbicide such as glyphosate is the requirement for weed growth to be 30-40 cm tall prior to spraying (McWhorter, 1981). If maize is used in a double cropping system following harvest of a winter grain, a delay of 2 or 3 wk is needed for sufficient johnsongrass growth for a planting-time treatment of glyphosate. This can lead to agronomic problems. First, the soil may dry during the delay following harvest of small grains. It often is difficult for the no-tillage planter to penetrate the hardened soil and plant the seed at the proper depth. Second, surface soil moisture can become limiting for seed germination.

In a test where glyphosate was applied as a post-planting time directed spray in no-tillage, the maize was planted early to avoid drought problems. However, only the johnsongrass between the rows was controlled and many maize plants adjacent to weed clumps had disease symptoms at the time glyphosate was sprayed. No difference in maize chlorotic dwarf and maize dwarf mosaic occurred in the sprayed plots as compared to control treatments.

An important consideration in using glyphosate is its expense. The cost of the chemical for a broadcast treatment at a recommended rate for johnsongrass control is ca. $117/ha based on recommended manufacturers' prices as of August 1982. Thus, treatment in field maize is difficult to justify economically. Use of wick applicators and recirculating sprayers can cut costs tremendously by conserving the chemical (McWhorter, 1981).

However, the requirement for extended johnsongrass growth above the maize canopy limits these uses for maize virus disease control.

In our tests (All, unpublished; All et al., 1977), other disease control strategies, including resistant hybrids, carboluran, and irrigation, have been tested individually or in combination within johnsongrass treated plots. There has been no indication of an additive or synergistic influence of johnsongrass control on disease impact within these integrated control treatments. However, when a susceptible hybrid (Dekalb 1214) was tested without johnsongrass suppression, the impact of maize chlorotic dwarf and maize dwarf mosaic was increased tremendously.

**CROP ROTATION**

Our results demonstrating erratic johnsongrass control in continuous maize are verified by McWhorter (1981). Typically, total eradication of johnsongrass is not achieved by growers, but instead it is suppressed below threshold levels as a weed. Observations suggest that the population threshold for johnsongrass contribution to maize chlorotic dwarf and maize dwarf mosaic problems is lower than its weed pest threshold (All, unpublished; All et al., 1981). Thus, in areas where there is a history of maize virus disease and johnsongrass problems, rotation of maize with a non-graminaceous crop such as soybeans [*Glycine max* (L.) Merr.] may have merit. Also, troublesome fields could be left fallow for half a season and an intensive effort at johnsongrass eradication could be attempted.

Periodic rotation of maize with soybeans has special merit because of recent advances in graminicidal herbicides for treatment in broadleaf crops. One chemical, Poast R (Sethoxydim, BASF Corp.), provides outstanding control of johnsongrass and may be used as a post-planting time broadcast spray in soybeans (Antognini, 1981). Thus, johnsongrass produced from rhizomes or from seed may be treated throughout the season until eradication is achieved. Growers can produce soybeans in the johnsongrass control year, then rotate back to maize when the weed is eliminated. There are no residue carryover effects with Sethoxydim. The chemical is expected to receive registration for use in soybeans in 1983 and several similar acting herbicides will receive labeling thereafter (Antognini, 1981).

Use of a preplant incorporated application of trifluralin (Treflan R) or promfluralin (Tolban R) for johnsongrass control is currently registered for soybeans. This application has given excellent suppression of the weed. However, double rates are required and carryover problems occur with residues the following season. Thus, maize plantings are not recommended for 2 yr following this treatment (McWhorter, 1981).

Another alternative is to leave fields fallow during the growth cycle of johnsongrass and attempt eradication with cultural and chemical procedures. Winter grains can be cropped in these areas and johnsongrass controlled following grain harvest. Use of repeated tillage and/or mowing coupled perhaps with herbicide treatment are all directed at weakening and destroying
johnsongrass plants and rhizomes prior to seed formation by the weed (Miller, 1978). Use of the above johnsongrass control programs to suppress maize virus diseases is currently hypothetical and awaits research evaluation.

CONCLUSIONS

It is apparent from our research with maize chlorotic dwarf and maize dwarf mosaic over the past 8 yr that an integrated control procedure is necessary for optimum management of disease problems. The nature of the control system adopted should vary according to local circumstances such as disease history, occurrence of johnsongrass, and the position of maize in the overall cropping program of the grower. The heart of a disease management program involves use of disease resistant hybrids coupled with early planting. These are low cost procedures with proven efficacy on disease. Other methods such as use of carbofuran (vector control), herbicide(s) (johnsongrass control), or irrigation (enhanced plant growth) entail substantial costs and their use either individually or in combination must be weighed against the yield benefits achieved. Also, factors such as multiple pest suppression by a pesticide and crop enhancement, in addition to disease suppression, should also be considered prior to adopting these strategies.

It is apparent that the threshold for johnsongrass as a source of maize viruses for disease occurrence in maize fields is lower than its weed-pest threshold in these fields. Control of johnsongrass to levels that markedly suppress disease is very difficult in continuous maize culture. Thus, crop rotation may be advisable in cases where maize virus problems have been serious for several years and where johnsongrass has been a persistent weed pest in maize. Use of soybeans in a rotation system has several advantages, especially with the advent of new “over-the-top” graminicides that can be used for johnsongrass eradication. In situations where rotation with non-gramineous crops is not feasible, an intensive johnsongrass eradication program could be achieved by leaving fields fallow for half a season and using multiple weed control techniques.

LITERATURE CITED

Breeding for and Genetics of Virus Resistance in Field Corn

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ABSTRACT


Breeding for resistance to virus diseases of corn (Zea mays) has been confined primarily to maize dwarf mosaic and maize chlorotic dwarf. Attempts to develop virus resistant versions of desirable inbreds have not been very fruitful but some backcrossing with alternating selfed generations have resulted in development of new resistant inbreds. Population improvement using some form of recurrent selection has been effective. I have attempted to present some of the problems encountered and to give my opinion on why certain results were expected.

I have discussed the merits and limitations of the use of various data that can be collected to measure the response to virus disease in different genetic models. I do not believe that we should automatically assume that data which measure responses to a disease are appropriate for use in all genetic models.

Improvement of a crop through breeding is a relatively long and continuous effort. However, it is a very important endeavor that provides more food for man both directly by increased potential crop yields and indirectly by reducing or eliminating losses to pests. The methods employed in plant breeding usually do not change dramatically within a year or two. Therefore, the recent paper by Findley et al. (1981) appropriately summarizes the present breeding work on virus diseases of corn (Zea mays L.). In an attempt to avoid duplicating the content of that manuscript, I have chosen to present my opinions on the problems that were encountered in breeding for resistance to viruses and then to summarize briefly what has been accomplished and is presently underway in the public sector. I will not attempt to summarize the effort by the private sector because that will be presented elsewhere in these proceedings (Duvick, 1983; Eberhart, 1983). However, I want to stress that those in the public and private sectors have worked together in providing farmers with hybrids that are resistant or tolerant to attack by the viruses.

