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PROCEEDINGS

International Maize Virus Disease Colloquium and Workshop

Edited by

L. E. WILLIAMS, D. T. GORDON, and L. R. NAULT

AUGUST 16-19, 1976

OHIO AGRICULTURAL RESEARCH AND DEVELOPMENT CENTER
WOOSTER, OHIO 44691
USA

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PREFACE

Maize is currently the world's third most important food grain and is a staple in the diet of 500 million people in the developing countries of Africa, Asia, and Latin America (CIMMYT REVIEW, 1976). Maize also serves as a food for livestock that provides the principal animal protein for these people.

Half of the area planted to maize worldwide is in these developing countries, but they account for only one-fourth of the world's production. Diseases rank high among constraints to greater maize yields in these countries. Maize streak in tropical Africa and corn stunt in tropical Latin America, for example, have been identified by CIMMYT as important constraints to improved yields.

In view of the importance of maize in world food production and of virus and virus-like diseases in limiting maize yields in the developing countries, an International Maize Virus Disease Colloquium and Workshop was held on August 16-19, 1976 at the Ohio Agricultural Research and Development Center, Wooster, Ohio. The purposes of the colloquium and workshop were to determine the importance of maize virus and virus-like diseases in the agriculturally developing countries and to explore means to control these diseases through an international cooperative program. Participants were maize workers from various countries in Africa, Asia, and Latin America and from the United States. The colloquium and workshop was jointly sponsored by the U.S. Agency for International Development and the Ohio Agricultural Research and Development Center.

The editors assume responsibility for changes to improve clarity and form of manuscripts and discussions.

L.E. Williams
D.T. Gordon
L.R. Nault

ACKNOWLEDGMENTS

Grateful acknowledgments are due to: G.B. Baird (USAID) for assistance in planning; C. Johnston (OARDC) for administrative leadership; E.H. Roche and K.H. Remy (OARDC) for technical assistance in publishing these proceedings; O.E. Bradfute, W.R. Findley, R.E. Gingery, J.K. Knoke, R. Louie, and Diane C. Robertson (OARDC-USDA maize virus disease research group) for assistance in planning and carrying out arrangements for the colloquium and workshop; E.A. Bosler, Joanne Hershberger, Bonnie Littleton, R.M. Ritter, W.E. Styer, and Laurie Waltz (OARDC) for assistance with arrangements for the colloquium and workshop and with the preparation of these proceedings; and V.D. Damsteegt, I.W. Deep, R.E. Ford, C. Johnston, B.L. Renfro, and R.W. Toler for serving as chairmen of the colloquium sessions.

We are grateful to the U.S. Agency for International Development and to the Ohio Agricultural Research and Development Center for their financial support.

D.T. Gordon, Chairman
L.R. Nault
L.E. Williams
Colloquium and Workshop
Planning Committee

United States Agricultural Experiment Stations' Involvement in Maize Improvement

Roy M. Kottman

Dean, College of Agriculture and Home Economics, The Ohio State University; Director, Ohio Agricultural Research and Development Center; and Director, Ohio Cooperative Extension Service, Columbus, Ohio 43210.

We here at the Ohio Agricultural Research and Development Center are deeply honored to serve as the host institution for this International Maize Virus Disease Colloquium and Workshop. My assignment at this time is to indicate to you something of the size and scope of the State Agricultural Experiment Stations' involvement in research on maize, or as I shall refer to it, on corn. As all of you are well aware, total production of corn in the United States this year will be well over six billion bushels. The North Central States, from Ohio on the east to North and South Dakota, Nebraska and Kansas on the west, and from Wisconsin and Michigan on the north to Missouri and Illinois on the south, produce 85 percent of the U.S. corn crop. Of the 50 states, Ohio ranks sixth in corn production, behind Iowa, Illinois, Indiana, Nebraska and Minnesota, in that order. Sixty-eight percent of our U.S. corn crop is fed to livestock and poultry, 28 percent is exported, and the remainder goes into various industrial uses or is processed for human consumption.

In addition to the more than six billion bushels of corn produced for grain, our nation in 1975 produced some 113,336,000 tons of corn silage, most of which was fed to beef and dairy cattle. Total value of the crop was $17,793,041,000. Comparison of this figure with our 1975 total U.S. farm income of $91,380,000,000 indicates that our U.S. corn crop represents 19.5 percent of total cash farm receipts in the country.

Publicly supported research on corn last year utilized the talents of 180 scientists in the State Agricultural Experiment Stations and 146 scientists in the U.S. Department of Agriculture (USDA), a total of 326 scientists whose efforts can be translated into some $21 million of research expenditures. This is only about 2.6 percent of the $806 million combined total outlay for research by the State Agricultural Experiment Stations and the USDA in 1975. Clearly, we depend on the private sector for a good bit of the research being done on corn in the United States. Even so, I believe an argument can be made that our publicly supported research effort on corn is far less than the value and importance of this crop would suggest as being anywhere near adequate.

In terms of research on diseases of corn, the Agricultural Experiment Stations in the 12 states of the North Central Region, in cooperation with the USDA, currently utilize the talents of 20 scientists plus a sizeable team of support staff. This research program involves an annual expenditure of approximately $1.5 million. Comparable data for the entire United States would be 44 scientists and an annual expenditure of $2.4 million.

Unfortunately, we do not have a computer print-out on manpower or dollars committed to research on corn viruses *per se*. Suffice it to say that aside from the combined efforts of the Ohio Agricultural Research and Development Center and of the USDA personnel located on this campus, there is far less research effort underway on corn viruses than their demonstrated and potential reduction of corn yields would dictate.

It is my hope, therefore, that this Colloquium and Workshop will kindle both interest and support for considerably more research effort on maize virus diseases, here in the U.S. and in all countries represented at this meeting.

Maintaining a Solid Foundation for Corn Production

Earl R. Glover

Deputy Administrator, North Central Region, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Illinois 61600.

I live in the heart of the Corn Belt of the United States of America. In fact, headquarters for the Agricultural Research Service's North Central Region is located in Peoria, Illinois. This is the center of the state where growers last year produced the largest average corn yield of any in the United States. That yield was 7.27 metric tons per hectare (116 bu/acre). The North Central Region last year produced 124 million metric tons of corn (4,866,492,000 bushels), which was 84 percent of the total U.S. corn production in 1975 and about half of the world production.

By the way, a farmer in McLean County, Illinois, established a new world's record for corn production last year. He grew 338 bushels of corn on 1 acre of land or 277 quintals per hectare. That, at least, sets a standard to aim at.

My position in this region provides me with a good overview of agriculture and our research in the North Central United States. About half of the 600 ARS scientists in this region are located on land grant university campuses, where many of them work cooperatively with state scientists.

With this background, I will emphasize selected corn research in this region and highlight some of the achievements at other locations where corn research is carried out by this agency.

Secretary of Agriculture Earl L. Butz said recently (1976 Corn Annual) that corn has been a catalyst in our agricultural and national development. He reminded us that corn has had a great deal to do with the size of our farms, the mix of livestock and crops, the mechanization of agriculture, and our total feed and food supply. Without corn, we could not have moved nearly as swiftly to the point where fewer than 5 percent of our people can supply the food for the other 95.

Corn yields worldwide have grown by more than 2 percent per year during the past two decades — to give an average yield of 2.7 metric tons per hectare. This is roughly 150 percent of the average yield of all grains, which again emphasizes the role corn can play in stimulating economic growth and stability and in providing a food source in many parts of the world. As you may not know, we may export nearly 30 percent of the record 1975 U.S. corn crop.

More extensive corn production in the United States has, however, brought problems: less crop rotation, higher plant densities, heavier rates of fertilizer, use of marginal land, and heavier equipment. These have greatly increased threats of infestations of weeds and insects, outbreaks of disease, losses of soil and water, compaction of soils, greater energy requirements, and pollution of lakes and streams. Because of increased demands for food, we cannot retreat to less intensive production, nor can we expand our acreage substantially. Some land just is not suited to corn production. The answers lie in improved technology that supports more intensive production.

Studies to gain improved technology for corn production are conducted by about 40 ARS scientists at 15 locations. Most of these scientists are in the North Central Region. Annual ARS support for corn research amounts to a little over $6.0 million.

As scientists concerned with corn diseases, you know far better than I that the double-barreled threat of genetic vulnerability and continuous corn is a primary challenge. I am pleased that much progress is being made to reduce this threat, but we have a long way to go before we pass the danger mark.

Scientists of this agency have launched a multipronged attack in cooperation with universities and industry. Through the use of recurrent selection they are utilizing exotic strains of corn to broaden the genetic base of hybrids and to reduce genetic vulnerability in hybrid corn. They are also determining how best to forecast outbreaks of disease; developing inbred corn that resists blights, anthracnose and downy mildew; studying exotic diseases to determine the conditions causing outbreaks and the resistance and susceptibility of corn; stepping up efforts to preserve corn germplasm reserves; and searching for safer and more effective chemicals to control weed and insect pests, disease vectors, and diseases.

You are aware that the southern corn leaf blight (*Helminthosporium maydis*) epidemic in 1970 emphasized the concern in this country about the genetic vulnerability of corn. This epidemic set off an intense series of research activities. Now, most corn hybrids are produced using N or normal cytoplasm that is resistant to the toxin produced by race T of the southern corn leaf blight pathogen. However, because the parental lines currently used frequently have similar genetic backgrounds, the vulnerability of present hybrid corn to insect pests and other disease organisms is still of major concern in 1976.

Agricultural Research Service scientists are reducing genetic vulnerability by using recurrent selection to improve breeding populations from which improved parental lines are selected. Small, step-wise changes are made in breeding populations by repeatedly selecting plants for desired traits and intensifying these traits by mating those selected. Results show that this cyclical selection procedure improves the performance of breeding populations. These improved populations provide inbred lines not related to others used. However,we cannot become complacent. Every effort must be made to widen the corn germplasm base and must include long-term research to incorporate exotic materials from diverse regions into adapted U.S. corn strains.

Much fundamental knowledge is needed about pathogens and conditions that cause disease outbreaks or epidemics. Scientists are studying several important diseases including southern corn leaf blight, anthracnose, and downy mildew from this standpoint. Downy mildew, for example, is a potentially serious disease for which control methods and resistance in commercial varieties are inadequate. Limited information is available on the epidemic potential of downy mildew (*Sclerospora sorghi*) in this country, and no information is available on the exotic strains of this pathogen.

Downy mildew was first observed in the United States in 1971 in the state of Texas. It has since spread to 12 states, as far north as Kansas and Illinois, eastward to Indiana, and southeast to Georgia. Failure of the disease organism to overwinter in the Corn Belt has probably been the major deterrent to the spread of the disease within this area.

Extensive screening of corn lines is now underway to find resistance to downy mildew. This effort is aided by a new mass screening technique developed by one of our plant pathologists in Texas. The technique is excellent for selecting downy mildew-resistant strains from a mixed population. Progress is also being made in developing inbred corn that resists northern corn leaf blight, anthracnose, and Stewart's wilt.

Increasing world trade and international travel multiply the risks to world crops from plant diseases that are now confined geographically. For this reason, we have 10 plant pathologists, an entomologist, a plant physiologist and support staff who work fulltime developing new knowledge on the vulnerability of major U.S. crops to exotic diseases.

Advances in technology in one farming operation often outdate technology in another. United States corn producers know this well. Technology represented by the picker-sheller has outstripped the older technology of storing and handling corn. Damage to kernels during harvesting has increased, and picking now begins while corn is still wet — frequently in hot weather at summer's end. This high moisture — often 30 to 35 percent when harvest starts — encourages the growth of molds, which can spoil many million bushels of corn every year. As you know, some of these at times produce toxins — for example, aflatoxin produced by the fungus, *Aspergillus flavus*.

Both temporary and long-term solutions to the problem of moldy corn are being sought by our scientists. Progress has been made. 1) Wet corn can be aerated with refrigerated or natural air, depending upon the season, for short-term storage until the grain can be dried or fed to livestock. Aeration should begin immediately after harvest. 2) Corn contaminated with aflatoxin can be detoxified by circulating gaseous ammonia in a specially sealed steel bin. Ammonia inactivates the toxin, as verified by chemical assays and by feeding tests with ducklings, broiler chickens, and rainbow trout. Feeding tests that may lead to approval of the process by the Food and Drug Administration are underway at Athens, Georgia. Ammonia also preserves corn. 3) A plant physiologist is seeking answers to the question: Can corn be bred for faster dry-down to speed up and lessen drying cost? Factors determining the rate of dry-down in corn have been very difficult to quantify. However, we know this is a trait that can be controlled genetically. Scientists are now attempting to identify inbred lines that contribute fastest rates of dry-down to resultant hybrids. Then it will be a question of whether this trait can be bred into hybrids without sacrificing other desirable characteristics.

As I stated earlier, the need for food and feed means intensive production, including corn after corn on the same land. What effect does continuous corn have, for example, on populations of corn rootworms — an insect that continues to develop resistance to the better insecticides?

More than one-fourth of the total U.S. corn acreage is now being treated annually for the control of corn rootworm. Half of those treated acres receive the chemical Furadan, to which corn rootworms are becoming resistant. Our entomologists tell me that none of the alternatives to insecticides — biological control, host plant resistance, crop rotation — seem particularly promising at this time. They have discovered a sex pheromone for the rootworm. Although the pheromone will not control the insect, it will help detect rootworms and enhance studies on what to do about the problem.

You and I know that years are involved in finding and testing biological controls against insects, and once they are developed, additional years are required before they can be approved and registered. Fifteen years elapsed between the

discovery, by an ARS entomologist, of the first viral insecticide and its registration. The virus is specific to and controls the insect known variously as the cotton bollworm, the tobacco budworm, and the corn earworm.

As another example, it will take an estimated 15 to 20 years before half of the U.S. corn acreage is planted to hybrids that resist both the first and second generations of the European corn borer. Breeding populations were made available to hybrid producers in 1975. The dual resistance was found by ARS scientists in exotic corn from Colombia, South America.

My discussion so far has dealt with problems of corn production. I would like to speak briefly about two significant developments involving the nutritive value of corn. This nation's largest dry corn miller began tooling up last year to produce corn germ flour based on a development by cereal chemists and engineers of this agency. The flour is refined from germ meal, historically an animal feed. It will be marketed as a meat extender and a protein fortifier in baked goods, snacks, and breakfast cereals. The flour is about one-fourth protein, one-half carbohydrate, and one-tenth mineral. Essential amino acids of the flour compare favorably with chicken egg protein.

We do not have to be satisfied, however, with today's nutrient levels of corn; not when we look closely at the genetic pool of improvement available to us. Early this decade, two of our scientists, a chemist and a botanist, studied the primitive Coroico corn from South America. They discovered that Coroico has an extra-thick aleurone layer, which is the site of B-vitamins and high-quality protein. The layer in this primitive corn is two to five cells thicker than in U.S. commercial hybrids. Besides making up more of the kernel, Coroico aleurone contains a higher proportion of protein, 35 to 38 percent, than the dent aleurone, which has 20 to 22 percent. Moreover, Coroico corn contains more of the essential amino acids lysine, arginine, methionine, and threonine. Plant breeders are hopeful that this trait can be passed along to improve future corn lines.

The United States relies on other nations for help, as in the case of South American corn. We also share our knowledge overseas. Let me briefly outline some of the direct technological assistance this agency has provided through the Special Foreign Currency Research Program and through funded programs of the U.S Agency for International Development (USAID).

The Agricultural Research Service has awarded 36 grants to foreign institutes of maize production. This has involved 79 scientists (36 American and 43 foreign) in nine countries (Brazil, Egypt, India, Israel, Pakistan, the Philippines, Poland, Spain, and Yugoslavia). More than $3 million equivalent in foreign currencies were expended. What were some of the results? 1) A study in Yugoslavia resulted in new corn germplasm resistant to the European corn borer. 2) A study in India on the species of *Helminthosporium* (leaf blight organism), which occur on corn and grasses, proved basic to U.S. corn research. 3) One of the most notable discoveries in India showed that certain wild grasses are hosts for *Helminthosporium* species, including those capable of causing disease similar to southern corn leaf blight.

Teams of scientists are working in both West and East Africa under the USAID program to improve production of maize. They have introduced recurrent selection for population improvement in both areas.

The team in West Africa has shown that maize is well adapted in a broad belt of semi-arid tropics, and that yields of more than 8 metric tons per hectare (127 bushels/acre) are possible. The team in East Africa was instrumental in developing Improved Equador 573 that is presently used as the male parent of 77 percent of the hybrid maize seed planted in East Africa, a total of 428,000 hectares (1,057,600 acres). Among other accomplishments, both teams have established regional variety trials, which give breeders a chance to check the range of adaptation and disease resistance of new varieties growing under different conditions.

Certainly not all of the research I have discussed will be of direct interest to you. Some may have direct application, and you may want to know more than the sketchy information I have provided. I will be more than pleased to put you in touch with the proper scientific authority.

As an American, I am aware of the role corn has played in stimulating the economic growth of the United States and the direct and indirect importance of corn to our food base. I hope corn can play that same role in other parts of the world.

CIMMYT's Role in Maize Improvement in the Less Developed Countries

R.D. Osler

Deputy Director General and Treasurer, CIMMYT, Apartado Postal 6-641, Mexico 6, D.F. Mexico.

Maize ranks third in world cereal production as well as in the less developed countries. It is also commonly recognized that maize grows over a wider range of environments than any other cereal and is thus exposed to more hazards and is a higher-risk crop than any other cereal. Yet, under good management, maize will produce equal or superior yields. With poor management all the cereals, maize included, produce about 1 ton/hectare, the national average yield in the less developed countries. On the other hand, experimental yield trials around the world have demonstrated the yielding capability of maize. Virtually every maize-producing country is reporting experimental yields two to four times their national average. Nevertheless, national average yields in the less developed countries have not kept pace with population increases. Many demographers have agreed that population growth during the past decade in the less developed countries has been approximately 30 percent. According to the 1974 FAO Production Yearbook, maize production in the less developed countries increased 35 percent in the early 1970's, compared to the first half of the 1960's. But, most of the increase was due to increased area. According to FAO, yield increased only 11 percent during that time.

Economists at CIMMYT studied maize production in 55 developing countries in which it is a major food and found that during the 1960's: 1) population in 45 of the countries increased faster than maize yields, 2) 20 of the countries were importing maize at the close of the decade, and 3) only eight countries had average maize yields of 1.5 tons/hectare or more. For the years 1971, 1972, 1973, developing countries harvested about 48 percent of the world maize area but they produced only 22 percent of the grain. Average grain yields were almost four times greater in the more advanced countries.

Against this background of food and fuel shortages and high costs of fertilizer and other inputs, CIMMYT's maize improvement program is built around two closely related concepts: 1) developing improved technology and 2) delivering the technology at the farm level in the less developed countries. Our research program cooperates directly with national programs providing germplasm and practical in-service training at CIMMYT for their young scientists. We now work with scores of institutions that have: 1) formally constituted programs involving public agencies, such as ministries of agriculture, universities, and other organizations; 2) private interests, such as seed companies or other independent groups or associations; and 3) no formal designation. Our materials and information are freely available to all.

In cooperating with these programs, the aim is to develop appropriate technology as a joint venture so that the new varieties or practices are tested as an integral part of the process of development and delivery to the countries. Our training stresses on-farm testing with instruction and use of improved technological practices under conditions similar to those in the trainees' home country.

Our technological emphasis is on six major problems. First, tropical maize plants are generally too tall and frequently lodge. We are developing short, stiff stalks that will make a more efficient plant — a plant that produces more grain per kilogram of nutrients per hectare. Second, numerous insects and diseases reduce maize yields worldwide. In our maize improvement scheme we are seeking generalized resistance to the pest complex, in addition to other desirable agronomic traits. Third, research stations in most maize-growing countries get double the maize yields obtained by their better farmers, and four times the national average. CIMMYT seeks better agronomic practices to go with the newer varieties to fit better the needs of the average farmer and thus close part of the yield gap. Fourth, presently available maize varieties lack "adaptation" for many areas. Adaptation means the ability of maize varieties to produce satisfactory yields of grain over a period of years and a range of locations and under different conditions of temperature, moisture, and pests. We are developing varieties with adaptation to provide more stable performance and hence, fewer risks. Fifth, maize protein quality is generally considered poor due to an inadequate balance of amino acids. We are developing maize varieties with high quality protein that will compete in yield with normal maize varieties. Sixth, available maturity ranges of improved maize varieties are not sufficiently broad to cover the demands of farmers in developing countries. Farmers in rainfed areas need a short-season maize to fit a short rainy season or rotation with other crops. Farmers with ir-

rigated land may need a full-season maize variety. As a means of better explaining CIMMYT's maize improvement program we have constructed what we have named "CIMMYT's Maize Pyramid" (Fig. 1).

I now plan to briefly discuss each of the eight steps of the pyramid. Before doing this, however, I wish to make a few introductory comments. To begin with, we visualize CIMMYT's maize program as a continuous gene flow starting with new germplasm and ending with actual production in the farmer's field. In addition, any pollination we make should be based on the needs of some farmer somewhere in the world, with emphasis on the development of cultivars with broad adaptation, and better, more efficient yields per unit of inputs in the farmer's field. What we are now calling the "CIMMYT Maize Pyramid" is a formalization of activities that have evolved over several years. One of the earlier examples involved testing of progenies from our advanced unit population Tuxpeno 1 in Zaire in early 1974 and the subsequent formation, increase, and recommendation of a variety named Salongo.

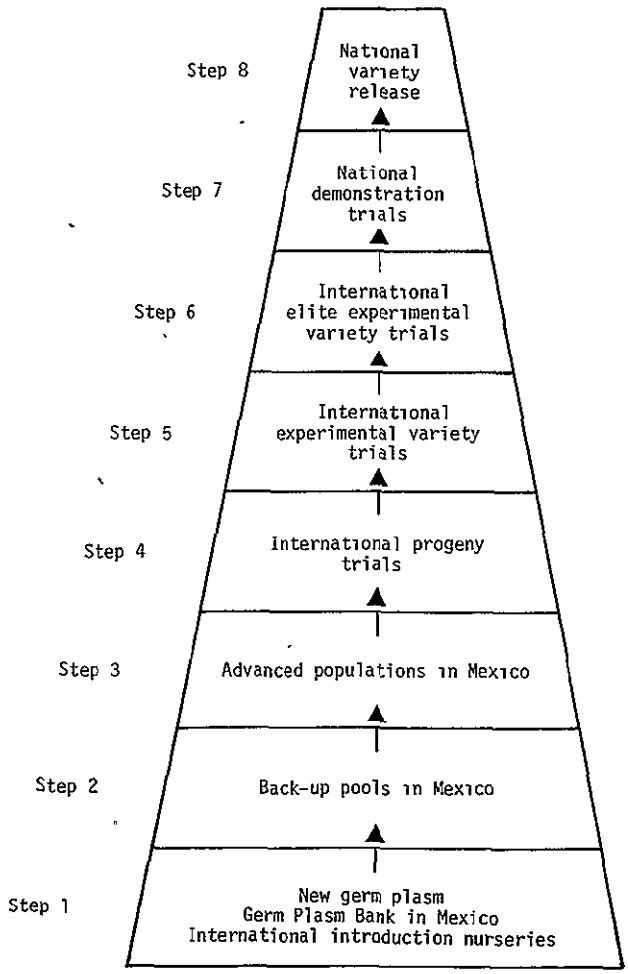

Fig. 1. CIMMYT's maize pyramid.