Unless otherwise noted, my comments should be assumed to pertain to maize dwarf mosaic caused by maize dwarf mosaic virus strain A (MDMV-A) or to maize chlorotic dwarf caused by maize chlorotic dwarf virus (MCDV). I would refer the reader to the article by Gordon et al. (1981) for the history of virus diseases. This article also clarifies some of the confusion in the literature as to which disease(s) was being investigated in a given study. From a practical standpoint, I have chosen to assume that if plants were classified on the basis of leaf discoloration and stunting, maize chlorotic dwarf was involved either with or without MDMV. If only mosaic symptoms were indicated, maize dwarf mosaic was assumed to be the only disease present, and if both mosaic and leaf discoloration/stunting symptoms were recorded, then both diseases (sometimes referred to as the virus complex) were considered present.

BREEDING FOR RESISTANCE

Effectiveness and efficiency of a program for resistance breeding to any disease or pest would be enhanced if the number of genes conditioning resistance and the type of gene action were known in advance. However, if breeding programs were not initiated until after this information was obtained, corn producers would suffer losses to a disease over a much longer time. Therefore, programs for resistance breeding do and should commence as soon as a serious disease problem is encountered. This happened with the virus diseases of corn in the 1960's. Not only did the corn breeders not have information on the number of genes or type of gene action, but they knew little about the causal pathogen(s). However, they could see disease symptoms severe enough to warrant a breeding program seeking resistance to whatever pathogen(s) was causing the yield losses.

Corn breeders, working cooperatively with plant pathologists and entomologists, realized that the first
step was to locate sources of resistance. Sites where the natural occurrences of the diseases were most severe were chosen for use in these endeavors. Portsmouth, Ohio, and Yazoo City, Miss., were the two earliest sites. Waverly, Tenn., and several other locations were soon selected as test sites. Along with site selection, a method to evaluate differences among genotypes needed to be formulated. Differences in disease incidence was one method, but many genotypes seemed to vary for disease severity. Therefore, rating scales (often 1-9) were described in which class 1 indicated no symptoms and classes 2-9 indicated progressively more severe symptoms, with the highest rating reserved for a dead or severely stunted plant. Josephson and Hilty (1970) described such a rating scale as follows: 1 = no apparent symptoms; 2 = top two or three leaves with mottling, no stunting (mottling of whorl only); 3 = entire plant above the ear mottled and/or discolored, no evident stunting; 4 = chlorosis and/or discoloration above the ear, little stunting; 5 = plant above the ear discolored, stunting, and ear reduced in size; 6 = upper three-fourths of plant chlorotic and/or discolored, stunting and ear reduced in size; 7 = entire plant discolored and stunted, small ear or none; 8 = entire plant discolored and stunted, no ear produced; 9 = plant completely collapsed, no ear.

Some researchers used such a scale for maize chlorotic dwarf but used a different descriptive scale for maize dwarf mosaic, and made separate ratings for the two diseases. Sometimes ratings for the virus complex were taken and then one scale would be used for all symptoms expressed by the plants. In these cases, plants with mosaic symptoms were often called "mottled", even though plant pathologists might not agree that mosaic and mottling are synonymous.

Generally each plant in a plot was rated and the data reported both the percentage of diseased plants and the disease severity index (DSI) (total numerical rating of all plants/total number of plants in plot). In this index, one value estimated both disease incidence and severity. In some instances, only a visual mean rating for a plot was taken to evaluate genotypes.

When ratings were taken for the virus complex, I would estimate that those plants with maize dwarf mosaic symptoms were rated mostly from 2 to 4 in the above scale, and plants with maize chlorotic dwarf symptoms were rated from 2 to 9. Therefore, with equal percentages of diseased plants, genotypes with maize chlorotic dwarf would have higher disease severity ratings than genotypes with maize dwarf mosaic. Thus, if disease incidences were equal, selection for those genotypes with lower disease severity ratings would eliminate more maize chlorotic dwarf susceptible than maize dwarf mosaic-susceptible genotypes.

Earlier work focused primarily on screening to identify sources of resistance. Unless plants were evaluated specifically for the mosaic symptoms of maize dwarf mosaic, the greatest selection was probably for differences among genotypes for response to maize chlorotic dwarf. Plants tested in later years may or may not have been inoculated with MDMV. However, plants with the leaf discoloration/stunting symptoms associated with maize chlorotic dwarf were selected higher (more severe) than plants with only maize dwarf mosaic symptoms. Thus, those genotypes exhibiting susceptibility to maize chlorotic dwarf would be eliminated more rapidly than those with susceptibility to maize dwarf mosaic except when maize chlorotic dwarf was not present or present at only a low incidence. This would be especially true if evaluation of genotypes was done only once and that was a few weeks after silking when maize dwarf mosaic symptoms are less pronounced than earlier and the leaf discoloration/stunting symptoms of maize chlorotic dwarf are quite evident. In actual practice and probably because more sources of resistance to maize dwarf mosaic were present, progress for developing hybrids with MDMV-resistance has advanced more quickly than developing MCDV-resistant hybrids.

After identification of resistance, breeding programs began to utilize these sources of resistance to improve otherwise desirable lines, create new resistant lines, and improve breeding populations. Breeding procedures to utilize genes for resistance have included backcrossing and subsequent selfing, progeny row selection to develop new lines, and recurrent selection to improve populations.

Recovery of established inbreds with virus resistance by backcrossing has been, at best, of limited success. In retrospect, this was probably to be expected except possibly for MDMV-resistance when manual inoculation of plants was used during the backcrossing cycles. Of course, the success of transferring virus resistance to established lines requires that one must be able to identify which plants to select for the next cycle, i.e., which plants of the backcross or of the selfing generation between backcrosses have the most genes for resistance. This selection is hampered by plants that have escaped infection and appear highly resistant (disease free) but which may or may not have genes for resistance. The greater the number of these escape plants selected, the less was the progress realized in transferring virus resistance to an otherwise desirable line.

Even though backcrossing to recover a virus resistant version of a given line has not been very successful, backcrossing with selfing between backcrosses has been an effective breeding procedure for producing new resistant lines in Ohio (Findley et al., 1977) and Tennessee (Naidu and Josephson, 1976).

Selection for resistance to the virus complex could be advantageous in that resistance would be for both maize chlorotic dwarf and maize dwarf mosaic. However, selection for resistance to the virus complex could slow progress if in 1 yr selection was for resistance to one virus (e.g., MDMV) and the following year the other was the predominant virus and selection was for this second virus. Of course, the number of genes for resistance to these viruses may be too large to expect a backcross program to be effective. Only limited data on number of genes controlling MDMV resistance are available and even less is known on the number of genes that control resistance to MCDV.

Although Scott and Rosenkranz (1974b) have shown
that resistance to maize dwarf mosaic and maize chlorotic dwarf are independently inherited, when selection and/or evaluation was for resistance to the virus complex under conditions of high incidence of both maize dwarf mosaic and maize chlorotic dwarf, one would expect an inbred classified as resistant to have resistance to both MDMV and MCDV. This seems to have been the case for many lines developed by L. M. Josephson in Tennessee and by others. For example, T232 has resistance to MDMV and MCDV (little, if any, leaf discoloration/stunting) and has been used to confer virus resistance to hybrids. However, at a site where the incidence of maize chlorotic dwarf is high, T232 exhibits very distinct veinbanding symptoms, a diagnostic symptom for presence of MCDV (Louie and Knoke, 1981). The presence of this symptom would indicate that T232 is susceptible to MCDV.

This conflicting interpretation as to whether T232 is susceptible or resistant to MCDV suggests two possibilities. It could be that MCDV causes veinbanding symptoms in all susceptible genotypes but some genotypes also express leaf discoloration/stunting symptoms. Under this assumption, T232 is susceptible to MCDV but has resistance to the leaf discoloration/stunting portion of the symptoms caused by MCDV, i.e., selection for “apparent resistance” has been for resistance to only a portion of the disease syndrome. The possible explanation that selection was for resistance to only a portion of the symptoms of MCDV infection seems reasonable until one considers other information. When one evaluates corn genotypes, the incidence of plants with veinbanding symptoms will be considerably higher than the incidence of plants with leaf discoloration/stunting symptoms. The reason that some plants of a given inbred or single cross are resistant to one portion of the symptoms and other plants of the same genotype growing in the same plot lacked this resistance is not readily apparent.

Another possible explanation of why T232 appears to be both susceptible and resistant to MCDV would be that a second pathogen causes leaf discoloration/stunting symptoms (possibly only when in combination with MCDV). However, no one has detected a separate pathogen to date. Thus, at least in my mind, the dilemma about whether T232 is resistant or susceptible to MCDV continues to be unresolved.

Data from diallel crosses have been used in an attempt to gain information on types of gene action involved in resistance to virus disease(s) (Johnson, 1971; Josephson and Naidu, 1971; Loesch and Zuber, 1972; Naidu and Josephson, 1976; Nelson and Scott, 1973; Zuber et al., 1973). Also, recently Scott and Rosenkranz (1981) have presented data from a diallel cross but did not calculate the usual diallel analysis. Generally, diallel analyses have shown high general combining ability (GCA) variances and a relatively low but often significant specific combining ability (SCA) value. Thus, the conclusion has often been made that resistance to virus disease is mostly additive type gene action with little nonadditive gene action. The merits of using a diallel cross as a method of determining the type of gene action for virus resistance will be considered in a later section of this paper, but usually the conclusion that additive gene action is important has led researchers to suggest that recurrent selection should be an effective breeding method. Scott and Rosenkranz (1974a) and Findley et al. (1977) have shown that recurrent selection has indeed been effective in population improvement for virus resistance.

Data from diallel crosses provide other valuable information needed in breeding and hybrid production programs. These data indicate how many parents of a hybrid need to have genes for resistance and provide information on whether some resistant lines confer higher levels of resistance to their hybrids than do other resistant lines. Johnson (1971) found that predictions of three- and four-way crosses were the most accurate when the virus responses of the nonparental single crosses were used. Zuber et al. (1973) reported a nearly linear response in increased maize dwarf mosaic ratings for both observed and predicted double-cross performances with each additional substitution of a susceptible inbred parent for a tolerant one.

The main problem that slows progress in breeding for MCDV-resistance has been that we cannot manually inoculate with MCDV. Thus, we must rely on the whims of a vector to inoculate our breeding material. Although MDMV can be manually inoculated, not all breeding programs make use of this procedure. The lack of manual inoculation slows progress in developing MDMV-resistance, but in programs where selections are for many characteristics, some breeders feel that they can make adequate progress without manual inoculation. However, I do not know of anyone that would not welcome a way to inoculate manually with MCDV.

I have attempted to point out some of the problems encountered in breeding for resistance to MDMV and MCDV. These problems probably slowed progress but did not prevent the identification or development of resistant inbreds and/or populations. This success has been recently summarized by Josephson and Scott (1981) in a listing of lines and populations with resistance to one or more of the virus diseases. As indicated in this listing, resistance has been found in a number of sources. Future tests will provide information on whether the genes for resistance from different sources are the same or different. Scott and Rosenkranz (1975) have shown that virus-resistant progenies can be obtained from a number of populations.

The rate of progress that has been made in developing virus-resistant material, in spite of the problems involved, reflects the seriousness of losses from virus diseases. Initially, progress was aided by the fact that at least some virus resistance was present in material already present in many breeding programs. Therefore, it was not necessary to make a worldwide search for resistance before breeding programs could be initiated.

For various reasons, the breeding effort devoted to virus resistance in the public sector has declined. In a telephone survey, I contacted a number of corn breeders to determine who was still breeding for virus resistance.
Of those who indicated they were breeding for resistance to viruses, I asked with which disease(s) they were working and what types of breeding activities they were conducting. Individuals contacted who gave a positive response (states where located in parentheses) were: H.S. Aycock (VA), A. J. Bockholt (TX), W. A. Compton (NB), W. R. Findley (OH), C. G. Poneleit (KY), J. R. Wallin and L. L. Darrah (MO), D. R. West (TN), and J. O. York (AR). A summary of the information obtained is presented in Table 1.

Certainly, I do not want to imply with our listing of successes that we can discontinue breeding for virus resistance. Quite the contrary, I feel that new procedures to create heavy infection with MCDV will open new horizons on levels of resistance that we can attain. For instance, a somewhat different procedure to infest plants with viruliferous leafhoppers has been presented at this colloquium (Havener, 1983).

**GENETICS OF RESISTANCE**

The genetics of resistance to virus diseases was summarized recently (Scott et al., 1981). Therefore, I have chosen to look at some of the published data and try to determine whether we really know much about the genetics of resistance to virus diseases in corn. I am probably really asking the question, “Can we use genetic or statistical genetic designs on data reflecting virus response when those designs were devised for continuous data?”

The answer to this question can vary depending on the type of genetic model being used, the type of data collected, and the material evaluated. I believe that the potential problems are more acute with classical genetic models than with quantitative genetic models. In addition, if hybrid vigor affects disease severity but not disease incidence, then genetic interpretations based on disease incidence should be better than those obtained when some rating scale was used to measure disease severity. However, if a diallel cross, not including parents, was being evaluated, this consideration should not be important because all entries would be hybrids. If a rating scale is used to indicate disease severity, the increments of the scale should be equal and the scale should include the entire range of responses to the disease. When DSI values are used, one must remember that a given DSI can be obtained by different combinations of disease incidence and disease severity. For instance, with 50 plants in a plot a DSI of 3.67 could reflect that all plants were diseased with an average DSI of 3.67, 10 plants non-diseased and diseased plants averaged 5.00, or 20 plants non-diseased and diseased plants averaged 9.00. The assumption that these combinations reflect the same type of gene action is probably not very realistic.

I will first discuss results from what I consider quantitative genetic models. For virus resistance studies involving corn, these include analyses of data from e^-.
In the previous section, I mentioned that data from diallel crosses (Johnson, 1971; Josephson and Naidu, 1971; Loesch and Zuber, 1972; Naidu and Josephson, 1976; Nelson and Scott, 1973; Zuber et al., 1973) suggested mostly additive gene action but sometimes nonadditive gene action. The usual practice of selecting parents that markedly differ for virus resistance to be used in the diallel would have the effect of inflating the GCA variances obtained because GCA's reflect magnitude of differences among means for all crosses involving each parent. Unfortunately, GCA's are not free of nonadditive types of gene action. Thus, even if virus resistance was controlled only by nonadditive type gene action, the GCA variance would not be zero.