Step 1. New germplasm. — The germplasm bank in Mexico contains approximately 12,000 entries (varieties, lines, wild types) that are continuously being classified for economic characteristics. International introduction nurseries consist of materials from other countries and newly arrived in Mexico. Each year the best new materials are integrated into the back-up pools.

Step 2. Back-up pools in Mexico. — Here germplasm has been classified into pools (genetic soups) according to agro-climatic characteristics (lowland tropics, highland tropics, or temperate zone), grain types (dent, flint, floury, yellow or white endosperm), and length of growing season (early, intermediate, and late). Use of such categories results in 34 pools that are required to cover the developing maize producing countries of the world — 12 for the lowland tropics, 14 for the highland tropics, and 8 for the temperate zone. Back-up pools are grown twice a year in Mexico with use of a half-sib selection method. Field trials of progenies and pools, in Mexico and at selected sites outside Mexico, determine if and when selected progenies are integrated into the advanced populations.

Step 3. Advanced populations in Mexico. — Here, materials continue to be grouped by agro-climatic regions. But unlike the pools, these populations have been subjected to various generations of testing, selection, and recombination for better plant type; better disease and insect resistance; and better yield over environments differing greatly in altitude, precipitation, temperature, day length, and indigenous insects and diseases. This step of the pyramid involves production in Mexico of 250 full-sib progenies within each advanced population.

Step 4. International progeny trials. — The 250 progenies produced in Step 3 are sent to collaborators at five sites, worldwide, to be grown in 250 5-meter rows, with six local checks, forming a 16x16 lattice with two replications at each site. A sixth site is at one of CIMMYT's stations in Mexico. Ten or so best progenies, identified by the collaborator at each site, form one experimental variety for the following year.

Step 5. International experimental variety trials. — In Mexico during the winter season, CIMMYT staff intercross the 10 or so best progeny from each site and use reserve seed to produce an experimental variety that will be tested by collaborators at 20-25 sites, worldwide, during the following summer season. These experimental varieties were actually selected by the national program that tested and identified the best progenies in Step 4. Data from these 20-25 sites determine the following year's elite experimental varieties.

Step 6. International elite experimental variety trials. — Again, drawing on reserve seed of varieties which performed well in Step 5, CIM-

MYT in Mexico ships enough sets for trials at about 125 sites the following summer season. For the first time, some trials are seeded in farmers' fields. In addition, the first seed increase of the most promising experimental varieties will be made by some of the national programs. This seeding serves as a means of gaining a year in distributing seed to farmers, in case the simultaneous tests prove that one or more of the new experimental varieties merit further testing in national demonstration trials.

Step 7. National demonstration trials. — National programs along with a strong element of local farmer participation decide whether an elite experimental variety justifies wider demonstration in farmers' fields. CIMMYT can supply up to 49 kilograms of seed that each government can further increase. In large countries, demonstration trials take place at hundreds of sites.

Step 8. National variety release. — Based on worldwide data and farmer reaction to local demonstrations, each national program decides whether to recommend and use a new variety. If new varieties are to contribute substantially to total production, they must be accepted by local farmers and made available widely. Any characteristic or requirement interfering with acceptance and use limits the contribution of a new variety or practice.

Before discussing other aspects of CIMMYT's maize program, I would like to describe where we now are.

1) A number of varieties evolved from CIMMYT materials are actually being grown in several countries. A number of national programs have decided to move ahead immediately with seed increase and subsequent release of varieties on the basis of performance of progenies or experimental varieties without waiting for more extensive testing.

2) Ninety-six experimental varieties were tested in 1975. These tests were conducted at 34 sites in 21 countries (13 in Latin America, 4 in Africa and the Mideast, and 4 in Asia). Considering only yield, in 17 of the 21 countries one or more experimental varieties was significantly better than the best check variety of hybrid included in the trial.

3) On the average, the 96 experimental varieties out-yielded their original parental population by 11.2 percent.

4) National program requests for seed for testing in 1976 have far outstripped CIMMYT's supplies in a number of cases.

5) Data received to date clearly illustrate that many progenies and several experimental varieties are better than the best check varieties in most important characteristics for which data were reported.

I must hasten to comment, however, it is also clear that much remains to be done in certain regions before better varieties will be available for farmer use.

In addition to research on critical technological problems in maize production, we work directly in training staff for national programs in developing countries. CIMMYT offers several kinds of training and experience to maize scientists, primarily from Asia, Africa, and Latin America: 1) in-service training: generally, 6 to 7 months residence in Mexico; 2) Master's degree program in cooperation with universities in various countries; 3) predoctoral fellows: 12 to 18 months in Mexico to do their thesis research under CIMMYT supervision; 4) postdoctoral fellows: 2 years' service as an associate on the CIMMYT staff; and 5) visiting scientists.

In-service training. — The present maize in-service training program is only 5 years old but already 227 participants have passed through the course, including 56 in 1975. The program receives about 50 trainees per year, one-fourth specializing in discipline oriented, on-station research skills and three-fourths in skills of production agronomy. This latter group is involved in research on station as well as farmers' fields and in the integration of the various factors impinging on efficiency of farm production.

In-service training is designed to develop skills in field research, production management, and laboratory techniques; to give experience in an interdisciplinary team effort; and to teach the relationship between improved technology and development. One feature of production training is the laying out of agronomic trials on private farmers' lands and organizing field days for farmers. This work is performed by trainees in Vera Cruz state under supervision of CIMMYT training officers and the Mexican extension service. One type of field trial taught to trainees is the "maize diamond" which contains four plots: 1) the farmer's variety grown with the farmer's production methods; 2) the farmer's variety grown with improved production methods; 3) an improved variety grown with the farmer's production methods; and 4) an improved variety grown with improved production methods. The range of yields within this demonstration, under Mexico's lowland tropical climate, is from 1.5 to 4.0 tons per hectare. This demonstration helps identify limiting factors in yield and permits farmers at a field day to select their own technology.

Training in national programs. — For a number of years we have recognized that neither CIMMYT nor its sister international centers could possibly train all the maize staff required by the less developed countries to efficiently and rapidly increase their national production capabilities. As one means of increasing CIMMYT's impact on

total training needs, we decided to concentrate more of our efforts on developing national "in country" training capabilities. Starting in 1974, CIMMYT offered in-service training for officers from national programs who were preparing to give short courses for production agronomists in their own country. Eight individuals have now been trained (three from Ecuador, three from El Salvador, one from the Philippines, and one from Pakistan). CIMMYT training staff in Mexico is occasionally lent to national programs outside Mexico to assist with local courses. During 1975, CIMMYT's maize training officer participated in short courses for 90 production agronomists in Pakistan and 25 in Tanzania. This training role is expected to increase.

Academic training. — During 1975, the maize program sponsored the training of one Master's degree candidate in Mexico, and 12 predoctoral fellows in the U.S.A.

In 1973, CIMMYT began a new approach to post-graduate degree studies in collaboration with Kansas State and Cornell Universities. This system consists of integrated thesis research by groups of graduate students, mainly from developing countries. The principal aim is to stress joint efforts in solving maize problems. For example, the Cornell team consists of six people from five countries representing the disciplines of agricultural economics, agronomy, plant pathology, plant breeding, biometry, and entomology. The Kansas State group had four students representing four countries: Pakistan, Zaire, Japan, and Cameroon; and three disciplines: breeding, agronomy, and plant pathology. Regular planning sessions are or were held every 2 weeks at both Cornell and Kansas State. During these meetings the advisor and students map their research course. The Graduate Committee for each student includes all the advisors for all students, thus aiding the interdisciplinary planning process. Thesis research is or was carried out at CIMMYT in Mexico and at Cornell and Kansas State, with the degrees being granted by the U.S. universities. The Kansas State group have completed their degrees, whereas those from Cornell plan to obtain a final cycle of data in Mexico in the summer and fall of 1976.

Although it is too early to evaluate this new approach, both U.S. institutions as well as CIMMYT presently conclude it should be continued and expanded. Since CIMMYT certainly cannot house all that is needed, the universities must find ways of doing the appropriate research on campus or at other institutions.

Postdoctoral fellows on the maize staff in Mexico have increased from five per year during 1970-73 to approximately 10 per year during 1974-76, and the number is expected to remain at the higher level through the remainder of the 1970's. At the beginning of 1976, the maize program had 11 postdoctoral fellows coming from seven countries (three from England, one from Germany, one from India, one from Japan, one from The Netherlands, two from El Salvador, and two from the U.S.A.).

During 1975, the maize program received 12 visiting scientists and 20 short-term visitors. Visiting scientists are senior crop researchers or experiment station managers who spend from 1 to 12 months at CIMMYT to become familiar with world germplasm and CIMMYT research methods, which may be used in their own national programs. Short-term visitors are agricultural policy makers and administrators who spend from a few days to a month at CIMMYT.

The network of alumni from this program is one of the strengths of CIMMYT's international work. The CIMMYT maize staff spent 1,207 man days in 1975 (about 3.5 man years) in consultation with governments of maize-producing countries of Asia, Africa, and Latin America, or in discussions of basic research at other research institutions. This consultation included participation in regional workshops, where research plans are made for the following year; observation of CIMMYT nursery materials grown by national programs; visitation of farmers' commercial fields to identify constraints; and meetings with national policymakers to discuss fertilizer supplies, maize prices, and other policy questions.

Maize Production and Improvement in the Tropics and Subtropics

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Maize or corn (*Zea mays* L.) is the third most important cereal crop of the world. It is grown and produced mostly in temperate areas, but is increasing markedly in the tropics and subtropics. Production has approximately doubled during the past 20 years in each of these three areas (Tables 1 and 2). In the true tropics the 108% production increase during the past 20 years is calculated to be divided equally (44% each) between area and yield increases (Table 1); within 34° latitude, the 99% production increase is also calculated to be nearly equal between area (42%) and yield (39.5%) increases.

Diseases rank high among the constraints to maize production in the tropics and subtropics and quite often determine whether or not corn is adapted to a particular area. Important diseases are the various stalk rots, the *Sclerospora*-incited downy mildew diseases, maize streak in Africa, and corn stunt in Latin America. Streak and stunt are the most important diseases in their respective regions. However, sorghum downy mildew (*Sclerospora sorghi*) may have replaced stunt as the major disease in Venezuela and is becoming increasingly important in some other Latin American countries. Sugarcane mosaic virus is prevalent throughout the corn growing areas of Asia. The improved varieties released to Asian growers are products of their own national programs and, thus, are considered highly vulnerable to attack by exotic viruses and the stunt agent.

TABLE 1. Approximate maize area planted and total production in tropical compared to temperate zones

	Zone		% Temperate /total
	Temperate	Tropical	
Area (000 ha)			
1952-56 (avg.)	61,391	24,627	71
1973-75 (avg.)	77,272	35,534	68.5
20-year increase (%)	26	44	
Production (000 metric tons)			
1952-56 (avg.)	129,408	25,438	83
1973-75 (avg.)	255,929	52,808	83
20-year increase (%)	98	108	

[a]Within 23.5° latitude.

Real danger exists to world maize production if a virulent strain(s) of maize streak virus moves beyond the African continent or if the corn stunt agent, maize chlorotic dwarf virus, or virulent strains of the maize dwarf mosaic virus move from the Western Hemisphere. My understanding is that the insect vectors and collateral hosts of these three diseases exist throughout the tropics and subtropics, and that the weather and cropping systems in these areas would promote high disease development.

CIMMYT has recognized streak, stunt, and the *Sclerospora*-incited downy mildew diseases as being among the most important diseases in these areas. They also realize the reduction in maize yields that could result if these diseases were to spread into new areas. Two laboratories each in Africa, Central America, and southeast Asia are collaborating with CIMMYT in an attempt to develop multiple resistance in their breeding populations to all three diseases. In this program identical sets of full-sibbed or self-pollinated progenies are planted in 2-hectare plots at six locations. Populations are then reformed at CIMMYT from the data supplied, and new progenies generated to form the next cycle of selection. This approach to reduce the vulnerability of world maize production to these three major diseases is applauded, but it represents only one facet in overall needs. Our concluding session permits the opportunity to discuss and plan other needs that can best be done on a cooperative basis.

TABLE 2. Approximate maize area planted and total production in tropical and subtropical compared to temperate zones

	Zone		% Temperate /total
	Temperate	Tropical	
Area (000 ha)			
1952-56 (avg.)	45,662	40,356	53
1973-75 (avg.)	55,300	57,506	49
20-year increase (%)	21	42	
Production (000 metric tons)			
1952-56 (avg.)	110,481	44,371	71
1973-75 (avg.)	220,553	88,177	71
20-year increase (%)	100	99	

[a]Within 34° latitude.

The Developing International Agricultural Research Networks

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The areas of concentration for U.S. development assistance programs are: food production and nutrition, population planning and health, and education and human resources development. Of these, food production and nutrition receives by far the greatest budgetary emphasis. This emphasis reflects the overwhelmingly rural nature of most developing countries, where it is not uncommon for 75 percent or more of the people to derive their livelihood from agriculture.

Seeking to better the position of the poor and knowing that the product of an unchanging system is constant, we seek opportunities to change the agricultural system, *i.e.*, to promote agricultural development.

The U.S. Agency for International Development (USAID) has development assistance programs in 64 countries. In each case, the agricultural development program is tailored to fit the country. A cataloguing of these programs would be unproductive, but I thought that a group of scientists and research administrators might be interested in two aspects of our agricultural programs that will probably increase in importance over the next few years.

Mosher (1) described five factors as essential for agricultural development: 1) markets for farm products; 2) production incentives; 3) transportation; 4) local availability of supplies and equipment; and 5) constantly changing technology.

Although these factors could be restated to have more or fewer components, they are useful for conceptualizing the agricultural process and planning its development.

As developing countries become more systematic in their development efforts, several countries are becoming more aware of opportunities to increase agricultural production through changes in the economic and political environment. They are upgrading their capabilities to analyze the interactions of factors affecting agricultural production, with the objective of more accurately predicting the effects of governmental policy alternatives. In cooperation with a few U.S. universities, AID is structuring a capability to help develop these analytical capabilities. Such systematic analysis tends to have its first effects on the marketing system. Changes in costs of inputs and outputs and in altered input-supply systems or output-marketing systems are frequent avenues for the impingement of national policy on agricultural production.

Changes in policies can be important for a developing country, but the more dramatic and sustainable changes in production result from changes in technology. Entomologists and pathologists will quickly remind us that a constant technology results in a regression of production, so we recognize that a constantly changing technology is central to the agricultural development process.

Leaders of developing countries have been impressed by changes in agricultural technology. Their impressions have formed in response to the technology advances made in the late 1960's but, unfortunately, many nonagriculturists continued to look for "miracle seeds" to solve their food problems. Part of our task is to convince nonagriculturalists that advances in technology will result from concerted, diligent efforts, and not imported miracles.

Researchers in developing countries know that imported technology is valuable, but they also know that it can seldom be directly applied. Instead, it has to be molded and polished to fit local conditions. The fitting of imported technology to the local environment requires the same resource as does the ability to generate technology — a research system. Structuring of a research system in the developing countries is thus being belatedly, but increasingly, recognized as an integral part of the development process.

Few countries can afford to trace the leisurely path of research development used by many of the more developed ones. Time is pressing, and resources are scarce. We thus find several of the more progressive developing countries structuring a research system that is immediately linked to farmer and extension service. The system tries to routinely identify and solve the cultivator's problems. In consideration of the demand for scarce resources, the research system subjects itself to a rigorous discipline of defining objectives, methods, and resources with frequent internal reviews of progress. It is a demanding, but rewarding, system as many scientists from developing countries will appreciate.

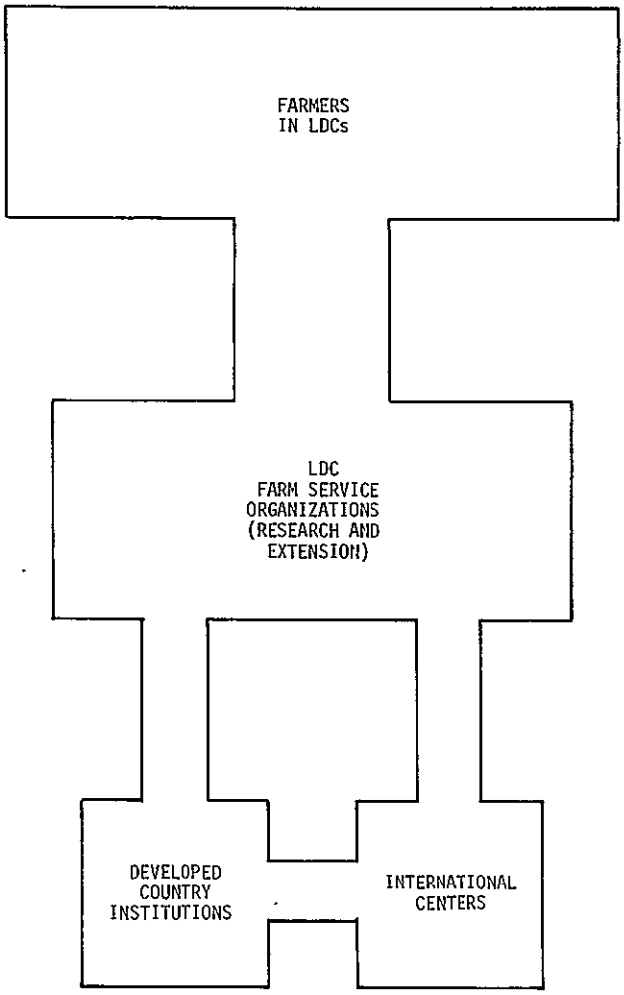

Fig. 1. Diagram representing research network extending from LDC farmer to international centers and institutions in the more developed countries

Resources are always scarce, and the developing countries are alert to the wealth of knowledge available in the international centers and institutions of the more developed countries. They are responding by linking their research system into a network of research capabilities that extend from the LDC farmer through the LDC research and extension system to the international research centers and the institutions in the more developed countries (Fig. 1).

Several of these networks are now functioning, and in USAID we see the strengthening of these networks as a major asset to the developing countries. Since the weakest link in these networks is often the national research and extension systems in the developing countries, we seek to strengthen this component. In many cases, the strengthening of a national research system can best be approached by channeling resources through a major research program, such as the one around maize.

The international maize research network is one of the oldest, most developed, and most effective. A few years ago it was remarkable to have a research team of a plant pathologist and an entomologist jointly planning and executing a research effort. Today it is common to have a research program being jointly planned and executed by scientists of 8 or 10 disciplines, who work in different departments, institutions, states, and countries. Information and research materials flow easily and swiftly among all participants, and every participant shares in the accomplishments of the total program. I think that agricultural research is proving to be one of man's first successful international efforts. I congratulate you for being a part of the international maize research program.

LITERATURE CITED

1. MOSHER, ARTHUR T. 1966. Getting agriculture moving. Agricultural Development Council, New York. 191 p.

Improvement of Maize Production in Developed and Less Developed Countries: Need for International Cooperation

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Maize is one of the more efficient converters of solar to nutrient energy. Among the cereals it ranks third in world production, exceeded only by wheat and rice.

During the past two decades there has been a marked increase in acreage planted to maize in many developing countries. This increase has not always been accompanied by greater yields per unit area of land. There are many problems to be overcome before maximum yields are approached. Diseases of the crop are only one of the several known limiting factors. Maize virus diseases may not always be the leading production problem, but they are omnipresent and take their toll. They potentially are always a major threat. However, even a relatively small percentage loss in this day of food shortage is intolerable, if we have means to prevent it. Perhaps this meeting will give us relevant information from around the world.

Twenty years ago, four diseases of maize in the United States were classified as being incited by viruses. The causal agent of one of these, corn stunt, has since been shown not to be a virus, but rather a mycoplasma-like organism. Up to that time, all of these diseases were of little or no economic importance. In 1960, however, it was pointed out that viruses of maize, because of their apparently high mutation rate and ability to spread rapidly, had potentials of inciting economically important diseases of maize in the United States. The relatively narrow genetic base of U.S. maize germplasm increased the likelihood of a widespread problem.

This problem developed a few years later when, in 1963, what appeared to be corn stunt became increasingly prevalent and severe in a number of southern states. About the same time, maize dwarf mosaic (MDM) was identified in the Ohio River bottomlands. It is believed now that maize chlorotic dwarf virus (MCDV) was present also and, when alone or in combination with MDMV in the same plant, caused serious losses in yields over wide areas wherever the collateral and overwintering host, Johnsongrass, was abundant. It should be emphasized that this latter virus (MCDV), since it was only vector transmitted, was not discovered until nearly a decade after the discovery of MDMV. The maize chlorotic dwarf virus proved to be a new type of maize virus. Subsequently, strains of both MDMV and MCDV were described. Corn mosaic, a disease first recognized in Hawaii and later in other tropical regions, also has recently been tentatively identified in the southern United States where its vector also appears to occur.

This relatively rapid change in only one facet of maize pathology may well be repeated one or more times in the developing countries where the production of maize is being newly exploited. Climates in many of these countries are favorable for rapid reproduction of the highly mutable causal agents and their vectors. Anyone with even limited acquaintance with maize virus diseases readily recognizes, and is struck by, the number and variety of virus-like symptoms on plants in tropical maize fields. Mosaics, ringspots, stripes, chlorosis, and necrosis are often not hard to find. Some of these are of an infectious nature and some probably are noninfectious and represent phenotypic expressions of abnormal physiology. There is, and will be, a need to identify properly these maize diseases and to characterize their incitants in these new geographical frontiers of maize production. Identification on the basis of symptoms alone is hazardous. Environment and host genotype interact and modify symptoms. Symptoms of a given disease are not consistent on all host genotypes in all environments.

Some confusion exists at present in the nomenclature of these kinds of maize diseases. The disease known as "corn mosaic" in one part of the world may not be the same disease with that name in another area. There is need to systematize the nomenclature, at least among the maize virus diseases. This will take sustained efforts and reliable data.

Another problem, perhaps the foremost, is the control of these diseases. This, it would seem, is most important in the less developed countries where food and feed supplies are often critically low. Host plant resistance must be identified and put into practical use, either directly or by transfer of genes conditioning resistance into adapted germplasm. Screening of genetic materials in a "troubled" area is an expedient method for a "stop-gap" effort, but is not as effective as when pathogens and strains are known and mechanisms of inheritance are determined in a long-range breeding program.

Identification of maize viruses is a primary task that can be accomplished in a number of ways. Such methods will be discussed during this Colloquium.