To determine the magnitude of bias that selection of parents, influence of hybrid vigor on disease expression, and use of a given rating scale have on the GCA and SCA variances obtained from diallel analyses probably cannot realistically be done. Perhaps the best we can do is to see if the results seem reasonable. When large estimates of GCA variances are obtained, this suggests that the genes for resistance can be accumulated and eventually fixed in a genotype. Of course, we know this is possible because they were fixed in the resistant inbreds that were chosen as parents. Thus, the only genetic information we are gaining from a diallel analysis is whether or not there is enough nonadditive gene action so that the SCA mean square is significant.

Generation mean effects analyses are based on data from parents, F1, and segregating generations. Data from Mississippi (Grogan and Rosenkranz, 1968) indicated no dominance, but generation mean effects analyses of data from Missouri (Loesch and Zuber, 1967) and Ohio (Dollinger et al., 1970), calculated and presented by Scott et al. (1981), suggested dominance was more important. Whether these differences are attributable to differences in genotypes tested, different rating scales employed, or different degrees of influence of hybrid vigor is not known. However, in the Missouri and Ohio data, the F1 was rated as more resistant than the resistant parent but in the Mississippi test the resistant parents rated very near to 1.0. In the Mississippi test, the F1 could not have been rated much more resistant than the resistant parent because values less than 1.0 (healthy plants) were not possible.

The influence of hybrid vigor on the expression of virus severity should not materially affect the estimate for additive gene effects because this estimate is equal to the difference between the backcross generations which should be essentially equal in vigor. Estimates of dominance, additive x dominance, and dominance x dominance effects involve parental means. Thus, these values could be biased by the influence of hybrid vigor on the expression of disease severity, especially in the case of maize chlorotic dwarf.

If some of the populations grown for generation mean analyses were not able to have their true phenotype described because of the limitations of the rating scale, the resistant parent and its backcross generation would be the generations most likely affected. This would reduce the additive effects and influence all other effects, but the direction of bias would depend on the type of gene action involved.

Utilizing chromosomal translocations and evaluating for both maize dwarf mosaic and maize chlorotic dwarf, Findley et al. (1973) found evidence for the presence of genes for resistance in both Oh07 and Mo22 on both arms of chromosome 6; the long arms of chromosomes 1, 2, and possibly 10; and the short arms of chromosomes 3, 7, 8, and 10. Oh07 appeared also to have a gene for resistance on the long arm of chromosome 7.

Using chromosomal translocations, Scott and Rosenkranz (1977) found that inbred Mp412 had a gene for maize chlorotic dwarf resistance on the short arm of chromosome 1, the long arm of chromosome 3, and probably also on the short arm of chromosome 4.

Scott and Nelson (1971) found that inbred GA209 had a gene for virus resistance to MDMV on both arms of chromosome 6. Scott and Rosenkranz (1973) reported that inbreds Ark H-24, Ark H-77, Mp339, Mp412, Mo18W, and probably Tx601 also had a gene for resistance to MDMV on each arm of chromosome 6.

When chromosomal translocations are utilized in a genetic test, it probably does not make much difference whether data for disease severity or incidence are taken because comparisons are made between two classes of plants.

Naidu and Josephson (1976), using data from a diallel cross, estimated that at least four genes were involved in conditioning virus resistance. Dollinger et al. (1970), using means and variances of the F1, F2, and backcrosses, obtained estimates of from one to three (possibly four) genes for resistance. In retrospect, both of these studies involved the virus complex (both maize dwarf mosaic and maize chlorotic dwarf) and thus do not provide information on the number of genes for resistance to either MDMV or MCDV independently.

To date, no estimate of number of genes for resistance to MCDV has been presented, except when chromosomal translocations were used. This is probably not surprising because we have not been able to inoculate manually with MCDV. Although inoculation by leafhoppers is effective enough to cause major yield losses, this inoculation has probably not been uniform enough for genetic studies. Under these conditions, one would expect some symptomless plants that escaped infection to be classified as resistant, and this in turn would contribute to misleading ratios that would not fit any genetic model.

Some data using classical genetic models to determine number of genes for resistance to MDMV have been reported. Before we proceed to review these reports, let us consider one important fact about the expression of maize dwarf mosaic symptoms, i.e., the percentage of maize dwarf mosaic-diseased plants observed in a given genotype can vary depending on when notes on disease incidence are taken. In general, the higher the level of resistance, the longer symptom expression is delayed. Kuhn and Jellum (1970) suggested use of a "disease index" based on plants diseased at 6, 11, 16, and 28 days after inoculations. Kuhn and
Smith (1977) indicated that when this system was used with 550 corn lines, they could be divided into three distinct categories: resistant, intermediate, and susceptible. Scott et al. (1969) and Findley et al. (1977) have also reported that the percentage of diseased plants increased with time after inoculation.

Scheifele (1969) suggested that in at least one inbred, resistance to MDMV-A was caused by a single dominant gene. Wernham and Scheifele (1968) and Scheifele and Wernham (1969) concluded that resistance to MDMV-A and MDMV-B was not conditioned by the same genetic system.

Roane et al. (1977) evaluated parents, F₁, F₂, and F₃ populations on a two-class rating scale (ratings 1-5 being resistant, 6-7 being susceptible) and concluded that Oh7B had one dominant gene for resistance to MDMV, but the number of resistance genes in T₈ was not determined. They found a high correlation between mean F₂ and mean F₃ plant virus disease ratings for Oh7B material but not for T₈.

Findley et al. (1977) reported that the percentage of maize dwarf mosaic-diseased plants in some of the segregating generations involving Oh07 suggested a single dominant gene at the first date of data collection, but at later dates all populations were quite susceptible. Data from segregating generations with Pa405 suggested one dominant gene with aphid inoculation and two dominant genes with mechanical inoculation. Their tests with MDMV strains B, D, E, and F suggested one dominant gene for resistance in Pa405.

Data based on a rating scale would appear to have serious limitations when used to determine the number of genes for resistance by using a classical genetic model. Dollinger et al. (1970) state: "The use of the Mendelian method of analysis depends upon the ability to assign individuals to classes with clear phenotypic distinctions which may reveal underlying genetic differences." Scott and Rosenkranz (1982) have proposed that when each allele for resistance has an equal effect, data taken on a rating scale would not be appropriate to determine the number of genes conditioning virus resistance unless at least three conditions are met. The conditions are as follows:

First, each increment of change in the rating scale must be equal to one change in the number of alleles for resistance. That is, each of the described differences in the rating scale must correspond to one allelic difference. Secondly, the scale used must have the number of classes necessary for the number of genes conditioning resistance. Thus, for an F₂ in which one gene controls resistance, a 9-class scale would be needed, but with four genes a 9-class scale would be required. Finally one and only one genotype can have a rating of 1 (symptomless plants). Thus, with four genes conditioning resistance, plants with as many as seven alleles for resistance would still have to express disease symptoms.

As a consequence of the apparent problems associated with data from a rating scale, Scott and Rosenkranz (1982) have proposed a new system to determine the number of genes conditioning resistance to MDMV. This system requires only data on the percentage of diseased plants in two or more segregating generations from a cross between a resistant and a susceptible inbred. This system utilizes the fact that segregating generations have different numbers of alleles for resistance. For instance, if the resistant parent has two genes for resistance, the percentage of plants with two or less alleles for resistance in the F₂, backcross to susceptible parent, and backcross to the resistant parent are 69, 100, and 25, respectively.