Thus, identification of maize virus and mycoplasma-like diseases, characterization of the morphologies of incitants, systematization of disease nomenclature, and control through host plant resistance are some of the problems calling for solution. These can be approached and more expediently solved through international cooperation.

Here at the Ohio Agricultural Research and Development Center (OARDC), a maize virus disease research team of specialized personnel trained in the disciplines of biochemistry, electron microscopy, plant virology, pathology, entomology, and maize breeding has already made outstanding contributions in this area of plant science. Much of the success of this group was due to a spirit of cooperation among the members of this group, members of the S-70 committee (regional committee dealing with maize virus diseases in the U.S.), and key researchers and contact people around the world. That type of cooperation might serve as a model for an international maize virus disease program. This research team at OARDC could well function as an integral part of an international cooperative effort to solve the many problems associated with maize virus and mycoplasma-like diseases, and to contribute substantially to basic knowledge of this subject.

There are a number of other agricultural research organizations, centers, and institutes, strategically located in various parts of the world, that are intimately associated with the crop production problems in the respective areas. These organizations have, in relatively short times, established excellent reputations. One could cite numerous instances of the efforts of these research bodies in increasing food and feed production in the world.

It is suggested that perhaps these institutions and organizations and OARDC might combine efforts to form a productive, international cooperative for solution of maize virus disease problems on a worldwide basis. Presently, there is no group dealing solely with this complex problem.

In addition to both the practical and fundamental research endeavors that an international cooperative of this kind could undertake, the OARDC might well serve as a training center for personnel from the many countries where maize virus disease problems occur. Training of personnel is already being done by some of the international centers and institutes, but this is mainly in disciplines other than maize virus diseases.

Details of such an international cooperative enterprise would have to be worked out; certainly a number of problems would have to be surmounted. It would seem, however, that many basic components of such a project are already at hand. It remains to coordinate these into a functioning whole.

It is imperative that we now dedicate ourselves to responsible and truly cooperative efforts around the world to combat hunger. Scientists, particularly those in agriculture, have worked in a spirit of cooperation and sharing of information. But, too often our information is only widely disseminated through scientific publications with its great loss of time before application. We need, through truly cooperative efforts, free exchange and input of ideas and data as research programs progress, and wider availability of pre-publication information.

Discussion Following Morning Session

R.I. Brawn: Dr Kottman, do you have any figures on private sector scientists working on corn?

R.M. Kottman: No, I do not. This is one of the jobs that we have approached in the Agricultural Research Institute. We made a guess on that in 1966 but I do not recall the figures on corn At that time I believe that private sector research was approximately the same size as was publicly supported research. I have a feeling that privately supported research has declined in this intervening period relative to the publicly supported research Publicly supported research itself has not done too well. I would say that we do not have nearly the amount of impetus that we ought to have in many areas. I think we would all agree with Mr Glover that we have great opportunities to provide assistance on many problems facing agriculture around the world, if we could somehow obtain a bit greater resources through either the private or public sectors or both. We are working on this again with members of the Agricultural Research Institute Hopefully, we can get enough enthusiasm going to pull together figures from various private companies. It is difficult — we have good cooperation from some companies and somewhat less from others. So, we shall have to work on it.

L.M. Josephson: I like the idea that Dr. Ullstrup mentioned about the people here in Ohio and those in S-70 serving as a nucleus for virus research, worldwide, as well as serving as a training center It is, probably, one of the most refreshing ideas that has come out in a long time We have been struggling in S-70 as to what the future would be Some states are more or less curtailing work they have been doing on corn viruses and look to Ohio to carry it on We in S-70 are still planning to continue our program, but there is a problem of how we are going to initiate new research We would like to do work on modeling and other research but no funds are available Probably, Dr. Kottman would have some good ideas of how we might go about getting funds from Federal agencies and private industry.

R.M. Kottman: I think it is a good idea, Dr. Josephson, to get a focus on a problem like this so that Congress can see and understand what we are talking about If we just talk about research in general, legislators, no matter what country we are talking about, have difficulty focusing on it. But if we take a crop like maize and talk about diseases, I think that they can understand.

We might put together a package that could be brought to their attention and be helpful in a joint venture with the USDA, the Ohio Agricultural Research and Development Center, and S-70 in bringing together a research project, which would encompass the North Central and Southern Regions. USAID, the Rockefeller Foundation, and others might be interested if we were to put together enough of a package that it would be significant in working with the scientists in this room from the several countries where assistance is needed Dr. Ullstrup should be congratulated for bringing this idea forward

I believe it is obvious that the increase in international travel and international exchange of goods will create more and more insect and disease problems. We had better get ahead of those problems, instead of getting behind, as we did with southern corn leaf blight In that instance we suddenly found ourselves with millions of dollars in losses. This could happen on an even broader scale with some of the corn virus diseases and they might well be more difficult to handle.

R.W. Toler: I would like to speak about an international group. Not only do we have some of the viruses mentioned from other countries, which we pick up occasionally in the United States, but it looks as though Texas serves as an introduction point. Dr. Bradfute and I found a rhabdovirus in corn in Texas that may have been introduced Now, it looks as though we might have another rhabdovirus that occurs in other parts of the U.S. The S-70 program, of which I was one of the original members, was first limited to the southern region but later it became an interregional project which included Ohio. This expansion has increased our research and brought free exchange of information, particularly in prepublication information on and approaches to identifying viruses by a teamwork approach. It has also improved our ability to identify germplasm which may have resistance I think that the idea of this type of cooperative program in moving to international cooperation at this time has merit.

Leafhopper-Transmitted Maize Rayado Fino Virus in Central America

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HISTORY AND DISTRIBUTION

The rayado fino disease of maize has been known in Central America since 1961. It was originally considered to be one of the four different types of "corn stunt" present in this area and transmitted by the leafhopper *Dalbulus maidis* (DeLong & Wolcott) [Ancalmo and Davis (1)]. Gamez (4, 5) demonstrated that the disease was caused by a virus transmitted by *D. maidis* in a persistent manner, and that the leafhopper could simultaneously transmit rayado fino virus (RFV) and corn stunt spiroplasma.

Rayado fino has been found in Mexico, Guatemala, El Salvador, Honduras, Nicaragua, Panama, Costa Rica, and Peru [Gamez (4, 5) and *unpublished*] The corn streak virus (BCSV) described in Brazil by Costa, *et al.* (3) and Kitajima, *et al.* (7) is serologically identical to RFV and similar in the symptomatology of infected plants, morphology of the virus particle, and ultrastructural changes induced in infected cells. BCSV and RFV are considered identical or closely related [Kitajima, *et al.* (7)]. The Colombian maize stripe virus (CMSV) [Martinez-Lopez, *et al.* (9)] is also similar to RFV in symptomatology of infected maize. Preliminary tests have shown these viruses to be serologically identical (Gamez and Martinez-Lopez, *unpublished*).

The virus and leafhopper vector have been observed from the warm coastal plains to the high, cool mountain valleys and plateaus of Central and South America.

SYMPTOMS

When infection occurs during the plant's early growth, symptoms appear within 8 to 14 days. At first, a few chlorotic dots develop at the base and along the veins of young leaves; in subsequent new leaves the dots gradually become more numerous and occasionally some fuse; finally, a characteristic fine stipple-striping of the vein prevails (Fig. 1). Long, continuous stripes are seldom found in the more tolerant varieties but are frequently observed in the more susceptible genotypes. The latter may also show varying degrees of stunting and chlorosis [Gamez (4)].

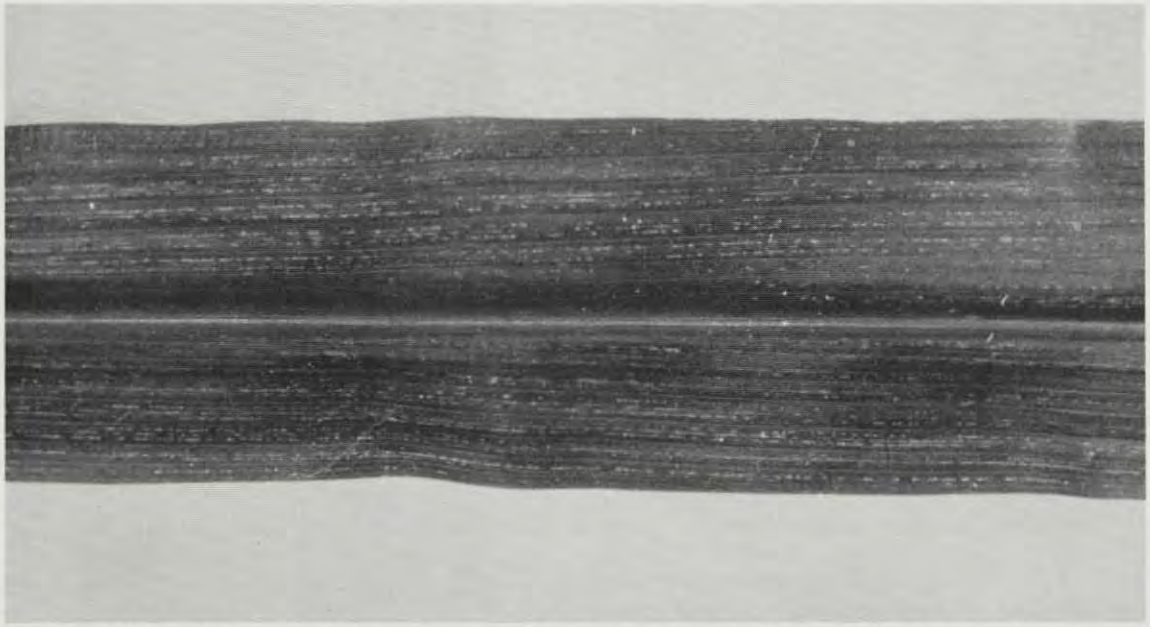

Fig. 1. Maize leaf infected with maize rayado fino virus showing characteristic fine, stipple-striping of veins.

Disease symptoms are more conspicuous during the first 3 or 4 weeks after infection; in tolerant varieties they gradually become milder and, eventually, as the plant nears maturity, are absent from newly formed leaves. Late infected plants may show only inconspicuous stipple-striping at the base of leaves or, frequently, no visible symptoms. Plants infected early produce ears of reduced size.

TRANSMISSION

The virus is transmitted by the leafhopper *D. maidis* [Gamez (4, 5)], its only known vector (Fig. 2). Attempts to transmit RFV by the planthopper *Peregrinus maidis* (Ashm.), by sap inoculating, or through seeds from infected plants were unsuccessful. Insects became viruliferous after injection of the virus into their haemocoel, or by artificially feeding them through membranes on virus preparations [Gamez (5) and *unpublished*, Paniagua and Gamez (10)]

Fig. 2. The leaf hopper, *Dalbulus maidis*, vector of maize rayado fino virus.

Transmission of RFV was characteristic of viruses that multiply in their leafhopper vector. Insects transmitted the virus after incubation periods of 8-37 days [Gamez (4, 5), Gonzalez and Gamez (6)]. Males had shorter incubation periods, were less efficient vectors and died sooner than females. Among females, 40 to 75% were active transmitters compared to 4.0 to 20% for males. No appreciable differences were observed between nymphs and young adults in their ability to transmit the virus [Gamez (5), Gonzalez and Gamez (6), Paniagua and Gamez (10)].

Infectivity was retained by *D. maidis* for 1-20 days; transmission by most individuals was intermittent and inoculativity decreased with time, but the virus was recovered from insects that had lost their abiltiy to transmit [Gamez (5)]. The shortest acquisition and inoculation feeding periods were 6 and 8 hours, respectively. The proportion of active transmitters in a colony varied from 11 to 34%. Colonies with 23 to 40% transmitters in the F_2 generation were developed by controlled matings and selection of viruliferous progeny. However, these more active colonies returned to the normal level of transmission following a few generations of random mating. Puncturing the abdomen of nymphs immediately before or after virus acquisition resulted in slight increases in the ability of *D. maidis* to transmit RFV [Paniagua and Gamez (10)]. Insects retained infectivity after moulting, but the virus was not transovarially transmitted to offspring of infected females.

RFV caused no observable deleterious effects on its vector, and longevity of transmitters and nontransmitters was similar [Gonzalez and Gamez (6)].

VIRUS PROPERTIES

Particles of RFV are isometric and 25-30 nm in diam [Gamez and Ramirez, 1975; Kitajima, *et al.* (7); Gamez, Fukuoka, and Kozuka, *unpublished*] (Fig. 3).

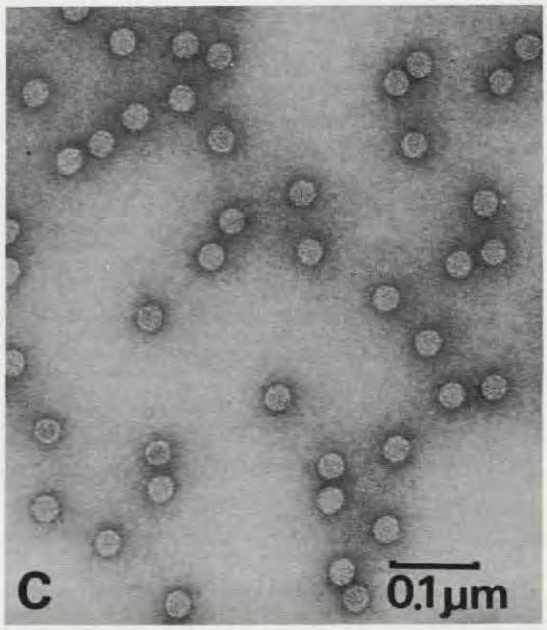

Fig. 3. Particles of maize rayado fino virus (25-30 nm diam).

Virus was extracted and isolated from infected maize leaves in 0.01 M phosphate buffer, pH 6.9, using methods involving a polyethylene glycol 6,000 (PEG) precipitation and sucrose density gradient centrifugation. Empty and full particles have been separated in density gradient columns. Infectivity of the preparations has been determined by vector injection or artificial feeding through membranes [Gamez and Ramirez, 1975; Gamez, Fukuoka, and Kozuka, *unpublished*].

Partially-purified preparations were used to prepare RFV antiserum that has permitted its detection in plants and the determination of its serological relationships to BCSV and CMSV [Gamez and Ramirez, 1975; Gamez and Martinez-Lopez, *unpublished;* Kitajima *et al.* (7)]. Electron microscopy of ultrathin sections of infected maize leaves revealed the presence of RFV particles in vacuoles of parenchymatous and epidermal cells [Kitajima *et al.* (7)]. Obvious alteration in cell organelles was not observed.

HOST RANGE AND EPIDEMIOLOGY

Only two species of Gramineae are known to be susceptible to RFV, *Zea mays* L. and *Z. mays mexicana* (Schrad.) Iltis. Other species of wild and cultivated grasses tested and found immune included: *Oryza sativa* L.; *Secale cereale* L.; *Triticum vulgare* L.; *Eragrostis* sp.; *Axonopus scoparius* (Flugge) Hitch.; *Digitaria sanguinalis* (L.) Scop.; *Paspalum conjugatum* Bergius; *Saccharum officinarum* L.; *Sorghum halepense* (L.) Pers.; *Tripsacum laxum* Nash; and *Coix lachryman-jobi* L. Other species of common wild herbs or grass-like herbs tested included *Setaria geniculata* (Lam) Beav.; *Cenchrus* sp. and *Cyperus tenuifolius* (Stend.) Dandy [Gamez (5), Paniagua and Gamez (10)].

Maize and teosinte are also the only plants upon which *D.maidis* is known to successfully complete its life cycle [Barnes (2), Pitre (11)]. Other grass species may serve as feeding hosts for the vector in the U. S. [Pitre (11)]. Certain undetermined species of wild grasses probably serve as hosts for *D. maidis* and RFV between maize planting seasons in tropical and subtropical areas of Latin America. However, maize is the only known host for both virus and vector in South America and most parts of Central America, since the distribution of teosinte is restricted to northern Guatemala and Mexico.

ECONOMIC IMPORTANCE

Yield losses on individual, early infected plants of local Central American varieties were 40-50% of the weight of the mature ear, but reached 100% in introduced foreign or newly developed maize varieties (A. Diaz, *personal communication*; Gamez, *unpublished*). Disease incidence was variable, depending on maize genotypes, geographic area, and seasonal and climatic conditions. Locally adapted varieties usually showed infection in 0-20% of the plants, but reached 100% in some of the more susceptible genotypes. Although RFV incidence appears to be gradually increasing in many areas of Central and South America, it is not as prevalent and economically important as the corn stunt disease.

CONTROL

Limited information is available on the behavior of maize materials to RFV. Observations carried out at experiment stations in Costa Rica and El Salvador showed that introduced lines and varieties were more susceptible than local ones. No immune varieties are presently available.

LITERATURE CITED

1. ANCALMO, O., and W.C. DAVIES. 1961. Achaparramiento (corn stunt). Plant Dis. Rep. 45:281.
2. BARNES, D. 1954. Biologia, ecologia y destribucion de las chicharritas, Dalbulus elimatus (Ball.) y Dalbulus maidis (DeL. & W.). Mexico, Secretaria de Agricultura y Ganaderia. Oficina de Estudios Especiales. Folleto Tecnico No. 11. 112 p.
3. COSTA, A.S., E.W. KITAJIMA, and S. C. ARRUDA. 1971. Molestias de virus e de micoplasma do milho em Sao Paulo. Resumos, 40 Cong. Soc. Bras. Fitopat.
4. GAMEZ, R. 1969. A new leafhopper-borne virus of corn in Central America. Plant Dis. Rep. 53:929-932.
5. GAMEZ R. 1971. Transmission of rayado fino virus of maize (Zea mays L.) by Dalbulus maidis DeLong & Wolcott. Ann. Appl. Biol. 73:285-292.
6. GONZALEZ, V., and R. GAMEZ. 1974. Algunos factores que afectan la transmision del virus del rayado fino del maize pro Dalbulus maidis DeLong & Wolcott. Turrialba 24:51-57.
7. KITAJIMA, E.W., R. GAMEZ, and M.T. LIN. 1975. Serological and histological comparison of rayado fino virus of maize from Costa Rica and the Brazilian corn streak virus. Abstracts, Ann. Meeting, Caribbean Div., Am. Phytopathol. Soc. p. 50.
8. KITAJIMA, E.W., T. YANO, and A.S. COSTA. 1975. Purification and intracellular localization of isometric particles associated with Brazilian corn streak virus. Cienc. Cult. (Sao Paulo). (In press).
9. MARTINEZ-LOPEZ, G., LUZ M. RICO DE CUJIA, and CONCEPCION DE LUQUE. 1974. Una neva enfermedad del maiz en Colombia transmitida por el saltahojas Dalbulus maidis (DeLong & Wolcott). Fitopatologia 9:93-99.
10. PANIAGUA, R., and R. GAMEZ. 1976. El virus del rayado fino del maiz: estudios adicionales sobre la relacion del virus y su insecto vector. Turrialba 26:39-43.
11. PITRE, H.N. 1967. Greenhouse studies on the host range of D. maidis, a vector of the corn stunt virus. J. Econ. Entomol. 60:417-421.

DISCUSSION OF R. GAMEZ PAPER

E.E. Rosenkranz: In Mississippi, we have been able to rear *Dalbulus maidis* and have them complete their cycle on *Tripsacum dactyloides*. This may be a different biotype of *D. maidis* from the one you have in Costa Rica. My culture was from the Boyce Thompson Institute. I do not know where Dr. Karl Maramorosch got his original leafhoppers.

R. Gamez: This is interesting, since *Tripsacum dactyloides* is, I understand, not very common throughout Central America. Your information is very valuable. I do not think it is in the literature, is it?

E.E. Rosenkranz: I do not believe so. *Dalbulus maidis* does not reproduce as well on *Tripsacum dactyloides* as on teosinte or maize, but it will complete its life cycle. I have maintained the leafhopper through the winter on *Tripsacum dactyloides*.

R. Gamez: I am certain that this leafhopper has hosts in addition to corn, because in our country we have only two seasons—rainy and dry. During the dry season, there is no corn whatsoever, but with new plantings, beginning the first of the rainy season, the virus and the leafhopper appear very rapidly.

G. Martinez-Lopez: What is the average temperature in the area where you find rayado fino and what is the altitude there?

R. Gamez: We have observed virus-infected corn at sea level elevations from Costa Rica to Nicarauga and bordering Guatamala. The average temperature is not less than 25 C, with the high between 35-40 C and the low somewhere around 18 C. These are not the areas where we find the highest incidence. Corn stunt is more prevalent in these areas. Maize rayado fino is masked by corn stunt in many cases. With increase in altitude, incidence of rayado fino increases. The virus is more prevalent in areas with average temperatures of 20-22 C; those occur at elevations from 1,000 to 1,500 meters above sea level. Naturally, latitude makes a difference and these regions are at 10° - 20° N latitude.

L.R. Nault: Drs. Bob Toler and Oscar Bradfute collected material with symptoms similar to those of rayado fino in Texas this summer. We transmitted a disease agent from their plants with *Dalbulus maidis*. Symptoms that you showed are similar to those on these plants. There was a latent period of about 6 days in the greenhouse in plants inoculated in the two-leaf stage. This latent period is much shorter than that for corn stunt spiroplasma. Would you comment on the appearance of these greenhouse plants in relation to what you see in corn infected with laboratory transmitted rayado fino virus?

R. Gamez: I am not at all surprised that you find rayado fino virus in the southern United States. Actually, I expected it to occur there. We have found the virus wherever we find *Dalbulus maidis*. I do not think this association is an accident. Maize and *Dalbulus maidis* have probably lived together for many hundreds of thousands of years. In other words, there is a close relationship between virus, vector, and host plant. With such a wide distribution, it is not surprising that with *Dalbulus maidis* in the southern United States eventually you will find rayado fino virus. As for the incubation period of the virus in the plant, with very susceptible materials under the proper environmental conditions I have observed symptom appearance within very short times after inoculation, for example 6 days as you mentioned.

R.W. Toler: Do you feel that the virus has been moving and spreading from where it was originally identified or have virologists only recently been looking for it in different locations?

R. Gamez: I think the virus has been around throughout this area for many years, and it just had not been detected and identified. My finding of this particular virus was a complete accident. I collected some leafhoppers in the field, because I wanted to start a colony for some work with corn stunt. The plants on which I reared these leafhoppers started showing the rayado fino symptom. From then on, I found the virus wherever I looked.

R.W. Toler: Do you think the virus has the potential to move to other areas and other countries?

R. Gamez: It is in all of these countries, already. According to the literature *Dalbulus maidis* will exist only in the lowland, tropical areas, but Dr. Martinez-Lopez has shown that it exists and thrives at 2,600 meters above sea level in the cool plains of Bogota in Colombia. It is likely that races of the leafhopper adapt to colder climates and are able to survive in cooler regions.

R.W. Toler: Do you think the distribution of the virus in known areas has been through the distribution of the leafhopper? Is it carried over from season to season in the leafhopper?

R. Gamez: Yes.

D.T. Gordon: Do you have any information on the relative importance of rayado fino virus and other viruses in Costa Rica?