Using this new system, Scott and Rosenkranz (1982) have concluded that inbreds GA209, Mp339, Mp412, T240, and Va35 have two, two, three, and one gene for resistance, respectively, to MDMV.

Louie et al. (1976) reported differences in response to MDMV infection among a given inbred from different sources. Thus, genetic studies using different seed sources could indicate different numbers of genes for virus resistance for a given inbred.

I do not think I have definitely answered whether or not we can use current genetic models for determining the type of gene action and the number of genes conditioning resistance to a virus disease. I hope I have pointed out some of the major problems arising from arbitrarily using these models with the assumption that they are as applicable to data taken on response to a virus as they are for a character such as plant height. I also hope that I have not discouraged research on determining the genetics of resistance to viruses of corn. As I see it, determining the genetics of resistance to viruses is just more of a challenge than studying the genetics of some other characteristics. Of course, we all appreciate challenges, so the potential for much good research still lies ahead of us.

LITERATURE CITED


**Zea diploperennis as a Source of Maize Chlorotic Dwarf**

**Virus Resistance: A Progress Report**

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Based on cooperative investigations of the Agricultural Research Service, Science and Education, U.S. Department of Agriculture, and The Ohio State University, Ohio Agricultural Research and Development Center, Wooster 44691.

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**ABSTRACT**


*Zea diploperennis* was used as a source of resistance to maize chlorotic dwarf virus (MCDV). Of the many hundreds of maize (*Zea mays*) lines tested to MCDV, all have shown at least some plants with the vein-clearing symptoms diagnostic for the disease.

Maize x *Z. diploperennis* backcrosses (BC) and backcrosses selfed (BC-S), involving maize as recurrent parent, were exposed to MCDV viruliferous leafhoppers (*Grinnellina nigrifrons*). The leafhoppers were fed on test seedlings for 48 hr. After 14-21 days, seedlings were examined for vein-clearing symptoms. Leaf samples from symptomless plants were tested by enzyme-linked immunosorbent assay (EIA), and those plants that reacted negatively were transplanted to the field or greenhouse. Viruliferous leafhoppers were again caged on the youngest leaves of older plants for 48 hr. After 14-21 days to allow for infection, leaf samples were again tested for MCDV by EIA. Plants with negative reaction were used either to pollinate other maize plants or were self-pollinated.

Inheritance data from the first tests of BC1 and BC1S generations plants indicated that resistance to MCDV was controlled by two dominant complementary genes. Later tests, including advanced backcross and backcross selfed generations, suggested several minor genes were also involved in resistance. A recurrent selection breeding procedure is proposed to concentrate genes for resistance to MCDV.

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**MATERIALS AND METHODS**

Maize x *Z. diploperennis* crosses were made in the greenhouse in 1979-80. Pollen production was induced in *Z. diploperennis* by exposing older plants to 9 hr of daylight 6 wk before the crosses were to be made. By the time pollen was available, only a few plants remained with fresh silk; however, most crosses produced some (2-25) viable seeds. In some of the later pollinations, seed set was enhanced by trimming silks of the maize plants to accommodate the shorter pollen germ tubes of *Z. diploperennis*.

Seedlings of first generation backcrosses with maize as recurrent parent (BC1), from seed produced in the field in 1980, were exposed to leafhoppers (*G. nigrifrons*) viruliferous with MCDV. Screening for MCDV reactions was done in the greenhouse in the winter of 1980-81. With a few exceptions, 18 seeds were planted for tests of each progeny. Following a virus acquisition access period (AAP) of 48 hr, leafhoppers were allowed to feed on test seedlings for 43 hr. Groups of nine pots with two test seedlings per pot, plus two pots with seedling checks, were caged with 250 to 300 viruliferous leafhoppers. After 14-21 days, seedlings were examined...
for vein-clearing symptoms. Leaf samples were taken from symptomless plants to test for virus by enzyme-linked immunosorbent assay (EIA). The EIA was performed as described by Knoke et al. (1983).

Plants with negative reactions in EIA were considered resistant and transplanted to the greenhouse and used to pollinate other maize plants and/or selfed. The second generation backcross (BC2) or selfed seedlings (BC1-S1) were exposed to viruliferous leafhoppers. Symptomless seedlings were assayed by EIA and those that reacted negatively for MCDV were transplanted to the 1981 field or greenhouse-sunyard. The transplants were inoculated again with MCDV at or near tasseling stage to insure that these plant populations did not include virus escapes. Leafhoppers were caged in groups of approximately 10 on the youngest leaf for a period of 48 hr following a 48 hr AAP. After 14-21 days the plants were again tested for MCDV by EIA.

Subsequent tests of segregating progenies for resistance to MCDV were conducted using procedures similar to those described above. Exceptions were that 15 leafhoppers were caged on two plants per pot and retests using leaf cages were made on younger plants, 6-8 wk old.

## RESULTS AND DISCUSSION

Of the 107 first generation backcrossed (BC1) plants from seed produced in the field in 1980, 22 appeared resistant (=immune) to MCDV. This proportion is not significantly different from a 1:3 ratio (X² = 1.125, P = 0.30-0.20). Ratios of resistant to susceptible BC2 and BC1-S1 plants from seeds produced in the greenhouse in the winter of 1980-81 were 100:270 and 30:24, respectively. Chi-square tests for 1:3 and 9:7 ratios indicated highly significant probabilities for goodness of fit (Table 1). These results suggest that resistance to MCDV was controlled by two dominant complementary genes.

However, in subsequent inheritance tests, resistance appeared to be controlled by more than two genes, as indicated by the lower number of resistant plants from seeds produced in the field in 1981 and following generations. Previously, assuming all maize lines were equally susceptible to MCDV, we predicted that segregation would follow the two-factor complementary dominant gene model, but percentages of resistant plants ranged from 4.2% in the BC2 generation to 24.8% in the BC1-S1 generation (Table 2).

It appeared that with two exceptions the seven progenies comprising the BC2 generation, tested after the 1981 field season, consisted of plants that had escaped infection rather than having resistance. In subsequent tests of resistance involving more stringent test conditions (described later), four progenies had no resistant plants and one of the three progenies with only one resistant plant produced none in the next generation. Plants in two other crosses also apparently escaped infection by MCDV. In these crosses, all plants of the same progeny were eliminated in only one of the two tests. In the other tests, 7 of 18 and 8 of 17 plants were resistant following examination for the vein-clearing symptom and by EIA. Plants that tested resistant but proved later to be susceptible were likely those that received lesser amounts of virus, or the more susceptible cell sites escaped inoculation by viruliferous leafhoppers.

Many of the plants with positive EIA readings, as determined by statistical evaluations of absorbance data, were considered to be resistant when evaluated by visual readings of the coloration within wells; i.e., well contents were colorless and yet the absorbance values were great enough to score the test as positive. These results suggest: a) a very low viral titer, b) spurious results, or c) inadequate non-infected maize controls. Our data were insufficient to eliminate any of these possibilities and we have chosen the strict criterion that in these uncertain positive reactions we accept them as

### TABLE 1. Reaction of maize x Zea diploperennis BC1-S1 and BC1 plants to maize chlorotic dwarf virus (MCDV).

| Generation | No. plants | Resistant | Susceptible | X² | 9:7 | P <
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>BC2</td>
<td>102</td>
<td>270</td>
<td>.428</td>
<td>.070-0.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BC1-S1</td>
<td>30</td>
<td>24</td>
<td>.011</td>
<td>0.90</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* a BC and S with subscripts indicate generations of backcrossing and selfing, respectively.  
* b Seedlings were exposed to leafhoppers viruliferous with MCDV for 48 hr and those plants determined as resistant showed no vein-clearing symptoms after 14-21 days and reacted negatively in tests with the enzyme-linked immunosorbent assay (EIA). The resistant plants were retested by caging viruliferous leafhoppers on the youngest leaf for 48 hr and by EIA 14-21 days later.  
* c Values of chi-square.  
* d Probability of chi-square.

### TABLE 2. Segregation for resistance to maize chlorotic dwarf virus (MCDV) in plants derived from various generations of maize x Zea diploperennis backcrossed progenies.

<table>
<thead>
<tr>
<th>Generation</th>
<th>No. plants</th>
<th>Percent resistant plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC1</td>
<td>9</td>
<td>67</td>
</tr>
<tr>
<td>BC1-S1</td>
<td>21</td>
<td>195</td>
</tr>
<tr>
<td>BC1-S2</td>
<td>37</td>
<td>112</td>
</tr>
<tr>
<td>BC2</td>
<td>6</td>
<td>137</td>
</tr>
<tr>
<td>BC2-S1</td>
<td>43</td>
<td>228</td>
</tr>
<tr>
<td>BC2-S2</td>
<td>15</td>
<td>66</td>
</tr>
<tr>
<td>BC3</td>
<td>45</td>
<td>359</td>
</tr>
<tr>
<td>BC3-S1</td>
<td>31</td>
<td>193</td>
</tr>
<tr>
<td>BC4</td>
<td>25</td>
<td>178</td>
</tr>
</tbody>
</table>

* a BC and S with subscripts indicate generations of backcrossing and selfing, respectively.  
* b Seedlings were exposed to leafhoppers viruliferous with MCDV for 48 hr and plants were classified as resistant which showed no vein-clearing symptoms after 14-21 days and retested negatively in tests with the enzyme-linked immunosorbent assay (EIA). Resistant plants were retested by caging viruliferous leafhoppers on the youngest leaf for 48 hr followed by EIA testing 14-21 days later.
effectively used recurrent selection to improve resistance to MCDV in *Z. diploperennis* appears to be controlled by relatively few genes, some with major effects. At least 10% of the plants segregated for resistance in most backcrossed progenies and up to 25% in selfed progenies. As self-pollination has not produced progenies approaching homozygosity for resistance to MCDV, some genes involved were assumed to have minor effects that are indiscrete in expression. These minor genes may also occur in such low frequencies that the number of individual plants required to provide a reasonable chance of including them may be prohibitive.

Recurrent selection has been shown to be an effective breeding method for concentrating genes for multi-locus controlled traits when a reasonably accurate phenotypic evaluation is possible. Jenkins *et al.* (1954) effectively used recurrent selection to improve resistance to *Helminthosporium turcicum* Pass. in inbred lines of maize. Similarly, this breeding method should be effective in improving MCDV resistance in maize. One factor operating in our favor is that resistance to MCDV can be determined prior to pollination. The method of Jenkins *et al.* consisted of bulking pollen in approximately equal proportions from 10 instant plants and placing it on the silks of the same plants. We propose to use a similar pollinating procedure, with seed from the hand pollinated ears mixed in equal proportions to produce the population of plants for the next cycle of selection.

As to the perenniality of these crosses, all F1 plants were perennial, as were some plants with an estimated 62.5% maize germplasm. It is possible to maintain these plants for several years by transplanting between field and greenhouse to avoid winter kill and to maintain favorable growing conditions. Transplanting is made relatively easy by removing top growth, which also stimulates regrowth. Regrowth occurs from above-ground nodes, as new tillers, and from rhizomes. Thus, once a desirable perennial phenotype is identified, it can be maintained as a breeding source for several generations. Tiller production by the perennial types considerably extends time of flowering, particularly pollen shed.

**LITERATURE CITED**


Developing Virus Resistant Commercial Maize Hybrids

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ABSTRACT


Commercial companies must have hybrids that not only perform well under optimum conditions but also that show consistent performance in stress environments. Hence, private companies include virus resistance as an important trait for developing hybrids for the areas where maize chlorotic dwarf, maize dwarf mosaic, and corn lethal necrosis can cause economic losses. Sources of resistance and screening methodologies have been developed by scientists at public institutions and refined by private breeders. Therefore, private breeders have been able to concentrate their research efforts on developing commercial hybrids with considerable success. With such close cooperation, response to threats from new diseases, such as corn lethal necrosis, has been extremely rapid.

Farmers seek maximum yields under optimum growing conditions but also strive for consistency of performance in stress environments such as disease epiphytotics. The latter is extremely important because cash flow is a significant factor in their farm businesses. As reported earlier in this colloquium, two virus complexes can cause serious economic losses in maize (corn) (Zea mays L.) in the USA; the maize chlorotic dwarf virus (MCDV)/maize dwarf mosaic virus (MDMV) complex in areas where johnsongrass [Sorghum halepense (L.) Pers.] is endemic (Gordon et al., 1981) and corn lethal necrosis (CLN), now confined to a small area in central Kansas and Nebraska (Uyemoto et al., 1980). [The CLN is caused by combined infections of maize chlorotic mottle virus (MCMV) with MDMV or wheat streak mosaic virus (WSMV) (Niblett and Clafflin, 1978)].

Commercial companies strive to provide new hybrids that meet the needs of the consumer. Disease resistance, including that to MCDV/MDMV and CLN, must be incorporated into hybrids that also have resistance to insect pests, good standability, and high yields under varying environmental conditions. We value information from public institutions on sources of resistance, inheritance of resistance, and screening techniques. With this information supplementing results from experiments of our plant pathologists, we plan our applied breeding programs to develop inbreds and hybrids needed for each ecological zone.

THE MCDV/MDMV COMPLEX

MCDV/MDMV infected plants can be found throughout the johnsongrass areas of the southern USA, and resistance to these two viruses in corn is due to completely separate genetic systems. Artificial inoculation techniques have been developed for MDMV (Matthews, 1970), and we have developed multi-row inoculating machinery for our large scale screening programs. On the other hand, mechanical inoculation will not work for the leafhopper-transmitted MCDV (Nault et al., 1979). At present the most practical alternative is to identify suitable locations with heavy johnsongrass growth for screening under naturally occurring virus infections. Several seed companies as well as the University of Tennessee have nurseries in the Waverly, Tenn., area where both MCDV and MDMV are present (West et al., 1982). The Portsmouth, Ohio, area has been used for many years by the Ohio Agricultural Research and Development Center (OARDC), and they have released lines such as Oh7B and Oh514 that have been used in commercial hybrids and as source materials for new proprietary inbreds.