R. Gamez: Yes. Rayado fino is an important virus in many cultivars, but it is not the most important problem of maize. Corn stunt is our most important problem throughout this area. Rayado fino would be second to corn stunt in this particular area, 1,000 meters above sea level. Other virus diseases, at least in Central America, do not appear to be as important as rayado fino or corn stunt. Maize dwarf mosaic virus occurs. We find it in different areas, but the materials we have appear to be rather tolerant. The incidence is low and it is difficult to find.

D.T. Gordon: How long have you seen maize dwarf mosaic virus in this area? Is it of recent occurrence or has it been there for some time?

R. Gamez: I isolated it when I first started looking about 10 years ago. So it probably has been around for a long time.

R.W. Toler: Do you have Johnsongrass in areas where you find maize dwarf mosaic?

R. Gamez: I do not think so.

R.W. Toler: Do you have any other strains of sugarcane mosaic virus in Costa Rica?

R. Gamez: Yes. We have the common sugarcane mosaic virus which is not, fortunately, very important.

L.R. Nault: Is there a break in corn crops in the Central American countries or do they overlap in time? In other words, what happens to the leafhopper? Where does it survive?

R. Gamez: No. Corn crops do not overlap. We have a dry season of approximately 4 months, and no corn is grown during that period. But there are many wild grasses growing at that time. Some maize is grown under irrigation in certain small isolated areas, but this does not really explain where the virus survives when there is no corn in the field. Obviously, there must be other hosts for the virus and the vector.

S.P. Raychaudhuri: For cereal viruses, grassy hosts are very important. Have you seen in nature any weed or grassy hosts around fields that could serve as a source of inoculum? This is particularly important in case of rice and wheat viruses.

R. Gamez: Grassy weed hosts were the first types that we tested for susceptibility to rayado fino virus and found them not susceptible. We have not looked for rayado fino viral symptoms in any crop other than maize. We are searching for additional host plants now. We have surveyed most of the common wild grasses in the highlands of Costa Rica, but we have not found hosts for both virus and vector.

V.D. Damsteegt: Is it true that *Dalbulus maidis* only lives 30 days or can it exist for a longer period?

R. Gamez: The life span varies mostly with temperature. It may live about 60-70 days.

B.L. Renfro: Has there been any concerted effort to breed for resistance to rayado fino?

R. Gamez: No.

B.L. Renfro: Are there indications that there is more than one gene involved? You are getting quite an array of reaction types.

R. Gamez: That is right. It is too bad that Mr. Diaz-Chavez of El Salvador was not able to attend this meeting, since he planned to present data on the behavior of a significant amount of genetic material that CIMMYT has been screening for corn stunt resistance in El Salvador. He did a thorough evaluation of rayado fino incidence, and he has some very interesting data. Aside from that there has not been any search for resistance.

B.L. Renfro: Could you concentrate genes for resistance?

R. Gamez: We have screened 200 to 300 materials that maize workers from Central America have sent us, and so far all these materials are susceptible.

L.R. Nault: Have you attempted to inoculate *Zea perennis*?

R. Gamez: No.

New Maize Virus Diseases in Colombia

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I wish to thank Dr. Paulina Beregoff Gillow of the Preventorio Arthur Stanley Gillow, Bogota, for the use of the electron microscope laboratory facilities.

This study was supported in part by the International Foundation for Science (IFS), Sweden.

ABSTRACT

Studies on maize virus and virus-like diseases in Colombia indicate that two predominant viruses are present. One of them, the virus del rayado colombiano del maiz, appears to be a new virus disease. It is transmitted by the leaf hopper *Dalbulus maidis* and produces bright yellow spots on leaves. The disease reduces plant size and causes high yield losses in susceptible cultivars, mainly in the highlands. The other produces mosaic symptoms in maize grown in the Valle del Cauca and may be a strain of maize dwarf mosaic virus. This virus also produces severe yield loss, particularly when the infection takes place early in the growing season. Besides these two virus diseases, the corn stunt disease agent was observed primarily in maize grown at 1,000 m above sea level but more recently at 2,800 m. Work has been done on characterization of the two viruses and on the identification of resistant or tolerant maize lines that may provide the means to overcome losses caused by these pathogens.

Maize is one of the most important crops in Colombia, where it is used for human food, animal feeds and industrial purposes. The crop is grown under a wide range of conditions: variable rainfall (with and without irrigation), from the high Andes Mountains to the lowland regions in the North Coast (0 to 2,800 m above sea level), and by farmers with all levels of technologies. The area planted to maize ranges from about 600,000 to 800,000 ha, with a tendency to plant less with a corresponding reduction in the amount of corn available (25).

Average national yield is low, mainly because of the low yields of farmers with small landholdings who do not use improved seeds and new technologies and who have to carry out most, if not all, planting activities with hand tools. Commercial farms that use appropriate agricultural machinery, fertilizers, improved seeds, herbicides, and insecticides have an average production of 2.4 metric tons/ha. In the Valle del Cauca such farms average 3.4 metric tons/ha.

The Instituto Colombiana Agropecuario, through its maize program, has invested a lot of effort that has increased yields from an average of 1.1 metric tons/ha during the 5-year period 1940-1945 to 1.4 metric tons/ha during the 5-year period 1970-1975 (a 27.27% increase) (25).

At this Institute, superior genetic materials were identified for each of the five regions (0-600, 600-1,200, 1,200-1,800, 1,800-2,400, and 2,400-2,800 m above sea level) and new varieties and hybrids were developed with resistance to rust, corn leaf blight, and some other diseases. However, insect resistance was largely ignored.

Recently, earliness, resistance to preharvest ear rots and some other diseases, and development of "multi-ear" varieties that can contribute to higher yields have been investigated.

In the last 3 years special attention has also been given to maize virus and virus-like diseases in Colombia. Two main virus problems have been found. The presence of what appears to be a new disease in maize, caused by a virus transmitted by the leafhopper *Dalbulus maidis* (DeLong & Wolcott) (Homoptera: Cicadellidae), has been observed in maize crops over 1,000 m above sea level. The name "virus del rayado colombiano del maiz" (VRCM) was proposed for this virus (6,10). Another disease, caused by a mechanically and aphid-transmitted virus, produces mosaic symptoms and the presence of ringspots in infected plants. This virus, which appears to be a strain of maize dwarf mosaic virus (MDMV), has been observed in maize crops in the Valle del Cauca (27,28,30). Besides these two viruses, the corn stunt agent has been found in the highlands, and some preliminary studies have been made (15,20).

VIRUS DEL RAYADO COLOMBIANO DEL MAIZ

Symptoms. — The virus del rayado colombiano del maiz causes conspicuous bright-yellow spots on leaves of more than 300 maize cultivars tested. The first symptoms appear as early as 3 or 4 days after inoculation as small spots of various sizes at the bases of new leaves. These spots become more numerous and fuse to form yellow stripes along the veins in subsequent leaves (Fig.

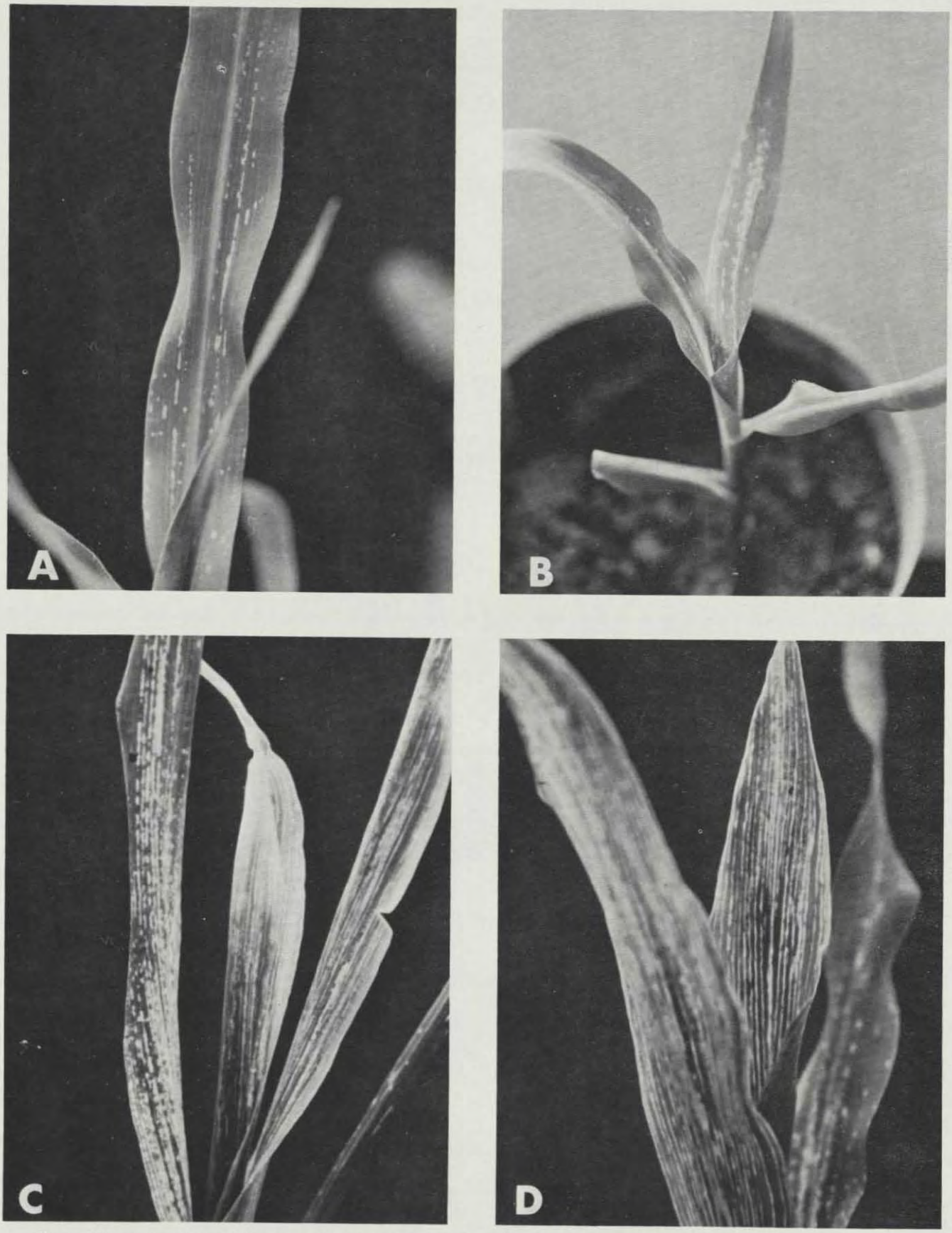

Fig. 1. Disease symptoms of the virus del rayado colombiano del maiz: A,B,) small spots at the base of the first leaves to develop symptoms; C,D,) bright yellow stripes in leaves of infected plants developing from fusion of small spots.

Fig. 2. Disease symptoms of the virus del rayado colombiano del maiz: A) darker-green areas in the non-chlorotic areas and large number of bright yellow spots; B) numerous yellow spots giving appearance of leaves almost completely yellow, with some wilting.

1). Sometimes the stripes are so numerous that leaves appear almost completely yellow (Fig. 2). In some plants severe destruction of the cells in the area of the lesion occurs and holes develop in the leaf blade (Fig. 3). These symptoms plus wilting are associated with the most susceptible cultivars (Fig. 2). In general, there is a darker green color of the non-chlorotic area (5, 10, 21, 22).

Different degrees of stunting occur in diseased plants, with stunting being more severe in plants infected earlier (10, 12, 13). Poor development of root systems occurs but is often not too conspicuous. Ears on diseased plants have very few or no grains (11, 12, 13).

There was no difference in the severity of symptoms at temperatures of 14, 18, 22, 26 or 30 C. But, high temperatures did reduce the number of infected plants, from an average of 23% at 14, 18, and 22 C to 19% at 26 C and 3% at 30 C, with average incubation periods of 18, 9, 9, 8, and 10 days, respectively (16, 17, 19). The average incubation periods in the plant at 18, 22 and 26 C were very close to those of the rayado fino virus of maize (RFVM), another maize virus transmitted by *D. maidis* (8 days at 25 C) (1, 2). Symptoms on leaves and the reduction in size of infected plants were more severe than the symptoms described

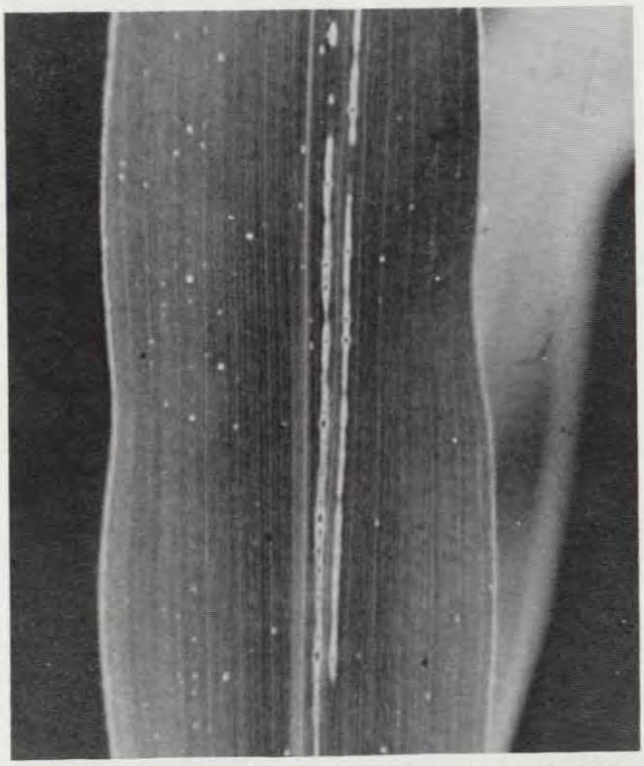

Fig. 3. Disease symptoms of the virus del rayado colombiano del maiz. Holes in the leaf blade resulting from severe destruction of cells associated with yellow spots.

for RFVM by Gamez (1, 2) and Gonzalez and Gamez (3).

Symptoms characteristic of VRCM in maize also have been observed on *Sorghum vulgare* var. *glutinosum* Pers. in the field, but they have not been observed on other crop plants or weeds growing in areas where the disease was observed in maize.

Transmission. — VRCM is not transmitted mechanically but it is by the leafhopper *D. maidis* in a persistent manner. There is no evidence of transovarial transmission nor multiplication in the vector. The virus has a long incubation period in the insect, with an average of 21, 22, 23, 24, and 24 days at 14, 18, 22, 26, and 30 C, respectively, and with minimum incubation periods of 11, 11, 13, 14, and 16 days (14, 16, 17, 19). Most of these incubation periods are longer than those reported for RFVM by Gonzales and Gamez (3), 10, 10, and 12 days for 20, 25, and 30 C, respectively.

The virus was transmitted by nymphs and adults and without differences in the efficiency by males or females (10, 11). Some insects were able to transmit the virus to all plants on which they fed, after the incubation period of the virus in the insect, but in others there was intermittence in transmission (10, 11).

About 10% of insects collected on infected plants in the field were able to transmit the disease (10), but this percentage was increased to 20-25% when the progeny of males and females that were efficient transmitters were used in transmission experiments.

VRCM can be transmitted simultaneously with the corn stunt disease agent (20). There is no evidence of seed transmission (11). The insect vector is not able to survive in absence of maize in the highlands of Colombia (26).

Purification — VRCM-infected leaves were ground in 0.1 M phosphate, 0.1 M M_gCl_2, pH 7.0 (3 ml/g tissue), squeezed through cheesecloth and centrifuged at 7,710 g for 10 minutes at 4 C. The supernatant was adjusted to pH 5.5. NaCl was added to 0.1 M and polyethylene glycol to 6%. After a 2-hour incubation, the virus was pelleted at 7,710 g for 10 minutes at 4 C. The pellets were suspended in grinding buffer, given a low-speed centrifugation, and subjected to rate-zonal density gradient centrifugation.

Infectivity of the virus band was tested by feeding virus-free insects through Parafilm membranes and placing them on healthy plants (7, 16).

The virus has also been purified by grinding in 0.1 M phosphate, pH 6.5, clarifying with $CHCl_3$ and n-butanol, and precipitating twice with polyethylene glycol (7) or by grinding in 0.05 M phosphate, 0.05 M EDTA, 0.2% 2-mercaptoethanol, pH 7.5 (10 ml/5 g tissue), squeezing through muslin, shaking with 2.6 ml $CHCl_3$, and centrifuging at 10,000 rpm for 20 minutes. The aqueous phase was centrifuged at 100,000 g for 2 hours and the pellet was suspended in grinding buffer (C. Ricaud, *personal communication*).

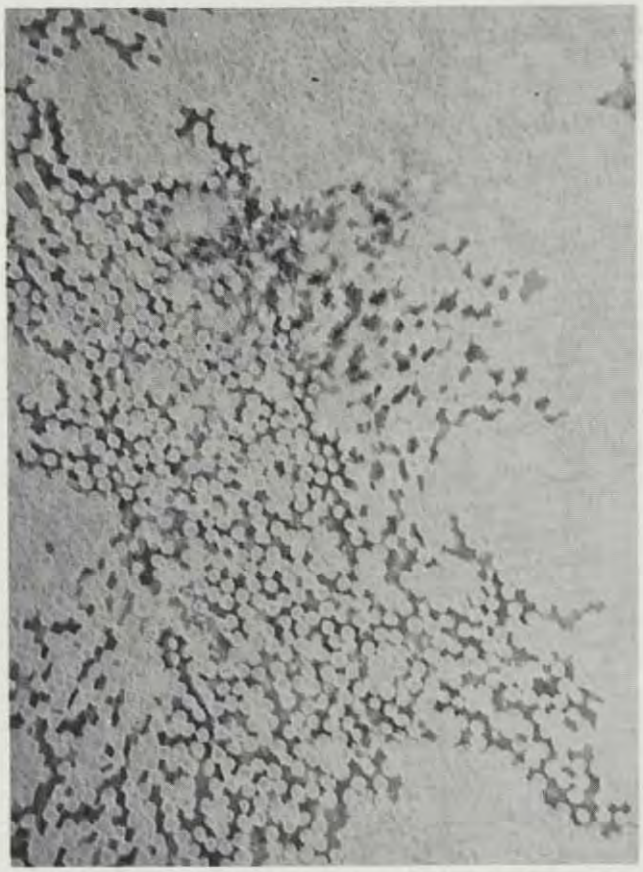

Fig. 4. Virus del rayado colombiano del maiz. Virus particles from a purified preparation in phosphotungstate (diam. about 30 nm).

Thermal inactivation point of the virus was between 60 and 65 C. Infectivity was retained at 20 C for 72 to 96 hours and at 4 C for 96 to 120 hours (18).

Virus particles were isometric and about 30 nm in diam (Fig. 4) [(8) and C. Ricaud, *personal communication*].

Electron microscopy of infected maize leaves revealed tubules containing a single row of spherical, virus-like particles (Fig. 5.).

Serology — In gel-diffusion tests, the virus reacted with VRCM-antisera to produce a distinct single band of precipitate, but it did not react with VRFM-antiserum provided by R. Gamez (7). However, Gamez got positive reactions with VRFM-antiserum and samples collected in Colombia (R. Gamez, *personal communication*). These results suggested the presence of VRFM in Colombia. Experiments to clarify these results are in progress. VRCM did not react with antisera to maize streak, maize stripe, or maize line viruses (C. Ricaud, *personal communication*).

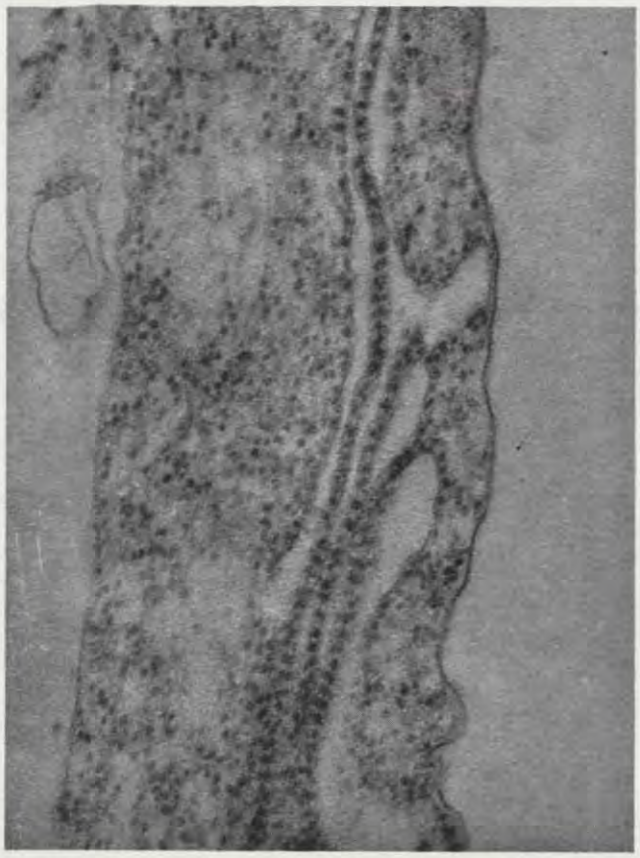

Fig. 5. Virus del rayado colombiano del maiz. Virus particles in thin sections from infected plants; present in a single row in tubules.

Economic importance — The virus has been observed in all maize growing areas in Colombia above 1,000 m but not in the lowlands. Incidence of infected plants in fields visited averaged 5%, with a maximum of 50% (9). But, this incidence was as high as 100% in some experimental plots surrounded by maize fields with high incidences of the disease at planting time (4). In general, the disease is more prevalent in highlands where there are lower temperatures. Disease losses are very high in susceptible cultivars, and in some there is wilting and quick death of infected plants (21).

In an experiment with the cultivar ICA V-504, Pineda (11, 12) and Pineda and Martinez-Lopez (13) divided the plant in six groups of three leaves to identify six stages of infection (Fig. 6), according to the leaves in which the first symptoms appeared. Assessment of disease losses of green forage indicated that there were high losses if plants were infected at stages one, two, and three, with losses as high as 81% for stage one and 46% for stage three (11). Assessment of disease losses in grain indicated that the disease was important at all six infection stages, with losses of 89, 84, 77, 50, 38, and 8% for stages one, two, three, four, five, and six, respectively (Fig. 7) (11).

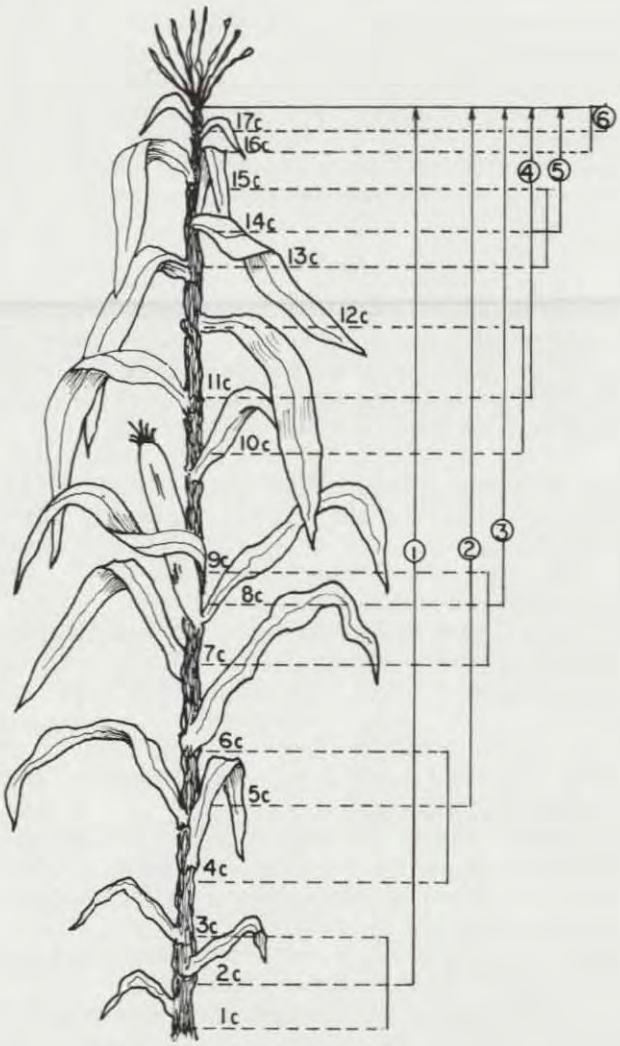

Fig. 6. Maize plant divided in six groups of three leaves to identify six stages of infection; stage one, early infection, and stage six, late infection.

Control measures — Much effort has been invested in evaluating cultivars of different regions to identify materials resistant to the virus, vector, or both. Salazar (21) and Salazar and Martinez-Lopez (22) evaluated 159 cultivars of highland maize in the field in an area of high incidence of the disease and selected 23 for greenhouse tests. In these tests all cultivars were susceptible to the disease, but in five the disease incidence was lower than in the control. There were significant differences in the incubation period of the virus in cultivars tested. But there were no significant differences in cultivar suitability to the vector. There were significant differences in the number of eggs layed and number of nymphs present (21). Some cultivars are being field tested again with some other lowland cultivars to identify good sources of resistance. Incidence of the disease was reduced under experimental conditions from more than 80% in 1973 and 1974 to less

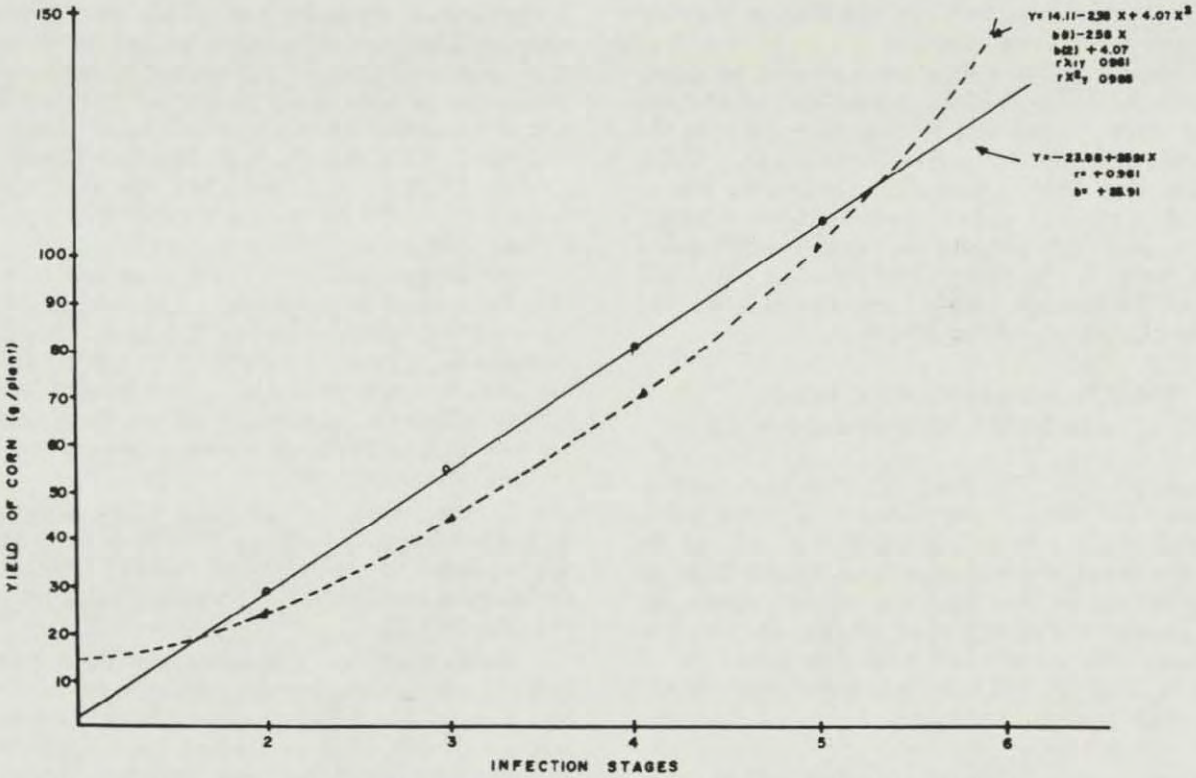

Fig. 7. Disease losses resulting from natural infection with the virus del rayado colombiano del maiz at six stages of plant development.

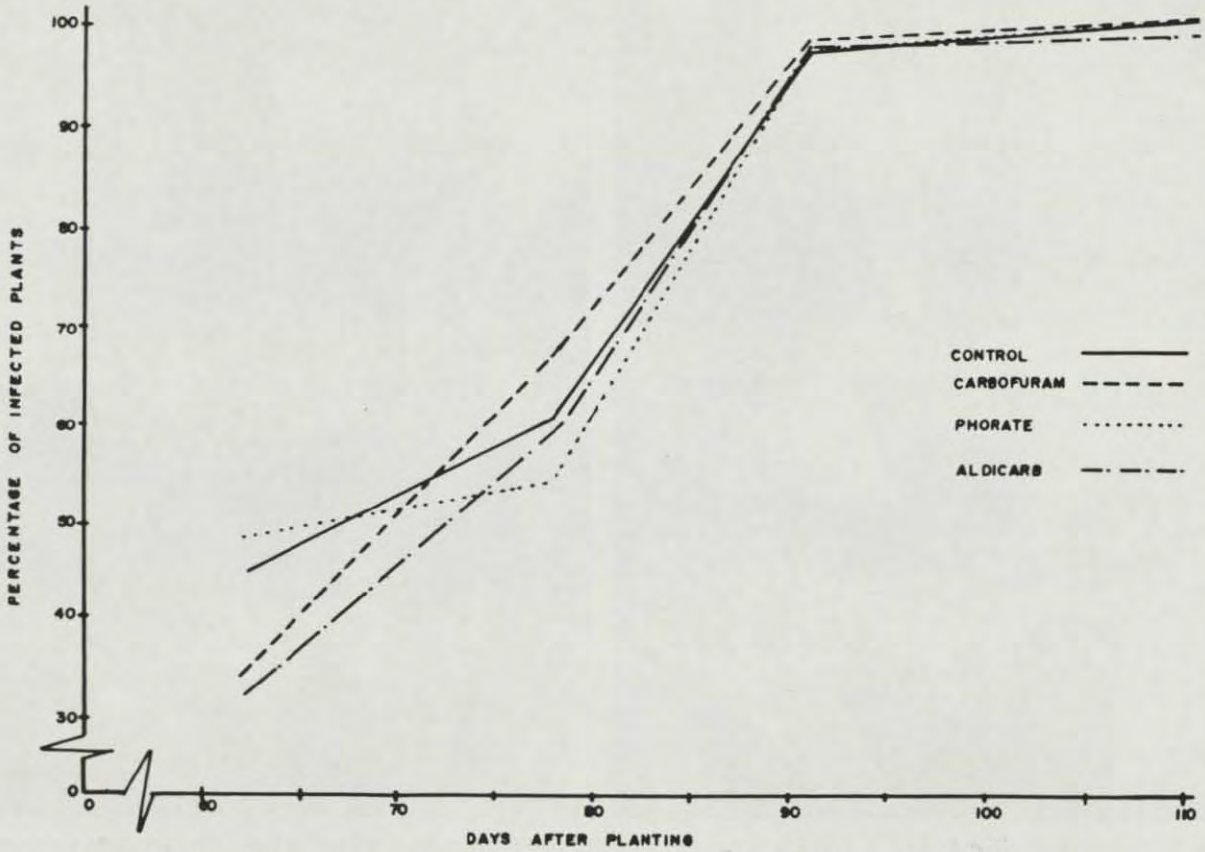

Fig. 8. Effects of three systemic insecticides on the development of virus del rayado colombiano del maiz.

than 2% in 1975 and 1976, by controlling planting dates and using crop rotation.

Incidence of virus was not reduced by using the systemic insecticides aldicarb, carbofuran, and phorate to control the vector. All three chemicals were bioactive for more than 72 days after planting under greenhouse conditions, but in the field in treated plots, surrounded by infected corn, it was not possible to detect differences among them. Sixty days after planting the incidence of the disease had an average of 41% and after 90 days it was 98% (Fig. 8) (4).

MAIZE DWARF MOSAIC VIRUS: A POSSIBLE NEW STRAIN

Symptoms. — The first symptom observed in the Valle del Cauca, particularly in some cultivars with the brachytic-2 gene, was a light mottle along the veins, more conspicuous at the base of leaves in which the first symptoms appeared. Later, mottle was observed in all new leaves, but sometimes was associated with the presence of ringspots (Fig. 9). These symptoms were associated with a slight reduction in size of diseased plants (28, 30).

Development of disease symptoms seems to be favored by temperatures of 26 and 30 C; at these temperatures about 70% of plants were infected, with a minimum incubation period of 4 and 3 days and averages of 5.2 and 3.6, respectively. Incubation periods were similar at 22 C but there was a reduction in number of plants developing symptoms. This reduction in infected plants was greater at 18 C, at which only one of 60 plants developed symptoms, with an incubation period of 10 days (28).

Symptoms similar to those observed in maize also have been observed in *Digitaria sanguinalis* Scop., *Echinochloa colonum* (L.) Link., *Rottboelia exaltata* L., *Sorghum vulgare* L., and *S. vulgare* var. *glutinosum* Pers. All of the above are hosts of MDMV. However, symptoms did not develop on *S. halepense* (L.) Pers. or some varieties of *Saccharum officinarum* L. (28, 29).

Transmission. — This virus was easily transmitted mechanically and as a stylet-borne virus by the aphids *Rhopalosiphum maidis* (Fitch) and *Myzus persicae* (Sulzer). It was not seed transmitted (28, 29, 30).

Purification. — The virus has been purified using various clarification procedures and combinations of NaCl-polyethylene glycol for precipitation (23, 27). The procedure that appeared to give the best yields was one used by Sanchez de Luque and Martinez-Lopez (23), in which infected

Fig. 9. Maize dwarf mosaic virus, a possible strain in Colombia. A) Mosaic symptoms and presence of ringspots on leaves; B) mosaic symptoms in the form of stripes along veins.

leaves were homogenized in 0.01 M phosphate, 1.0% 2-mercaptoethanol, and 6% n-butanol, pH 6.5. After centrifugation at 8,000 rpm for 10 minutes, virus was precipitated from supernatant with 0.3 M NaCl and 5% polyethylene glycol. Pelleted virus was resuspended in grinding buffer.

Thermal inactivation of the virus was between 50 and 55 C. Infectivity was retained for 8 to 9 hours at 24 C and for 45 to 46 hours at 4 C (27).

Virus particles were flexuous filaments about 708 nm long and 13 nm diam (Fig. 10) (23).

Fig. 10. Maize dwarf mosaic virus, a possible strain in Colombia. Virus particles from a purified preparation in phosphotungstate (708 nm long and 13 nm diam).

Serology. — Purified virus has been used for antiserum production but no serological tests have been made.

Economic importance. — The virus has been observed in the Valle del Cauca, the area in Colombia where most commercial maize farms are located and where the higher yields are obtained. In an evaluation of disease losses, using the hybrid ICA H-210, it was observed that losses were higher than 50% when disease symptoms developed in any of the first 12 leaves but only 15% when they appeared later during the growing season (28, 30).

Control measures. — To try to control losses caused by this virus, some effort is being invested to evaluate cultivars adapted to the 800-1,200 m above sea level region to identify resistant materials. To date, 566 cultivars have been observed in the field under natural infection. In these tests 63 cultivars were identified as symptom-free (28). In greenhouse studies with controlled inoculations, all 44 cultivars studied developed symptoms (28). It will be necessary to continue these studies to definitely identify resistant cultivars.

In areas where this disease is a problem, it will be very difficult to control because some identified hosts are prevalent weeds in the area and aphid vectors are present all year.

CORN STUNT DISEASE

When studies on the relationships between the VRCM and its vector, *D. maidis*, were in progress, it was observed that the corn stunt disease agent was also present in the Sabana de Bogota, at an altitude of 2,640 m above sea level with an average temperature of 16 C (15, 20). The disease was characterized by the development of purple to red coloration of the tip and edges of lower leaves; wide, chlorotic stripes or general chlorosis of young leaves; rupture of the leaf blade; shortening of internodes; and male sterility of infected plants (20).

Preliminary greenhouse studies were made to confirm the presence of this disease under the conditions of the Sabana de Bogota — conditions that did not appear to be appropriate for the development of this disease or its insect vector. An incubation period of 46 days for the disease agent in the plant with 82% of the insects able to transmit the disease was indicated. No differences in the ability of males or females to transmit the disease were apparent. Some evidences of intermittent transmission were observed (20). Presence of this disease had been reported in Colombia by Smith and Niederhauser (24) in the Valle del Cauca since 1956, at 1,000 m above sea level.

With the insect vector present in all maize-growing regions in Colombia and the apparent ability of the disease agent to develop in all, it will be necessary to initiate work to try to identify resistant cultivars. The disease may become a limiting factor in maize production.

LITERATURE CITED

1. GAMEZ, R. 1969. A new leafhopper-borne virus of corn in Central America. Plant Dis. Rep. 53:929-932.
2. GAMEZ, R. 1973. Transmission of rayado fino virus of maize (Zea mays) by Dalbulus maidis. Ann. Appl Biol. 73:285-292.
3. GONZALEZ V., and R. GAMEZ. 1974. Algunos factores que afectan la transmision del virus del rayado fino del maiz por Dalbulus maidis (DeLong & Wolcott). Turrialba 24:51-57.

4. MARTINEZ-LOPEZ, G. 1975. Relacion entre el control quimico del saltahojas Dalbulus maidis (DeLong & Wolcott) y la incidencia del virus del rayado colombiano del maiz. Page 28 in Congreso de la Sociedad Colombiana de Entomologia 3, June 24-27, 1975. Medellin, Colombia. (Abstr.).
5. MARTINEZ-LOPEZ, G., and L.M. RICO DE CUJIA. 1974. Dalbulus maidis (DeLong & Wolcott) the vector of a new disease in corn in Colombia. Proc. Am. Phytopathol. Soc. 1:35 (Abstr.).
6. MARTINEZ-LOPEZ, G., and L.M. RICO DE CUJIA. 1975. El virus del rayado colombiano del maiz (Zea mays L.). Not. Fitopat. 4:27-32.
7. MARTINEZ-LOPEZ, G., and L.M. RICO DE CUJIA. 1976. Estudios serologicos del virus del rayado colombiano del maiz. In Congreso de la Asociacion Colombiana de Fitopatologia y Ciencias Afines 2, September 1-3, 1976. Bogota, Colombia. (Abstr.). (In press).
8. MARTINEZ-LOPEZ, G., and L.M. RICO DE CUJIA. 1976. El virus del rayado colombiano del maiz es un virus isometrico de alreadedor de 30 nm de diametro. In Congreso de la Asociacion Colombiana de Fitopatologia y Ciencias Afines 2, September 1-3, 1976. Bogota, Colombia (Abstr.) (In press).
9. MARTINEZ-LOPEZ, G., and O. MARTINEZ. 1976. Algunos estudios sobre la incidencia del virus del rayado colombiano del maiz en Colombia. In Congreso de la Asociacion Colombiana de Fitopatologia y Ciencias Afines 2, September 1-3, 1976. (Abstr.). (In press).
10. MARTINEZ-LOPEZ, G., L.M. RICO DE CUJIA, and C. SANCHEZ DE LUQUE. 1974. Una nueva enfermedad del maiz en Colombia transmitida por el saltahojas Dalbulus maidis (DeLong & Wolcott). Fitopat. 9:93-99.
11. PINEDA L., B. 1975. Estudios sorbe algunas de la relaciones entre el virus del rayado colombiano del maiz, su vector y la variedad de maiz ICA V-504. Tesis M.Sc. Bogota. Universidad Nacional de Colombia Instituto Colombian Agropecuario, 110 p.
12. PINEDA L., B. 1975. Yield losses in the maize ICA V-504 caused by the virus rayado colombiano del maiz under field conditions. Proc. Am. Phytopathol. Soc. 2:78 (Abstr.).
13. PINEDA L., B., and G. MARTINEZ-LOPEZ. 1974. Resultados preliminares sobre evaluacion de perdidas causadas por el virus del rayado colombiano del maiz. Fitopatologia 10:10 (Abstr.).
14. PINEDA L., B., and G. MARTINEZ-LOPEZ. 1975. Periodo de incubacion del virus del rayado colombiano del maiz en el vector, el saltahojas Dalbulus maidis (DeLong & Wolcott). Page 52 in Congreso de la Sociedad Colombian de Entomologia 3, June 24-27, 1975. Medellin, Colombia. (Abstr.).
15. RICO DE CUJIA, L.M. 1975. The corn stunt disease agent in the Sabana de Bogota. Proc. Am. Phytopathol. Soc. 2:78 (Abstr.).
16. RICO DE CUJIA, L.M. 1976. Efecto de la temperatura sobre la incubacion del virus del rayado colombiano de maiz en la planta y en el vector. Tesis M.Sc. Bogota. Universidad Nacional de Colombia, Instituto Colombiano Agropecuario. 52 p.
17. RICO DE CUJIA, L.M. 1976. Efecto de la temperatura sobre la incubacion del virus del rayado colombiano del maiz. In Congreso de la Asociacion Colombiana de Fitopatologia y Ciencias Afines 2, September 1-3, 1976. Bogota, Colombia. (Abstr.) (In press).
18. RICO DE CUJIA, L.M. 1976. Punto de inactivacion termica y longevidad in vitro del virus del rayado colombiano del maiz. In Congreso de la Asociacion Colombiana de Fitopatologia y Ciencias Afines 2, September 1-3, 1976. Bogota, Colombia. (Abstr.) (In press).
19. RICO DE CUJIA, L.M., and G. MARTINEZ-LOPEZ. 1976. Efecto de la temperatura sobre la incubacion del virus del rayado colombiano del maiz en la planta y el vector. Revista ICA: (In press).
20. RICO DE CUJIA, L.M., and G. MARTINEZ-LOPEZ. 1976. El achaparramiento del maiz en la Sabana de Bogota. Not. Fitopat. (In press).
21. SALAZAR P.H. 1975. Identificacion de fuentes de resistencia al virus del rayado colombiano del maiz en materiales de maiz (Zea mays L.) de clima frio. Tesis M.Sc. Bogota, Universidad Nacional de Colombia, Instituto Colombiano Agropecuario. 92 p.
22. SALAZAR P. H., and G. MARTINEZ-LOPEZ. 1974. Reconocimiento de fuentes de resistencia al virus del rayado colombiano del maiz. Fitopatologia 10:11 (Abstr.).
23. SANCHEZ DE LUQUE, C., and G. MARTINEZ-LOPEZ. 1976. Estudios de purificacion y microscopia electronica de un virus en maiz en el Valle del Cauca. In Congreso de la Asociacion Colombiana de Fitopatologia y Ciencias Afines 2, September 1-3, 1976. Bogota, Colombia. (Abstr.) (In press).
24. SMITH, D.L., and J.S. NIEDERHAUSER. 1958. Occurrence of corn stunt virus in Central America and Colombia. Plant Dis. Rep. 42:512.
25. TORREGROZA C., M. 1975. Principales aspectos del cultivo del maiz en Colombia. In Reunion Anual de Maiceros CIMMYT, September 15-20, 1975. Mexico.
26. TROCHEZ P., A., L. POSADA, and G. MARTINEZ-LOPEZ. 1975. Estudio sobre las plantas hospedantes del saltahojas Dalbulus maidis (DeLong & Wolcott) (Homoptera: Cicadellidae) en la Sabana de Bogota. Page 17 in Congreso de la Sociedad Colombiana de Entomologia 3, June 24-27, 1975. Medellin, Colombia. (Abstr.).
27. VARON DE AGUDELO, F.H. 1975. Characterization studies on what appears to be a new virus disease in maize in Colombia. In Annual Meeting Caribbean Division. Am. Phytopath. Soc., Cali, Colombia, December 4-7, 1975. (Abstr.).
28. VARON DE AGUDELO, F.H. 1976. Estudios sobre una nueva enfermedad virosa del maiz en Colombia. Tesis M.Sc. Bogota, Universidad Nacional de Colombia, Instituto Colombiano Agropecuario. 131 p.
29. VARON DE AGUDELO, F.H. 1976. Reconocimiento de planta hospedantes de un virus en maiz en el Valle del Cauca. In Congreso de la Asociacion Colombiana de Fitopatologia y Ciencias Afines 2, September 1-3, 1976. Bogota, Colombia. (Abstr.) (In press).
30. VARON DE AGUDELO, F.H., and G. MARTINEZ-LOPEZ. 1975. Enfermedad virosa del maiz en el Valle del Cauca. Fitopatologia 10:9 (Abstr.).

DISCUSSION OF G. MARTINEZ-LOPEZ PAPER

V.D. Damsteegt: Have you done serology with rayado fino and Brazilian corn streak? These viruses apparently have the same size particle, and some of the symptoms appear similar.

G. Martinez-Lopez: No. To date, we have only done tests with our antiserum and virus and with antiserum provided by Dr. R. Gamez. I believe that Dr. Gamez used antiserum against our virus in Colombia and got reactions with some samples from Central America.

R. Gamez: We ran tests recently with antisera prepared by Dr. Kitajima in Brazil, Dr. G. Martinez-Lopez in Colombia, and myself in Costa Rica. We tested these antisera with viruses originating from Brazil, Colombia (both the highlands at Bogota and the lowlands at Cali), and different parts of Central America. Reactions obtained with these three antisera against the different isolates were nearly identical. By these tests, it appeared that we were dealing with exactly the same virus from Brazil, Colombia, and Central America. These data are only preliminary, and I would like to confirm them especially in view of the lack

of reaction that Dr. Martinez-Lopez observed with the antiserum. Also, there are obvious similarities in symptomatology. We also have the same vector and what appears to be the same type of particle. But, I still think that we should do more work before we make further conclusions.