Several companies have been able to develop commercial hybrids with excellent virus resistance (Tables 1 and 2). Resistance to the two viruses seems to be independently inherited. Hybrids such as Funk's G-4525 and DeKalb's XL72B have low virus ratings at Waverly but have only moderate tolerance to MDMV strain A. Hybrids such as Funk's G-4606 and Pioneer's 3195 have resistance to MDMV-A, but virus ratings are much higher at Waverly where MCDV appears to be the major pathogen. In contrast, Funk's G-4740 and G-4776 appear to have resistance to both viruses. West et al. (1982) reported that in 37 samples collected at Waverly tested by the enzyme-linked immunosorbent assay (ELISA) (Engwall and Perlmann, 1972), only MCDV and MDMV strain A were detected. However, from the differential responses between certain hybrids inoculated with MDMV-A in the greenhouse and those infected in the field, we suggest that MCDV may be the more important component at Waverly. With the high levels of natural virus infection at Waverly, LSD's for field ratings were smaller than those for artificially inoculated MDMV-A plants rated in the greenhouse.
TABLE 1. Disease ratings for maize chlorotic dwarf virus (MCDV)/maize dwarf mosaic virus strain A (MDMV-A) complex from 1980-81 and 1981 obtained by Funk Seeds International.

<table>
<thead>
<tr>
<th>Hybrid</th>
<th>Company</th>
<th>MDMV-A infection rates</th>
<th>MCDV/MDMV disease index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Susceptible checks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N28 x Mo17</td>
<td>Pioneer</td>
<td>4.4</td>
<td>3.5</td>
</tr>
<tr>
<td>3369A</td>
<td>Pioneer</td>
<td>5.5</td>
<td>5.0</td>
</tr>
<tr>
<td>XL82</td>
<td>DeKalb</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>G-4636</td>
<td>Funk</td>
<td>5.0</td>
<td>3.5</td>
</tr>
<tr>
<td>MDMV-A resistant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G-4606</td>
<td>Funk</td>
<td>1.8</td>
<td>1.5</td>
</tr>
<tr>
<td>PX95</td>
<td>Northrup King</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>3147</td>
<td>Pioneer</td>
<td>2.5</td>
<td>3.5</td>
</tr>
<tr>
<td>3195</td>
<td>Pioneer</td>
<td>1.0</td>
<td>5.0</td>
</tr>
<tr>
<td>MCDV resistant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G-4525</td>
<td>Funk</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>XL72B</td>
<td>DeKalb</td>
<td>5.4</td>
<td>5.0</td>
</tr>
<tr>
<td>3160</td>
<td>Pioneer</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>MCDV/MDMV resistant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G-4740</td>
<td>Funk</td>
<td>1.5</td>
<td>1.0</td>
</tr>
<tr>
<td>G-4776</td>
<td>Funk</td>
<td>0.8</td>
<td>1.0</td>
</tr>
<tr>
<td>LSD (P=.05)</td>
<td></td>
<td>2.6</td>
<td>3.2</td>
</tr>
</tbody>
</table>

* MDMV-A ratings were obtained from artificial inoculation of maize hybrids in greenhouse trials, Bloomington, Ill., based on percent infected plants, with 1 = 0 to 11% infected and 9 = 89 to 100% infected.

* Disease ratings obtained from natural infection in a johnsongrass infested nursery near Waverly, Tenn. Rating index 1-9, with 1 = healthy plants and 9 = dead plants.

Because gene action for resistance to MCDV seems to exhibit very little dominance, both parents of a hybrid must be resistant. Several genes seem to be involved in resistance to both viruses (Findley et al., 1973). We have used traditional breeding methodologies involving F2 and backcross source material to develop the resistant inbreds that produce hybrids with acceptable yields and other agronomic traits. However, we have not been able to reach the levels of performance for all traits of importance because of the large number of genes involved. Hence, we have tried recurrent selection to develop new

TABLE 2. Disease ratingsa of maize hybrids tested for resistance to the maize chlorotic dwarf virus (MCDV)/maize dwarf mosaic virus (MDMV) complex near Waverley, Tenn., in 1981.

<table>
<thead>
<tr>
<th>Hybrid</th>
<th>Company</th>
<th>MCDV/MDMV Disease index</th>
</tr>
</thead>
<tbody>
<tr>
<td>SX544</td>
<td>Aztec</td>
<td>Medium Season Hybrids</td>
</tr>
<tr>
<td>XL72AA</td>
<td>DeKalb</td>
<td>6.6</td>
</tr>
<tr>
<td>XL72B</td>
<td>DeKalb</td>
<td>5.6</td>
</tr>
<tr>
<td>3328</td>
<td>Pioneer</td>
<td>3.6</td>
</tr>
<tr>
<td>G-4525A</td>
<td>Funk</td>
<td>3.1</td>
</tr>
<tr>
<td>3147</td>
<td>Pioneer</td>
<td>Full Season Hybrids</td>
</tr>
<tr>
<td>3160</td>
<td>Pioneer</td>
<td>2.3</td>
</tr>
<tr>
<td>XL394</td>
<td>DeKalb</td>
<td>2.5</td>
</tr>
<tr>
<td>G-4787W</td>
<td>Funk</td>
<td>3.2</td>
</tr>
<tr>
<td>G-4740</td>
<td>Funk</td>
<td>2.9</td>
</tr>
</tbody>
</table>

* Ratings obtained from West et al., 1982. Rating index 1-9, with 1 = no virus symptoms and 9 = dead plants.

* In 37 samples tested with enzyme-linked immunosorbent assay, only MDMV-A and MCDV were detected.

TABLE 3. Disease ratingsa for two cycles of full-sib selection for resistance to the maize chlorotic dwarf virus/maize dwarf mosaic virus complex near Waverley, Tenn., in 1982.

<table>
<thead>
<tr>
<th>Synthetic</th>
<th>Cycle</th>
<th>Disease index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deep South</td>
<td>C0</td>
<td>4.2</td>
</tr>
<tr>
<td>Rust Resistant</td>
<td>C0</td>
<td>4.5</td>
</tr>
<tr>
<td>Tuxpeno</td>
<td>C0</td>
<td>5.0</td>
</tr>
<tr>
<td>Hybrid Checks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G-4733</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>G-4740</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>G-4525</td>
<td>2.0</td>
<td></td>
</tr>
</tbody>
</table>

* Rating index 1-9, with 1 = healthy plants and 9 = dead plants.
TABLE 4. Disease ratings of maize hybrids and inbreds tested for resistance to maize dwarf mosaic virus strains A (MDMV-A) and B (MDMV-B) and to corn lethal necrosis (CLN) in 1980-81 (average rating) and 1981. Data obtained by Funk Seeds International.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>CLN susceptible</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N28 x Mo17</td>
<td>Pioneer</td>
<td>4.4</td>
<td>3.5</td>
<td>2.0</td>
<td>4.0</td>
</tr>
<tr>
<td>3369A</td>
<td>Pioneer</td>
<td>5.5</td>
<td>5.0</td>
<td>3.8</td>
<td>4.5</td>
</tr>
<tr>
<td>G-4636</td>
<td>Funk</td>
<td>5.0</td>
<td>3.5</td>
<td>1.5</td>
<td>2.0</td>
</tr>
<tr>
<td>CLN resistant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A632 x Pa405</td>
<td>DeKalb</td>
<td>3.5</td>
<td>2.5</td>
<td>0.8</td>
<td>1.5</td>
</tr>
<tr>
<td>B68 x Mo17</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XL25A</td>
<td>DeKalb</td>
<td>3.5</td>
<td>2.5</td>
<td>0.8</td>
<td>1.5</td>
</tr>
<tr>
<td>3195</td>
<td>Pioneer</td>
<td>1.0</td>
<td></td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>G-4531</td>
<td>Funk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3011X</td>
<td>Funk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSD (P = .05)</td>
<td></td>
<td>1.7</td>
<td>3.0</td>
<td>1.2</td>
<td>1.9</td>
</tr>
</tbody>
</table>

* Rating index 1-9 obtained from artificial inoculation in greenhouse trials, Bloomington, Ill., based on percent infected plants, with 1 = 0 to 11% infection and 9 = 89 to 100% infection.