R.W. Toler: In regard to the high temperature effect, you indicated that the disease was not important or at least it did not show symptoms above 26 C. Was this a masking of symptoms or did the virus not multiply?

G. Martinez-Lopez: We have the same effect of the high temperature on the development of symptoms in the plant and the incubation of the virus in the insect. At this temperature, only a very small number of plants will show symptoms; and the incubation period in the insect will be longer, and very few will be transmitters. If you take plants out of 30 C and put them in 15-20 C, they will not show the symptoms.

R.W. Toler: Did you check the plant for virus multiplication at this temperature?

G. Martinez-Lopez: No. We did not try to recover virus from plants, since it is not transmitted mechanically. We would have to use the insect vector. If we feed the insect on leaves that do not have very severe symptoms, we do not get transmission. As a matter of fact, we have not been able to get as high efficiency of transmission in our populations as Dr. R. Gamez has with rayado fino virus. With a wild population, about 10% of the leafhoppers are able to transmit the virus. Of the leafhoppers that have been feeding on infected plants, only 10% are able to transmit the disease agent. We have been trying to select for higher efficiency of transmission. The highest level we have obtained is 20%. Even when using as many as 30 leafhoppers per plant, we never get more than 10-15% of the plants infected.

R.W. Toler: Do you have any sources of resistance from North American germplasm?

G. Martinez-Lopez: Up to now, we have only used corn materials grown in Colombia. Among these corn varieties, we have considerable material that has very low incidence of disease and appears promising. We are now testing some of these materials in the field. We did one greenhouse test in which some of these materials had a long virus-incubation period in the plant. In some of these tests there also appeared to be an effect on the vector; the percentage of leafhoppers that remained alive for long periods was lower. We need additional tests to confirm this latter observation.

G.E. Scott: When you define a plant as susceptible, does that mean it has at least one yellow spot or do you take measurements on the degree of symptom expression? In other words, how do you define susceptibility?

G. Martinez-Lopez: I am not talking about the actual number of spots. What we call susceptible is a plant that shows a large number of lesions and is very easily seen in the field. In the greenhouse, it is easy to detect and count numbers of spots. But under field conditions, there are other problems and these symptoms may be masked.

V.D. Damsteegt: Were the three systemic insecticides that you found ineffective applied to a small plot in an isolated area surrounded by all kinds of insects? Or, did you apply them to a large adjacent area as well as corn plots so as to eliminate large insect reservoirs?

G. Martinez-Lopez: No. We did not try field tests. We ran greenhouse tests on the insecticides, and these tests worked very well. We killed more than 90% of the insects in less than 2 hours. However, under field conditions, 2 hours were enough for the plants to become infected by virus carried by leafhoppers coming into the field.

J.M. Fajamisin: Symptoms and effects of this disease on the development of the plant appear similar to those of maize streak.

G. Martinez-Lopez: I have shown these symptoms to people working on viruses of maize in Africa, and they have said the same thing. I sent some samples to Rothamstead, Great Britain where serological tests against maize streak virus were performed. There was no reaction to maize streak virus nor to one or two other maize viruses in Africa that have the same size particle.

V.D. Damsteegt: I agree that this disease looks like maize streak. However, in our lab we have tried several insects indigenous to the U.S. to see if they would transmit maize streak virus. We tried *Dalbulus maidis* and *Dalbulus elimatus*, using injections and feeding through membranes, to demonstrate transmission. They did not transmit maize streak virus. There is no record of *Cicadulina mbila* or any other vector of maize streak virus in the U.S.

G. Martinez-Lopez: We have some *Cicadulina* species in Colombia but not the particular ones that are involved in transmission of maize streak virus.

J.M. Fajamisin: What is the chance of finding rayado fino in Africa? Is it possibly already there but has gone undetected? Do you think that *Dalbulus* spp. also occur there?

R. Gamez: I am not aware of any report of *Dalbulus maidis* in Africa. It is true that symptoms of maize streak and those of rayado fino are virtually identical, at least in certain genotypes. But, it is also true that the virus-vector relationship of the two viruses are entirely different.

V.D. Damsteegt: The particles also are different. The particle of maize streak virus is a spherical dimer and smaller than rayado fino virus.

G. Martinez-Lopez: Turning to a different subject, we have seen rayado fino symptoms on sorghum growing with corn in the same field. The symptoms on sorghum were identical to those on corn.

L.R. Nault: Earlier when we were discussing corn stunt, I asked if you had seen symptoms of high contrast stripes, chlorosis, reddening or plant stunting and if you had looked for a spiroplasma. You answered that you had not and that the symptoms seen in Colombia appeared more like what we have described for maize bushy stunt in the U.S. Would you comment further?

G. Martinez-Lopez: I have looked at the symptoms of corn stunt in your plants. These symptoms do not look like what we have in Colombia and more closely resemble the maize bushy stunt symptoms.

Maize Mosaic and Other Maize Virus and Virus-Like Diseases in Venezuela

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ABSTRACT

Maize is affected by several virus and virus-like diseases in Venezuela. Maize mosaic virus (MMV) is the most prevalent. Two strains of MMV were identified on the basis of symptomatologies. The causal virus particle is a rhabdovirus with a size of 255 x 90 nm. It is transmitted by the leafhopper, *Peregrinus maidis*, in a persistent manner. Hoja blanca del maiz (HBV) is probably a new viral disease of maize. Causal virus particles are isometric and about 55-60 nm in diam. The disease is also transmitted by *P. maidis*. Maize dwarf mosaic virus (MDMV), a strain of sugarcane mosaic virus (SCMV), is also present. Strain A is the most common. This virus is an elongated flexuous rod, 750 x 13 nm, and transmitted by aphids. Corn stunt, a disease caused by a spiroplasma, has a very low incidence and is vectored by *Dalbulus maidis*. Probably, the Rio Grande strain is the one present in Venezuela.

Maize (*Zea mays* L.) has played an important role in the development of Venezuela. We know from botanical and archeological evidence that maize originated in the Americas, and has been the basic food plant in the New World since prehistoric times. It was first believed that maize had a South American origin, but later archeological findings indicated that it was first domesticated in Central America. From this area it spread, in prehistoric times, to the south and east and was intoduced into Venezuela through Colombia. The first prehistoric corn found in Venezuela, so far, is dated around the year 130 B.C. (16).

Maize was the main food of indigenous tribes and still is a basic staple used by Venezuelans. Maize can be produced at sea level in approximately 4 months, but it may take as long as 13 months at elevations near tree-line, which in our case is about 10,000 feet (3).

Maize is predominately used in Venezuela for human consumption. However, large amounts are used for animal feeding and lesser amounts for industrial purposes, such as oil extraction, dextrins, alcohol, etc.

One of the main advantages of maize is that it grows well in a variety of climates, in areas too dry for rice or too wet for wheat. In the tropics warm conditions are not appropriate for growing wheat, so Venezuela's main cereal is maize. Cereals represent 7.5% of the agricultural sectors of the country and maize is the predominant cereal planted. The area under maize cultivation in 1974 was 462,383 ha and the production was 553,761 metric tons. Average production during the previous 10 years was about the same. During 1974, Venezuela imported 414,029 metric tons of maize for its necessities. This heavy deficit prompted a wide program to expand and develop this crop. This year (1976) there is an estimated area of 574,470 ha planted and production is expected to be around 750,000 metric tons.

A national program for maize estimated an annual increase of 6.1% in area planted with an 8.4% increase in production to reach 755,000 ha under cultivation by 1980, with a harvest of around 1,100,000 metric tons. Presently, yields are relatively low with an average of 1,200 kg/ha (approximately 20 bu/A) (1). The reason for this average low yield is primarily due to the fact that approximately 250,000 ha are cultivated on marginal lands under very primitive conditions. Most maize planted in Venezuela is of local varieties, but there is work underway to develop higher yielding, disease-resistant varieties. Under experimental conditions, these newer varieties yield between 5,000 and 7,000 kg/ha. It is expected that with better hybrids and use of more sophisticated cultural practices, yields could be increased soon.

A relatively large proportion of maize is lost each year to diseases and pests. From this point of view, viral diseases of corn are very important in Venezuela. The large populations of insect vectors of these viruses result in a high percentage of plants being infected. Plant virology is relatively a new science in Venezuela; so far, four viruses and a spiroplasma have been identified as maize pathogens.

VIRUS AND VIRUS-LIKE DISEASES

Maize mosaic virus. — This disease was probably recognized first by Kunkel in 1921 in Hawaii (4). Maize mosaic virus (MMV) or "enanismo rayado del maiz", as this disease is known in Venezuela, was first reported in this

country in 1939 by Muller under the name of "raya blanca" (11). In 1960, Herold, Bergold, and Weibel observed the viral particle with the electron microscope. This was the first demonstration of a rhabdovirus causing a disease in plants (7). MMV was also studied by Malaguti in 1963 in connection with its epidemiology and incidence in Venezuela (11).

Affected plants are more or less stunted, depending on the age at which they are infected. Stunting is due to the shortening of internodes, since their number remains constant. Average length of internodes in healthy plants is around 14.14 cm but in MMV-infected plants it is 5.43 cm (11). Light-green to yellow spots are the first disease symptoms observed. Later, leaves show parallel stripes along the veins but are of normal size (Fig. 1). Plants infected with MMV set no ears or very small ones with no commercial value.

We believe that there are two different strains of this virus in Venezuela, since two distinct symptoms can be consistently observed in fields. In both cases, striping and stunting are present. However, the width and number of stripes per cm are different. We call these strains maize mosaic virus-raya fina (MMV-RF) and maize mosaic virus-raya gruesa (MMV-RG). Maize leaves infected with MMV-RF show 13-15 stripes/cm (Fig. 2). Leaves of plants infected with MMV-RG have only 1-3 (but wider) stripes/cm (Figs. 3, 4). The number of stripes/cm was very consistent in all varieties examined. We base our affirmation that MMV-RF and MMV-RG are strains of the same virus on the fact that in both cases rhabdoviruses were found associated with the disease. Also, both were transmitted by the same leafhopper, *Peregrinus maidis* (Ashm.), and in serological tests an identical reaction was found for MMV-RF and MMV-RG, when antisera against MMV-RF were used (Fig. 5).

These viruses are easily identified by symptoms they produce on infected plants. Mixed infections are common in the tropics, but they can be identified by symptomatology since one virus does not obscure symptoms of the other. Observation of virus particles by electron microscopy is helpful in diagnosis. The virus is a poor antigen but antiserum has been developed and can be used for identification in immunodiffusion assays (Fig. 5). *Rottboellia exaltata* L. and *Septaria vulpiseta*, two common grasses in Venezuela, are susceptible to this virus. Also, *Zea mays mexicana* (Schrad.) Iltis and a *Sorghum* sp. were infected under experimental conditions (4).

Fig. 1. Stunted maize plant, infected with maize mosaic virus-raya fina.

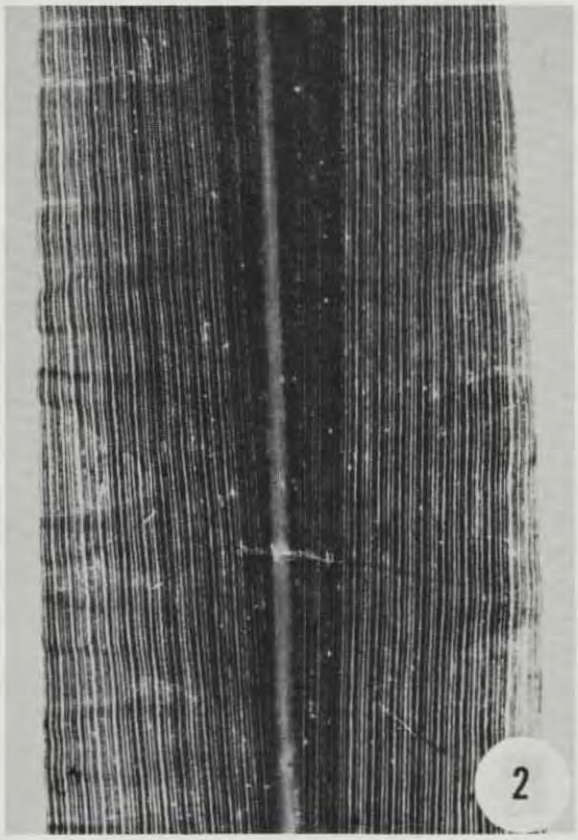


Fig. 2. Maize leaf infected with maize mosaic virus-raya fina, showing thin stripes (usually 13-15 stripes/cm).

Rhabdoviruses were found associated with both strains of MMV. Particles were difficult to measure due to their elasticity and plasticity *in vitro*. Size reported for MMV-RF was 255 x 90 nm in negatively stained preparations, and 242 x 48 nm in plant thin-sections (Fig. 6) (6). The particles are covered by an envelope with thread-like protrusions projecting outward. Sub-adjacent to the envelope is a helical structure running the length of the particle and surrounding a cylinder of undifferentiated material (Fig. 7) (6). Particles tend to break down easily at one end, resulting in a bullet-shape morphology. Aggregation tends to occur at broken ends, forming star-shaped groups of viruses. Particles are found in large numbers at the periphery of the nuclear membrane and they accumulate in the cell cytoplasm, usually surrounded by a membrane (Fig. 8) (10). After purification and extraction of nucleic acid, it proved to be a single-stranded RNA by the diphenylamine test and by digestion with ribonuclease. The particle also contained a high proportion of lipids (8).

Both strains of the virus were transmitted by *P. maidis*. *P. maidis* from healthy colonies on maize became viruliferous after feeding on infected plants or injection with clarified, infectious sap. In both cases infected plants had the same symptoms as those of the plant from which the sap was extracted or the plant upon which the insects had fed. Percentage of insects able to transmit the virus under laboratory conditions was about 5%. The acquisition period was 1 day or less, and the incubation period ranged between 11 and 30 days. MMV-RF multiplied in vectors that persistently transmitted the virus (5). Percentage of insects able to transmit the virus increased greatly when injected with the virus. The number of viruliferous insects varied with each experiment. Usually, 10-60% (avg. of 25%) of insects became infectious after injection with clarified, infectious plant sap. It is interesting that when *P. maidis* was injected with vesicular stomatitus virus (VSV), an animal rhabdovirus, it also multiplied in the leafhopper. This opens the possibility for studies on the multiplication of both animal and plant rhabdoviruses in the same type of cells (9). After viruliferous insects were fed on healthy plants, it usually took 30 days for symptoms to show. After extensive trials, no seed or mechanical transmissions of virus were obtained.

All commercial maize hybrids used in Venezuela are susceptible to MMV. Malaguti (11) tested 29 varieties in the field and found 9 to 50%

Fig. 3. Maize plant infected with maize mosaic virus-raya gruesa, showing stunting and stripes on leaves.

Fig. 4. Maize leaf infected with maize mosaic virus-raya gruesa, showing thick stripes (usually 1-3 stripes/cm).

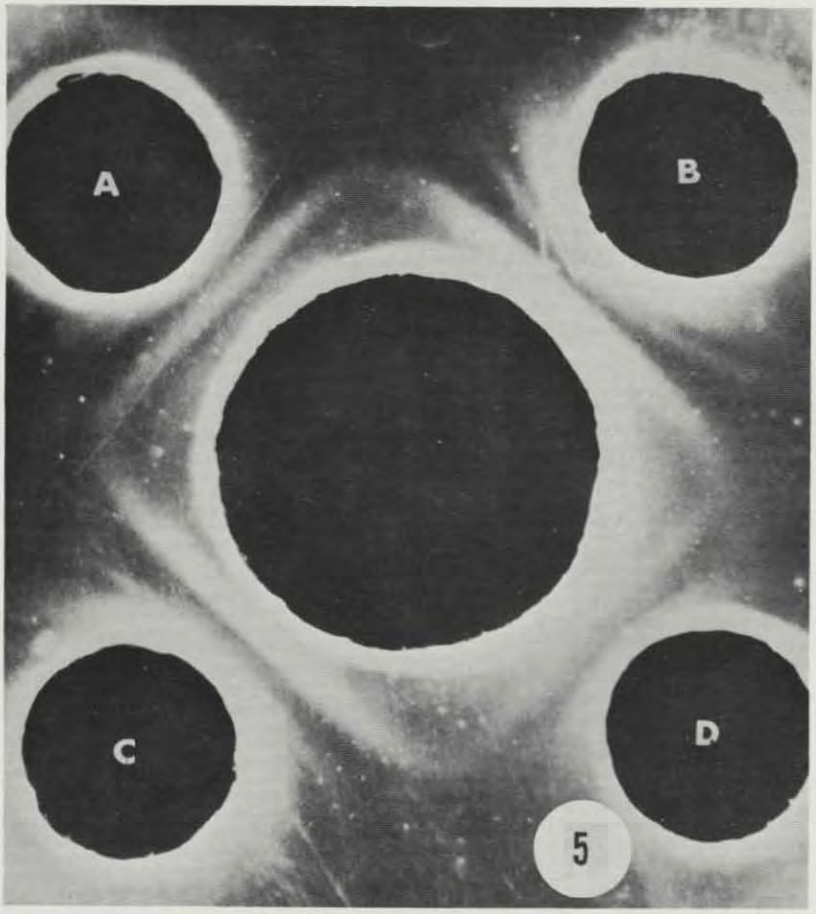
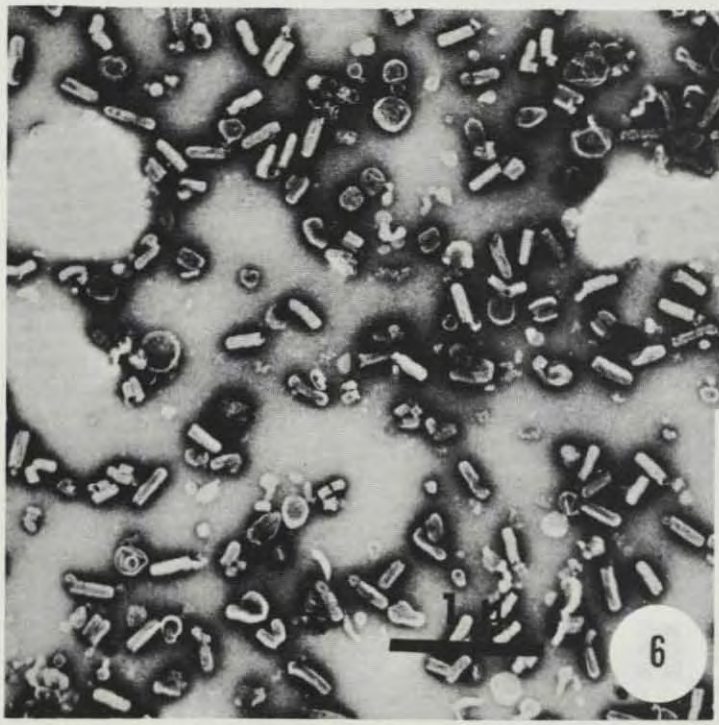


Fig. 5. Immunodiffusion test indicating relationship of MMV-RV and MMV-RG. Central well with MMV-RF antiserum, wells B and C with MMV-RF infected plant sap, and wells A and D with MMV-RG infected plant sap.



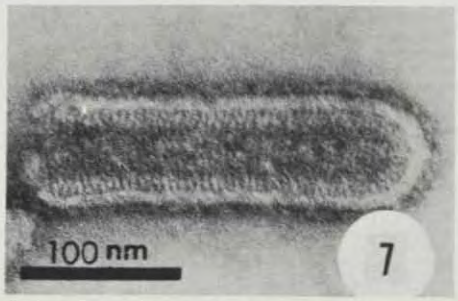


Fig. 6. Electron micrograph of purified maize mosaic virus particles. Large number of particles broken on one end.

Fig. 7. Electron micrograph of a maize mosaic virus particle. Particle has a bacilliform shape, with outer envelope, helical structure, and an inner cylinder.

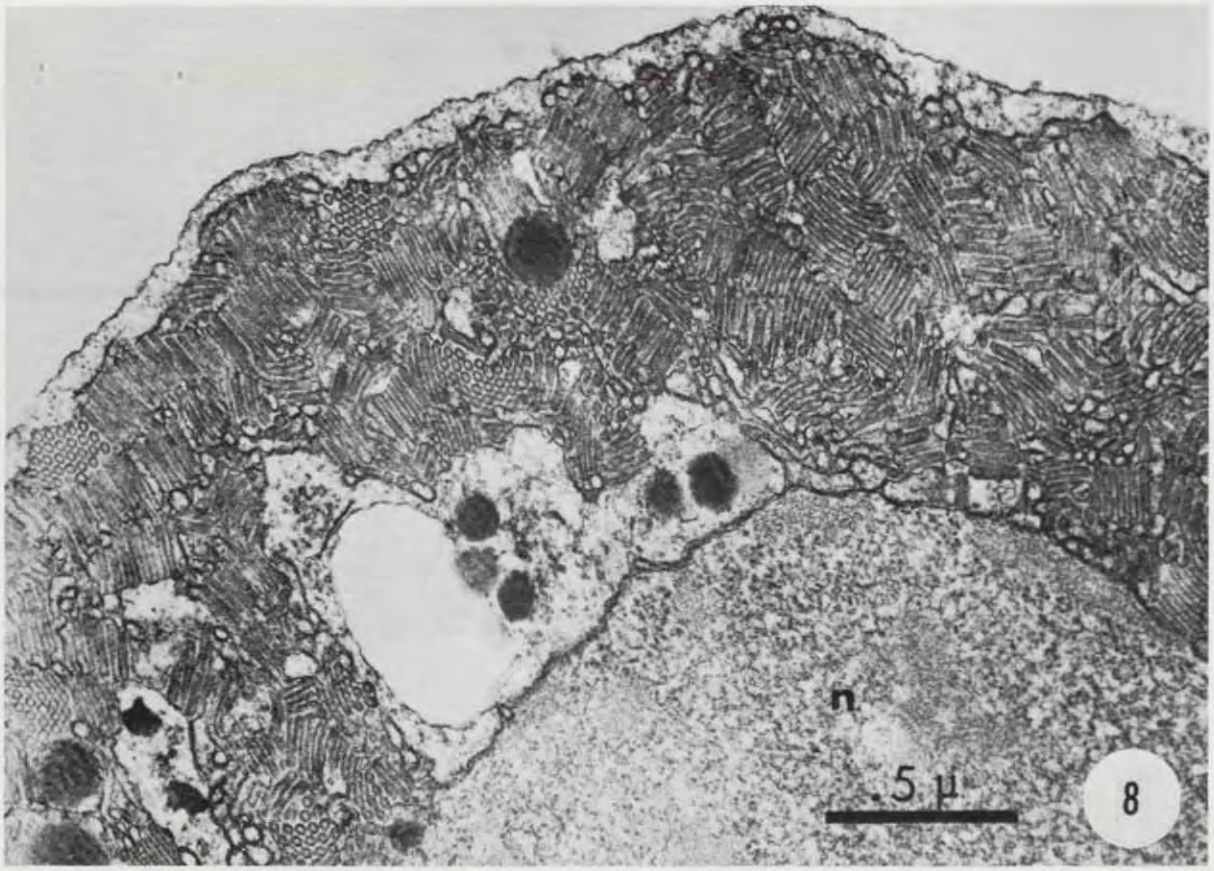


Fig. 8. Electron micrograph section of a maize cell infected with maize mosaic virus. Virus particles are located in the perinuclear space. Some virus particles are budding through the inner nuclear membrane.

infected plants in 12 repetitions performed throughout the year. Therefore, it appears that there are differences in susceptibility of cultivars. Hybrids derived from the variety Poey seem to show some resistance (G. Malaguti, *personal communication*). Resistance was reported in the variety Hawaiian Sugar, a variety traced back to the tropical sweet corn USDA 34 (2). This variety was tested in Venezuela in the field and from preliminary results seemed to be resistant (G. Trujillo, *personal communication*).

The disease is more prevalent during mild weather, from September to January. During the drier and wetter months, incidence of the disease is low, probably because these extreme weather conditions are not favorable to the vector (11). Incidence of the disease varies from year to year and can reach a high incidence. In 1949, an estimated 60% of plants were affected in some areas. To study the percentage of infection, six different fields were observed throughout the year in 1975. These fields were located at the experimental station in the Faculty of Agronomy, Universidad Central de Venezuela in Maracay. There, maize is planted the year around for research purposes. Perhaps, these results cannot be extrapolated to other areas of Venezuela, but they gave an indication of the relative importance of different viruses (10). Maize mosaic virus was the most prevalent of maize virus diseases observed. The highest percentage of plants infected was due to MMV-RF (25.21-61.23%); the percentage for MMV-RG was 2.60%-15.62% (Table 1).

Incidence of maize mosaic can be considerably reduced if old corn fields and other Gramineae are eliminated from the area previous to new plantings. Resistant varieties are not available at this time, but the finding of resistant genes is a hopeful sign that they can be developed. Insecticides could be of some value, especially to avoid secondary infections, since the virus is carried in a persistent manner by its vector.

Maize white leaf. — This disease, known in Venezuela as "hoja blanca del maiz" (HBV), was first reported by Trujillo *et al.* in 1974 (15). Affected plants are partially stunted with yellow to light-green uninterrupted bands. These bands are narrow on early infected leaves but become wider on subsequent leaves until the whole blade becomes pale in color. In old, infected plants, new leaves are yellow and no ears are set (Figs. 9 and 10). The virus is not mechanically transmitted.

Dip preparations of infected plants regularly revealed spherical viral particles of approximately

TABLE 1. Percentages of maize plants (Universidad Central de Venezuela in Marcay) showing symptoms of different viral diseases[a]

Disease	Fields					
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
Maize mosaic (MM) (raya fina)	50.00	41.25	28.20	61.23	25.21	26.20
Maize mosaic (MM) (raya gruesa)	7.47	5.08	2.60	7.12	15.62	13.10
Hoja blanca (HB)	2.80	12.10	8.60	6.31	10.51	8.52
Maize dwarf mosaic (MDM)	3.60	2.00	2.80	2.32	6.21	6.39
MM (RF) + HB	5.60	8.52	8.60	6.50	2.80	0
MM (RF) + MDM	3.60	1.65	0	0.96	2.80	2.13
MM (RG) + HB	0	0	0	0	2.80	0
MM (RG) + MDM	0.47	0	0	0	0	2.13
MM (RG) + MMV (RF)	0.93	0.47	2.80	1.28	0.70	0
MDM + HB	0.93	3.60	2.80	0.96	2.10	2.13
Healthy	23.83	26.13	43.60	13.32	31.35	39.40

[a]Data taken 2 months after planting.

55-60 nm diam (15). These were absent in healthy plant material. The majority of particles appeared to be dense, but occasionally "empty" ones were observed. These "empty" particles probably lack nucleic acid (Fig. 11).

The disease is readily transmitted by *P. maidis*. Both maize and *R. exaltata* are hosts of this virus. HBV was not transmissible to rice (*Oriza sativa* L.), which is susceptible to another virus disease "hoja blanca" with similar symptomatology (14). After a 5-day acquisition period, the vector required 14 to 18 days before it could transmit the disease in a persistent manner. *Sogata orizicola* Muir, the vector of hoja blanca of rice, and *Dalbulus maidis* (DeLong & Wolcott) failed to transmit the disease, even when injected with clarified, infectious plant sap. Transmission percentages increased to 20-50% when the injection technique was used with *P. maidis*.

Percentage of infection in fields was relatively low, ranging from 2.8 to 12.1% (Table 1). However, HBV was always present in plots visited, and since the vector is very common, it could well be a serious problem for maize in the future.

Probably HBV is a new corn virus, even though the particles are similar to the ones reported for maize rough dwarf virus (MRDV) (14). Symptoms are rather different and different vectors are involved. Furthermore, when antiserum against MRDV was used, no reaction could be detected.

Maize dwarf mosaic virus. — Maize dwarf mosaic virus (MDMV) is a strain of sugarcane

Fig. 9. Maize plant infected with hoja blanca virus, showing stunting and bands on leaves.

Fig. 10. Maize leaves infected with hoja blanca virus, showing clear bands, increasing in size and number in younger leaves.

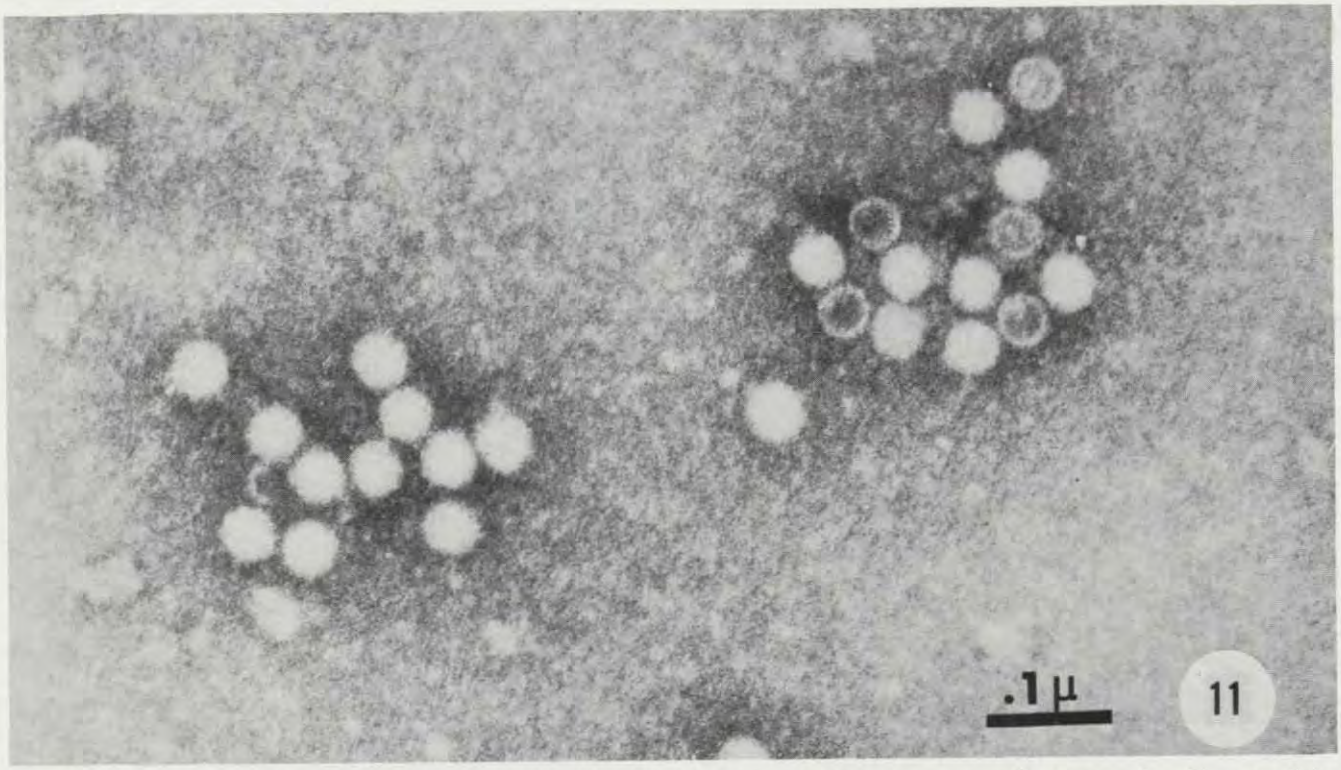


Fig. 11. Electron micrograph of virus particles of hoja blanca disease of maize from a root dip preparation. Some particles have an "empty" core, indicating a lack of nucleic acid.

mosaic virus (SCMV). The virus was first reported in maize and sorghum in 1969 in Venezuela (13). The main symptom of this virus on maize is a typical mosaic with dark- and light-green mottled areas. Plants are sometimes stunted, especially if they are infected early in the growing season (Figs. 12, 13). In Venezuela the A strain is most commonly identified. So far, this is the only mechanically transmitted viral disease of maize found in Venezuela. The virus was transmitted to several indicator plants of the Gramineae family.

The viral particle is a flexuous filament of 750 x 13 nm and can easily be detected in dip preparations by electron microscopy (Fig. 14).

MDMV is aphid transmitted and in Venezuela the main vector is *Rhopalosiphum maidis* (Fitch). Since sugarcane is a common crop in this country and large amounts of Gramineae are growing wild, there is enough infected material around from which the virus can be taken into nearby maize fields. Even so, percentage of infection was relatively low, between 2.0 and 6.3% (Table 1). Sometimes higher percentages were observed, especially when corn was planted near sugarcane.

The best way to control this disease is use of resistant varieties. Elimination of reservoir hosts as well as other Gramineae surrounding the corn field helps reduce its incidence.

Corn stunt. — Corn stunt or "achaparramiento del maiz," as this disease is known in Venezuela, was first reported in this country in

Fig. 12. Maize plant infected with maize dwarf mosaic virus, showing dwarfing and mosaic.

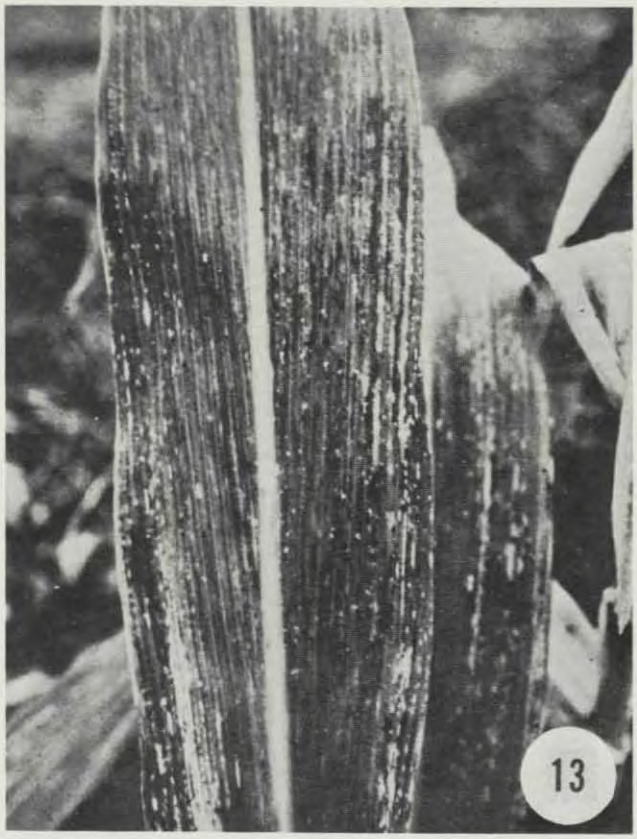


Fig. 13. Maize leaf infected with maize dwarf mosaic virus, showing mosaic.

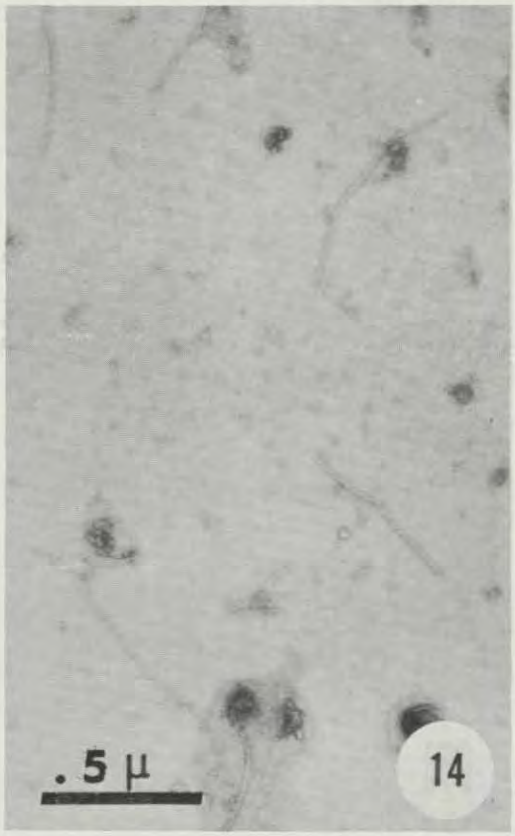


Fig. 14. Electron micrograph of maize dwarf mosaic virus particles in a dip preparation from infected roots.

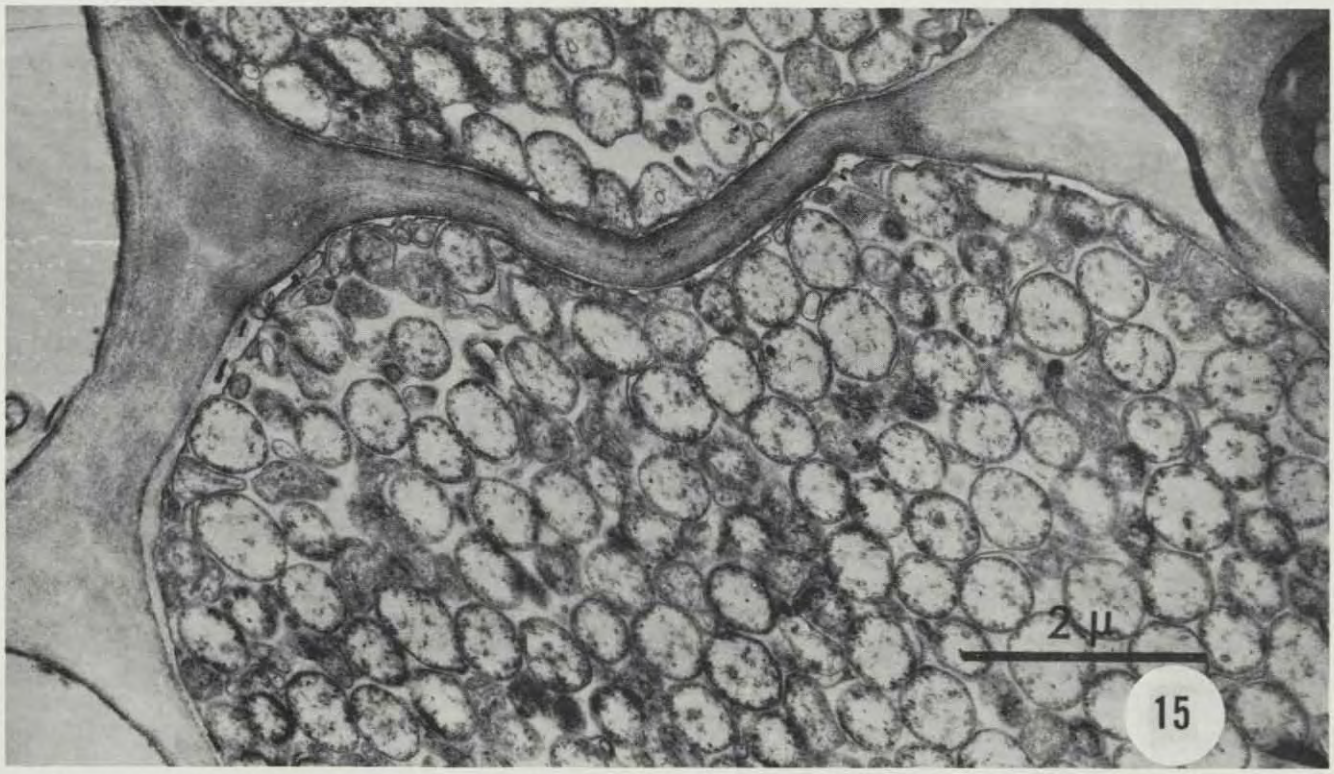


Fig. 15. Electron micrograph of a section through vascular tissue of a maize plant infected with corn stunt. Spiroplasmas can be seen in lumen of vessels.

1969 (12). The disease was identified by means of symptomatology and transmission by the vector *D. maidis*. In the field, affected plants can be distinguished 50-70 days after planting. They are slightly stunted and the stem is thinner than in healthy plants. Leaves show chlorotic spots at the base; later, these spots become bands that cover the whole leaf. Older leaves have a red coloration. Symptoms usually found in Venezuela seem to correspond to those of the Rio Grande strain (12).

The causal agent is a spiroplasma, which could be seen in sections of infected plants observed by electron microscopy. Spiroplasmas were abundant in the vascular tissue and absent in comparable healthy tissues (Figs. 15, 16).

This disease is not very important in Venezuela; occasionally it is found but the percentage is very low. Although corn stunt was reported in this country 7 years ago, its incidence does not seem to have increased, even though most of the varieties planted are susceptible. Due to its low incidence, no evaluation of its losses have been made and no control methods tried.

SUMMARY

TABLE 2. Virus and virus-like diseases of maize in Venezuela, pathogen morphology, and vectors

Disease	Symptoms	Pathogen morphology	Vector
Maize mosaic - raya fina	Stunting of plants; parallel, thin stripes on leaves; 10-15 stripes/cm	Bacilliform with rounded ends (255 x 90 nm)	*Peregrinus maidis*
Maize mosaic - raya gruesa	Stunting of plants; parallel, thick stripes on leaves; 2-3 stripes/cm	Bacilliform with rounded ends (255 x 90 nm)	*P. maidis*
Hoja blanca of maize	White or yellow bands, wider in new leaves	Isometric (50-60 nm)	*P. maidis*
Maize dwarf mosaic	Mosaic	Flexuous filament (750 x 13 nm)	*Rhopalosiphum maidis*
Corn stunt	Chlorotic spots, proliferation of ears, and stunting. Older leaves red.	Spiroplasma	*Dalbulus maidis*

LITERATURE CITED

1. Anuarico estadistico agropecuario. 1974. Direccion de estadistica. Ministerio Agricultura y Cria, Caracas, Venezuela.
2. BREWBAKER, J.L., and F. AQUILIZAN. 1965. Genetics of resistance in maize to a mosaic-stripe virus. Crop Sci. 5:412-415.
3. GRANT, C.J., W.H. HATHEWAY, D.H. TIMOTHY, C. CASSALETT, and L.M. ROBERTS. 1963. Races of maize in Venezuela. Natl. Acad. Sci. Pub. 1136. 92 p.
4. HEROLD, FRIEDA. 1972. Maize mosaic virus No. 94 *in* C.M.I./A.A.B. Descriptions of plant viruses. Kew, Surrey, England. 4 p.
5. HEROLD, F., and K. MUNZ. 1965. Electron microscopic demonstration of virus-like particles in Peregrinus maidis following acquisition of maize mosaic virus. Virology 25:412-417.
6. HEROLD, F., and K. MUNZ. 1967. Morphological studies of maize mosaic virus I. J. Gen. Virol. 1:227-234.
7. HEROLD, F., G.H. BERGOLD, and J. WEIBEL. 1960. Isolation and electron microscopic demonstration of a virus infecting corn (Zea mays L.). Virology 12:335-347.
8. LASTRA, R., and J.R. ACOSTA. 1975. Purification and partial characterization of maize mosaic virus. Page 244 *in* Proc. 3rd Int. Cong. for Virol. Madrid, Spain. (Abstr.).
9. LASTRA, R., and J. ESPARZA. 1976. Multiplication of vesicular stomatitis virus in Peregrinus maidis (Ashm.) vector of a plant rhabdovirus. J. Gen. Virol. 32:139-142.
10. LASTRA, R., and G. TRUJILLO. 1976. Enfermedades del maiz en Venezuela causadas por virus y microplasmas. Agron. Trop. (In press).
11. MALAGUTI, GINO. 1963. Enanismo rayado del maiz en Venezuela. Agron. Trop. 12:175-193.
12. MALAGUTI, G. and A. ORDOSGOITTY. 1969. El achaparramiento del maiz en Venezuela. Agron. Trop. 19:85-97.
13. ORDOSGOITTY, A., and G. MALAGUTI. 1969. El mosaico de la cana de azucar en siembras comerciales de maiz y sorgo. Agron. Trop. 19:189-195.
14. TRUJILLO, G. 1968. A possibility for selection of rice varieties resistant to hoja blanca. Plant Dis. Rep. 53:440-443.
15. TRUJILLO, G.E., J.M. ACOSTA, and A. PINERO. 1974. A new corn virus disease found in Venezuela. Plant Dis. Rep. 58:122-126.
16. WAGNER, E., and A. ZUCCHI. 1966. Mazordas de maiz prehistorico de Venezuela occidental. Bol. Informativo, Dept. Antropologia IVIC. 3:36-38.