* Rating index 1-9, with 1 = healthy plants and 9 = plants killed by CLN following artificial inoculation in field trials near Republic City, Neb., and Norton, Kan.

Source materials with high levels of disease resistance. This permitted us to concentrate subsequent selection on yield, standability, and other agronomic traits. Two cycles of full-sib recurrent selection tested at Waverly show progress in improving resistance (Table 3). The S2 lines were extracted from the Deep South Synthetic after the first cycle of selection for virus resistance.

CORN LETHAL NECROSIS

CLN is a very interesting disease because of the economic damage that results from a combination of two viruses, MCMV and MDMV or WSMV acting synergistically to make the combination much more severe than either virus alone (Uyemoto and Claflin, 1981). Although MDMV has been present in Nebraska and Kansas for many years, significant yield losses were not observed until MCMV appeared in this area in the mid-1970's. Until MCMV was found in Kansas, it was previously known to occur only in Peru. Thus far, the spread of the disease has been limited, but with the widespread occurrence of MDMV and the wide geographic distribution of vectors of MCMV [cereal leaf beetle, Oulema melanopa (L.); corn flea beetle, Chaetocnema pulicaria Melsheimer; flea beetle, Systena frontalis (F.); and corn rootworm, Diabrotica spp.], it appears that the potential range of CLN could be sizeable.

Research by University of Nebraska and Kansas State University plant pathologists (Doupnik et al., 1981; J. K. Uyemoto, personal communication) and by private companies indicates that some inbreds and hybrids with high levels of resistance to MDMV also have good resistance to CLN (Tables 4 and 5). B68, XL25A, and 3195 have good resistance to both strains of MDMV and

TABLE 5. Disease ratingsa of maize hybrids for resistance to corn lethal necrosis in Harlen County, Neb.

<table>
<thead>
<tr>
<th>Hybrid</th>
<th>Company</th>
<th>Disease ratingb</th>
</tr>
</thead>
<tbody>
<tr>
<td>A619Ht x A632</td>
<td></td>
<td>4.0</td>
</tr>
<tr>
<td>B73Ht x Mo17Ht</td>
<td></td>
<td>3.0</td>
</tr>
<tr>
<td>B68 x Mo17</td>
<td></td>
<td>2.0</td>
</tr>
<tr>
<td>Exp 6969</td>
<td>DeKalb</td>
<td>1.8</td>
</tr>
<tr>
<td>PX79</td>
<td>Northrup King</td>
<td>1.8</td>
</tr>
<tr>
<td>3194</td>
<td>Pioneer</td>
<td>2.0</td>
</tr>
<tr>
<td>LSD (P = 0.05)</td>
<td></td>
<td>0.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hybrid</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>B73Ht</td>
<td></td>
</tr>
<tr>
<td>Mo17</td>
<td></td>
</tr>
<tr>
<td>B64</td>
<td>2.3</td>
</tr>
<tr>
<td>B68</td>
<td>2.0</td>
</tr>
<tr>
<td>Pa405</td>
<td>2.0</td>
</tr>
<tr>
<td>LSD (P = 0.05)</td>
<td>0.8</td>
</tr>
</tbody>
</table>

* Ratings obtained from Doupnik et al., 1981.

b Disease rating index 1-5 with 1 = no virus symptoms and 5 = dead plants.
to CLN. In contrast, Va50 and G-4636 are very susceptible to CLN, but have good resistance to MDMV-B and some resistance to MDMV-A. Screening for resistance to MCMV alone has not been done on a large scale; however, since symptoms are relatively less obvious with MCMV alone, screening may not be effective. Hence, it seems necessary to screen for resistance to combined infections by both pathogens for best results.

We are grateful for assistance from Kansas State University and University of Nebraska plant pathologists in developing screening techniques and identifying sources of resistance to CLN. Funk corn breeders have obtained excellent success in screening for CLN at Republican City, Nebraska, and Norton, Kansas, as shown by the range of the rating index and the LSD. We use the mechanical inoculating machine and a mixture of MDMV and MCMV inoculum. Our plant pathologists have been producing the MDMV inoculum, but we have obtained the MCMV inoculum from the University of Nebraska. Funk has supported the CLN research at the University of Nebraska for the past 3 yr with a small grant. With seedling inoculation, susceptible inbreds such as Va50 will be completely killed prior to flowering. With greater vigor, susceptible hybrids are not usually killed, but few ears are formed.

Pa 105 has excellent CLN resistance but does not provide satisfactory performance in hybrid combinations in Kansas and Nebraska. This line should be valuable as a non-recurrent parent in backcross projects. B64 and B68 have good CLN resistance and can be used directly to produce satisfactory commercial hybrids such as B68 x Mo17. The related inbreds B64 and B68 were developed from B14 at Iowa State University in a backcross project to obtain resistance to the European corn borer [Ostrinia nubilalis (Hubner)]. The non-recurrent germplasm 41.2504B (PI 270297) was obtained from Argentina flint material through H. K. Hayes, University of Minnesota, and F. Dicke, formerly with the ARS-USDA stationed at the Ohio Agricultural Experiment Station, Wooster.

In the Funk screening program, we have found that nearly all B14 derived lines are susceptible to another important disease in Nebraska, Goss' bacterial wilt and blight [Corynebacterium nebraskense (Schuster, Hoff, Mandel and Lajan) Vidaver and Mandel], and the CLN resistant inbreds B64 and B68 are not exceptions. However, we have developed and are testing an experimental hybrid, derived from B64 source materials, with resistance to both CLN and Goss' wilt. Because of the high frequency of CLN resistant inbreds derived from backcrossing projects, we conclude that relatively few genes are involved. It appears that resistance has a moderate level of dominance as resistant hybrids can be obtained from the combination of a resistant line crossed to a moderately resistant line; e.g., B68 x Mo17.

Nault et al. (1982) reported that a recently discovered perennial teosinte, Zea diploperennis (llitis, Doebly and Guzman), has a high level of resistance to both MCDV and MCMV. We have obtained seed of this material for introgression into our elite breeding materials. We find that most unimproved exotic materials have many undesirable genetic factors tightly linked to genes for resistance which cause serious problems in developing high performing hybrids in short-term backcrossing projects. On the other hand, such material can be valuable sources of resistance in synthetic populations after several generations of random mating to break up some of the repulsion phase linkages.

**SUMMARY**

The major commercial seed companies have extensive programs to develop hybrids with resistance to the major virus diseases in the USA, viz MCDV/MDMV and CLN. From these programs, they have developed several resistant proprietary hybrids which are already in the marketplace. Research by public institutions has provided the foundation for these applied breeding programs and has greatly accelerated the development of commercial hybrids by the hybrid corn seed industry.

**LITERATURE CITED**