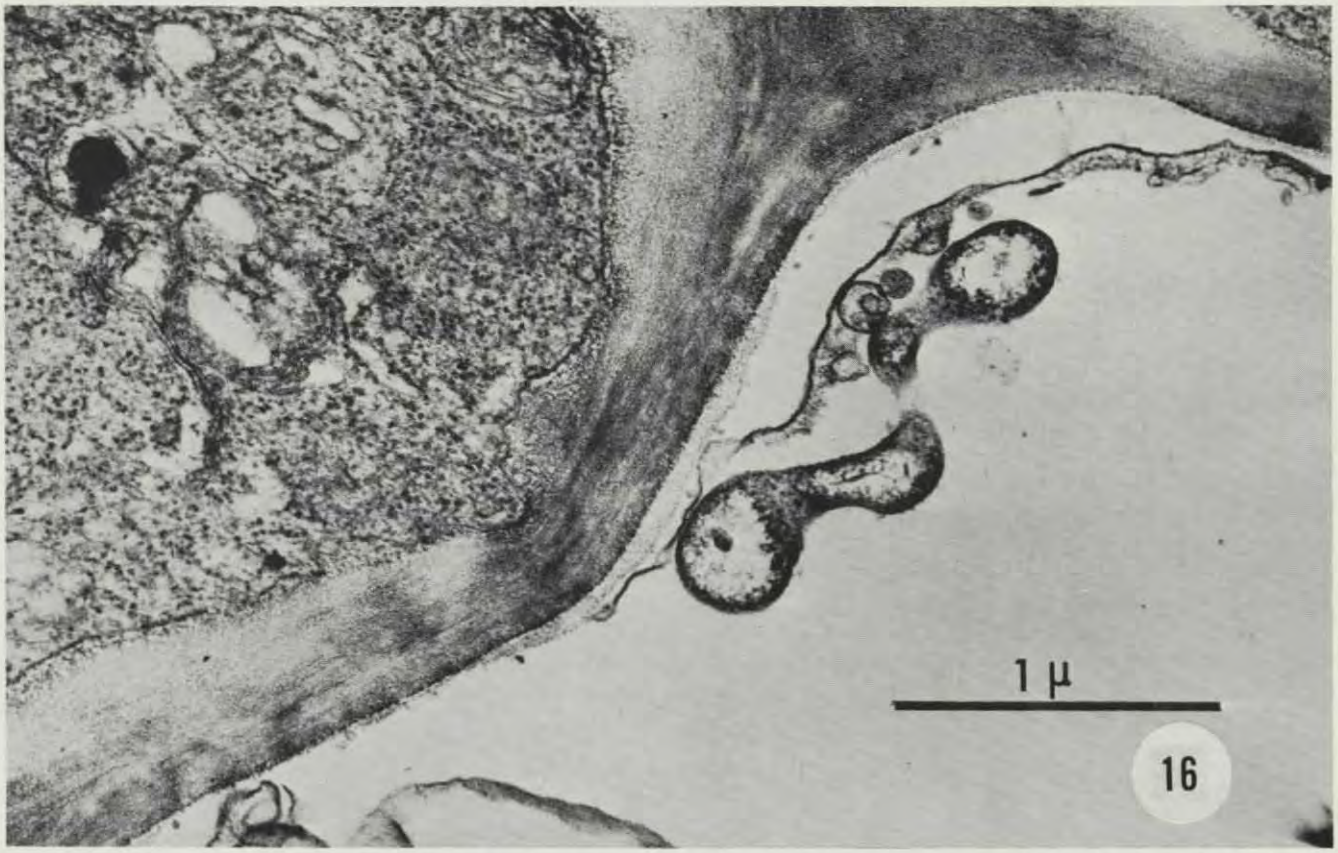


Fig. 16. Electron micrograph of vascular tissue from corn stunt-infected maize, showing a spiroplasma (sectioned longitudinally) with typical spiral shape.

DISCUSSION OF R.J. LASTRA PAPER

A.J. Ullstrup: Is there any relationship between the hoja blanca virus in corn and that in rice?

R.J. Lastra: That is a good question. We tried to transmit the maize virus with *Sogata orizicola*, the vector of rice hoja blanca virus, by injecting insects with the maize hoja blanca virus. We did not get transmission. Even though particles and symptoms are similar, the vectors are completely different.

R.W. Toler: This past winter I had some maize mosaic resistance trials in Hawaii in cooperation with Dr. J. L. Brewbaker. These trials involved maize germplasm from North America. There were a number of inbreds from North Carolina, South Carolina, Alabama, Louisiana, Tennessee, and Texas that had good resistance.

R.J. Lastra: As I have said, maize mosaic virus is important in Venezuela. Although Dr. Bradfute found the virus in the U.S., I do not think it is important here. I do not know about the other countries. Do you have information concerning maize mosaic in Hawaii?

R.W. Toler: It is extremely important and is the number one disease of corn in Hawaii. Without insect control or host resistance, you cannot grow corn there, especially during the winter months.

L.R. Nault: What do you consider to be the other hosts for the virus and vector?

R.J. Lastra: Corn is grown the year around in Venezuela with irrigation. We also see this virus in *Rottboellia exaltata* and *Sertaria vulpiseta*. They are two of the major grasses in Venezuela. We also can transmit the virus with the vector to teosinte and sorghum in the laboratory.

R.D. Osler: How severe is maize mosaic in a normal farmer's field?

R.J. Lastra: Sometimes, I have seen up to 60% infection in normal fields. Usually, corn on perimeters of the fields is very heavily infected. Incidence also depends on the year, part of the country, and if corn is planted the year around. Under irrigation the disease is very heavy.

L.E. Williams: Did you ever look at wheat to see if it has hoja blanca?

R.J. Lastra: We do not grow wheat. We used to grow wheat high in the mountains at around 8,000 meters, but the land there is very eroded. We are beginning to introduce some wheat varieties for cultivation. Presently, we must buy our wheat.

V.D. Damsteegt: Do you see any conflict between your rayado fino strain of maize mosaic virus and the rayado fino virus that is everywhere else?

R.J. Lastra: They are different viruses, because of differences in particle morphology and vectors. They also cause different diseases.

V.D. Damsteegt: I realize these differences, but what I meant was the synonomy of the name rayado fino. In terms of corn losses in Venezuela, how do virus diseases compare to your downy mildew epidemic?

R.J. Lastra: Until now, virus diseases were the worst problem. But in some parts of the country last year downy mildew was worse than virus diseases. This was the first year that we have a bad downy mildew epidemic. I do not know what losses will occur this year.

R.W. Toler: In Texas we commonly have plants infected with both downy mildew and virus.

Maize Virus and Virus-Like Diseases in Peru

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HISTORICAL BACKGROUND AND CURRENT STATUS OF MAIZE PRODUCTION

Although proof of existence of maize, presumably about 60,000 years ago, was presented by Barghoorn, Wolfe and Clisby (2), Peru in South America has been suggested as an independent domestication center in addition to the Middle American Center in Mexico and Central America. Such a proposal was made first by Grobman, *et al.* (7).

Corn appeared on the Coast by the 9th century B.C., usually together with an elaborate form of pottery for ceremonial use. Indians of the Andes and of coastal valley in Peru appear to have attained an early integration of maize along with other motivation factors. In another way it is difficult to explain their intimate relationship with maize expressed in so many ways through their artistic, religious, and social activities.

Historically, the first references to maize grown in Peru come from the Spanish conquerors. Spanish chroniclers, starting as early as 1533 and writing through the 16th century and early part of the 17th century, contributed abundantly to our knowledge of maize cultivation in historical times; that is, from the nearly 100 years preceding the Spanish conquest to the post-colonial period immediately following.

It cannot be definitely stated when and where corn cultivation began in Peru but it may be hypothesized that it started in the Incipient Agriculture Epoch, at least before 1,000 B.C., in the middle altitudes of the Sierra. Highland popcorn races appeared on the Coast in the Formative Epoch. The first hybrid populations for which there is evidence were present on the South Coast by about 500 B.C. At least eight races were in existence by the end of the Formative Epoch, and by the end of the Classic Epoch (0-1,000 A.D.) some ten new races had been added. During the next 500 years of the Post-Classic Epoch, at least four additional races were formed.

The disruption of the Inca Empire by the Spanish conquest and the civil war that followed had as a sequel a collapse of the oranized agriculture of that time and a change in its patterns from state planning and control of its major features to an individualistic subsistence type. Removal of large masses of the Indian population for labor in mining and lack of interest of the early conquerors in farming contributed to a deterioration of the irrigation and terracing systems in many areas of Peru and a retrogression of agricultural production in general. This situation continued through the Colonial and a great part of the Republican Periods, establishing a clear-cut contrast with the organized state of agricultural development in the pre-Columbian times.

MODERN MAIZE CULTURE

In 1975, the cultivated area of maize in Peru was 474,250 ha with 705,309 metric tons of production, making it the most important crop of that year. The area had been increased by 30% in comparison with that in 1970. This increase was due to government restriction of meat consumption and a growing demand of a rapidly developing poultry industry that had necessitated importation of this cereal.

Flint races accounted for 46% of the total production. Flints are cultivated in the lowlands for easy transportation into and commercialization in cities where they are processed into balanced food for poultry. Floury races accounted for the other 54%.

The low average yield of 2.8 ton/ha is due to the competition from other more profitable crops, shortage of fertilizer, and high production costs.

Valleys of the Northern and Central Departments produce most of the maize of the Coast. Two crops per year for grain and up to four for forage are possible in the valleys with sufficient water for effective irrigation.

Important corn-growing areas are found in the valleys of the Sierra and all of the middle altitude areas of the Andean Cordilleras. The greatest concentration is found in the Department of Cuzco, particularly in the Urubamba Valley famous for its large-kerneled Cuzco corn. High densities of planting, irrigation, and a perfect climate are responsible for the high yields of varieties, such as Yellow and Giant Cuzco. In the jungle, small-scale plantings of corn are made on newly cleared hillside lands and river beaches.

USES OF MAIZE

Maize is used in Peru primarily for human consumption and secondarily as animal feed and fodder. It now is and has been consumed by humans since ancient times in a great variety of forms, the most common of which are: 1) kcancha, a toasted or parched floury or sweet corn used before meals as bread substitute; 2) mote, made by boiling dry floury grain; 3) chicha or corn beer, made by fermenting an aqueous extract of pregerminated corn grain; 4) choclo, which is boiled green corn on the cob, for which floury varieties are used; and 5) many simple native food preparations based on floury corn, such as tamales, humita, chochoca, etc.

Parts of the corn plant other than the grain are also used for human consumption. Stalks of Sierra corn are chewed at harvest time for their high sugar content. Corn is utilized as a feed grain in most low elevation areas in Peru, particularly on the Coast. Flint and semident corn races are preferred for this purpose. Very little corn is used for feeding animals in the Sierra. Corn planted more densely for fodder is called chala. The most important green fodder and silage-producing areas for feeding dairy herds raised in pens with almost no pasturing are located on the Central Coast, particularly around Lima and other large cities. Dry corn stalks, after the ears have been harvested, are also used for animal fodder throughout the Coast and Sierra. Dried corn cobs are used as fuel in many rural areas (6).

VIRUS DISEASES

Corn virus diseases are suspected to have been present in corn fields since ancient times; however, the first report dates from 1947 as corn mosaic transmitted by *Rhopalosiphum maidis* (Fitch) (2, 6). Apparently, the same disease was reported by Bazan de Segura in 1959 (3); however, apart from the disease description, evidence of the virus identification was not presented.

Maize chlorotic mottle virus [MCMV]. — In 1971, a virus disease was noted in corn fields in the valleys of Lima, Huaral, Chancay, Lurin, and Canete of the Department of Lima, affecting hybrid PM-204 and the floury varieties Pardo, Chancayano, and Diente de Mula (4).

After mechanical inoculation in the greenhouse, symptoms started to appear in 7-10 days consisting of fine streaks that coalesced to form a chlorotic mottling (Fig. 1). Necrosis appeared over the chlorotic areas, then epinasty and death of leaves.

In the field, growth was stunted with the formation of short internodes (Figs. 2 and 3). Male inflorescences were also affected with hard pan-

Fig. 1. Mottling of maize leaves caused by maize chlorotic mottle virus.

Fig. 2. Maize plants stunted by the maize chlorotic mottle virus.

icles, short rachis, and few spikelets (Fig. 4). Fewer ears and ear malformations were produced.

The host range of MCMV includes *Zea mays* L.; *Zea mays mexicana* (Schrad.) Iltis; *Panicum virgatum* L.; *Sorghum halepense* (L.) Pers.; *S. vulgare* Pers.; *Setaria glauca* (L.) P.B.; *Bromus arnenis* L.; and *Triticum aestivum* L. 'Blue Boy'. It failed to infect 40 other inoculated hosts, including cereals, grasses, and some dicotyledons.

Aphid transmission tests were negative when *R. maidis*, *Aphis gossypii* Glover, and *Myzus persicae* Sulzer were used. *Dalbulus maidis* (DeLong & Wolcott) was also tested with negative results. No transmission was obtained through 800 seeds from infected plants.

Fig. 3. Symptoms of maize chlorotic mottle virus-infected corn in the field.

Fig. 4. Male inflorescences affected by maize chlorotic mottle virus.

The thermal inactivation point of the virus is 80 to 85 C and the dilution end point $1:10^{-6}$. It is very stable with a longevity *in vitro* of more than 288 hours.

The virus is polyhedral, about 30 nm in diam (Fig. 5). It did not react with antiserum to cocksfoot mild mosaic, phleum mottle, rice yellow mottle, brome mosaic, and sowbane mosaic viruses. These viruses were tested because of their similar physical properties and host range to MCMV.

Yield reduction on inoculated plants in an experimental plot reached 59%. In nature, the virus infects mainly floury and sweet varieties, causing losses in the order of 10-15%.

The virus may form synergistic complexes with the rayado fino virus. It also has been found associated with a disorder called bent shoot. In both cases there was no ear formation.

Sources of resistance have been found through evaluation of varieties in the germplasm bank of the Cooperative Corn Program of Peru under greenhouse and field conditions (Table 1). In 1972, an experimental plot was set up to test six floury and sweet corn varieties for resistance to MCMV. A new hybrid, Pardo x Blanco Urubamba, was found to be resistant but the other five were susceptible.

TABLE 1. Varieties resistant to maize chlorotic mottle virus in the Cooperative Corn Program of Peru

Place of collection	Number
Amazonas	13
Apurimac	37
Arequipa	2
Arequipa	8
Arequipa	12
Arequipa	13
Ayacucho	29
Ayacucho	31
Lima	46

Rayado fino virus [RFV]. — This disease was described by Gamez (5) and identified by him in Peru. Symptoms first appear as chlorotic streaks or short lines distributed more or less uniformly over the leaf. These streaks become more numerous and may fuse into short stripes (Figs. 6 and 7). Symptoms in seedlings are more conspicuous (1).

Fig. 5. Virus particles of maize chlorotic mottle virus (approximately 30 mm in diam.)

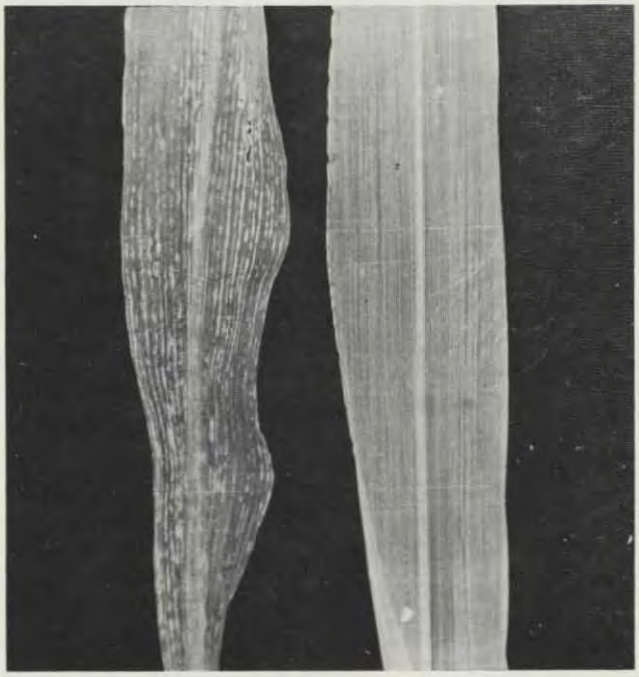

Fig. 6. Rayado fino virus symptoms on maize leaves.

Sometimes the disease can be found associated with maize chlorotic mottle virus. From a diseased plant, mechanical transmission of MCMV was obtained as well as insect transmission of rayado fino using *Dalbulus maidis* (DeLong & Wolcott).

VIRUS AND VIRUS-LIKE DISEASES SUSPECTED OF BEING PRESENT

Sugarcane mosaic virus. — In the areas where corn was cultivated next to infected sugarcane, the disease was described but the virus not identified.

Maize dwarf mosaic virus, strain A. — Johnsongrass showing streaking and mottling has been noted. Serological tests against antiserum of maize chlorotic mottle virus were negative, giving evidence of the presence of another virus, possibly maize dwarf mosaic virus.

Corn stunt. — Corn stunt is suspected to be present. Symptoms of stunting and purple leaf discoloration have been seen, and the insect vector *D. maidis* is present in corn fields.

UNNAMED VIRUS-LIKE DISEASES

1) Ringspots that give negative results when tested for fungi and bacteria. Neither were they mechanically transmissible.

2) Bent shoot that causes bending of the plant and prevents ear production. Sometimes found to be associated with maize chlorotic mottle virus.

Fig. 7. Rayado fino virus symptoms on maize plants in the field.

MAIZE RESEARCH PROGRAMS IN PERU

Research on virus diseases started in 1971 and was continued in 1972, when the collection of varieties of the Cooperative Maize Program of Peru was evaluated. At the same time resistance to *Helminthosporium turcicum* Pass. and *Puccinia sorghi* Schw. was investigated. Insect transmission of maize chlorotic mottle virus was begun in 1973 in a search for resistance to this virus in sweet and floury corn.

Unfortunately, research was interrupted and so far has not been resumed because of lack of funds. It is hoped that the funding problems will be solved and research re-initiated in the following areas: 1) identification of all virus and virus-like diseases affecting Peruvian corn, and 2) establishment of an International Cooperative Program for the exchange of resistant germplasm between Peru and other countries of the world.

LITERATURE CITED

1. A compendium of corn diseases. 1973. Am Phytopath. Soc., Inc., St. Paul Minn. 64 p.
2. BARGHOORN, E.S., M.K. WOLFE, and K.H. CLISBY. 1954. Fossil maize from the valley of Mexico. Bot. Leaflets, Harvard Univ. 16:229-240.
3. BAZAN DE SEGURA, C. 1959. Principales enfermedades de las plantas en el Peru, Asociacion de Agricultores de Canete e Ica, Canete, Peru. 70 p.
4. CASTILLO, J., and N. SALAS. 1971. Informes de viajes a valles del Departamento de Lima. Direccion General de Investigaciones Agrarias.
5. GAMEZ, R. 1969. A new leafhopper-borne virus of corn in Central America. Plant Dis. Rep. 53:929-932.
6. GARCIA RADA, G. 1947. Fitopatologu Agricola del Peru. Imprenta El Condor. Lima, Peru. 413 p.
7. GROBMAN, A., W. SALHUANA, R. SEVILLA, and P.C. MANGELSDORF. 1961. Races of maize in Peru: their origins, evaluation, and classification. Natl. Acad. Sci. Natl. Res. Council. Pub. No. 915. 374 p.

DISCUSSION OF J. CASTILLO-LOAYZA PAPER

V.D. Damsteegt: You said that the inactivation temperature for maize chlorotic mottle virus is 80-85 C. That is unusually high. I cannot think of many viruses that have such a high inactivation temperature.

J. Castrillo-Loayza: Yes, it is high. The laboratory work, purification and electron microscopy, were done by Dr. T.T. Hebert at North Carolina State University.

R. Gamez: What was the size of the particle of maize chlorotic mottle virus?

J. Castillo-Loayza: 30 nanometers.

R.W. Toler: Have you found any vectors?

J. Castillo-Loayza: No. We have tested aphids and *Dalbulus maidis*. We have not found any vector so far, but we believe that one is involved.

D.T. Gordon: Has this virus been reported to occur in other countries?

J. Castillo-Loayza: I do not know of any. So far it has been found only in Peru.

R.I. Brown: I have seen the ring spot symptom you mentioned in my travels in a number of places, at least what I think looks like it.

J.M. Fajamisin: I have also seen that symptom frequently. Do you sometimes see water soaking?

J. Castillo-Loayza: Yes, but this is not the same.

A.J. Ullstrup: Many, many kinds of spots—chloroses, necroses—result when you inbreed corn. All corn breeders know this. Some of these spots are associated with a single, recessive gene and others with a single, dominant gene. We found a symptom recently that looks very much like the one in question. It is associated with a single, partially dominant gene. Some people have reported in the literature that these are virus diseases, but they have never been able to transmit the pathogen. It seems to me that proof of a viral cause of such a spot must be pathogen transmissibility by insects, grafting, or mechanical transmission. There is at least one report in the literature of a "disease" like this being lethal. We found one a few years ago. It involved a single, recessive gene on chromosome 1. These spots are very confusing, and you will encounter them all the time in corn breeding.

V.D. Damsteegt: In epidemiology plots at Frederick, Maryland this year we had maize dwarf mosaic alone and in combination with maize chlorotic dwarf. At the plot edge where the corn had been inoculated, every plant showed spotting on the lower but not on the new leaves. In contrast, the healthy corn had green lower leaves. I do not know if the spotting involved a genotypic reaction of that variety in response to an early virus infection or to some other factor.

R.W. Toler: I have seen two types of spots similar to this. One was caused from insecticide damage that occurred under particular humidities and temperatures. The other, for the lack of a demonstrated cause, we called a physiological leaf spot.

A.J. Ullstrup: I have seen this water-soaking symptom. It is particularly noticeable early in the morning. Later in the day as the temperature increases and the humidity goes down, it disappears. The spot looks oily.

Maize Virus Diseases in the United States

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A portion of the research reported in this paper was financially supported by Grant No. 316-15-55 and Cooperative Agreement No. 12-15-75-2-14 from the Cooperative State Research Service, U.S. Department of Agriculture.

Approved for publication as Journal Article No. 187-76 of the Ohio Agricultural Research and Development Center, Wooster, Ohio.

ABSTRACT

An economically important maize virus disease, which resembles corn stunt, is prevalent in the USA from states bordering the Gulf of Mexico to southern Ohio, Indiana, Illinois, and Missouri and from southern Atlantic coast states to Texas. Principal symptoms of this disease are plant stunting and reddening or yellowing of leaf blades. The disease first became economically important in the early to mid-1960's. Prior to 1972, three strains of the corn stunt agent and maize dwarf mosaic virus (MDMV) had been implicated in the disease. However, corn stunt spiroplasma or mycoplasma-like bodies, reported incitants of corn stunt, and MDMV could not be consistently identified in maize plants with the above symptoms. In surveys since 1972, the recently discovered maize chlorotic dwarf virus (MCDV) was identified in about 91% of diseased samples from 16 states where the stunting disease is most prevalent. In these surveys MDMV strain A was associated with mosaic symptoms and was present in areas of the USA where *Sorghum halepense*, the overwintering host, occurred. In contrast, MCDV was present only in those states where *S. halepense*, the overwintering host, and *Graminella nigrifrons*, the principal leafhopper vector, occurred together. Corn stunt spiroplasma or maize bushy stunt mycoplasma (MBSM) were identified in the surveys from about 2% of diseased samples and only in samples from Louisiana and Texas. In these surveys four viruses and MBSM, all new to maize in the continental USA, were discovered. Wheat streak mosaic virus was the only other previously identified maize virus encountered.

Corn stunt, initially identified in the mid-1940's in Texas (1,14), was first reported to be an economically important disease of maize (*Zea mays* L.) in the USA after an outbreak in Mississippi in the early 1960's (7). In subsequent years the disease was reported throughout many southern states (9). In some areas of the South the disease became so severe that corn production was eliminated or greatly curtailed. Principal symptoms of the disease were stunting of the plant and a reddening or yellowing of leaf blades (22). With some plants upper internodes failed to elongate, whereas with others, upper internodes only partially elongated.

The initial identification of the pathogen in Mississippi by Maramorosch (17) indicated involvement of the corn stunt virus. This identification was based on the persistent transmission of the pathogen by the leafhopper, *Dalbulus maidis* (DeLong & Wolcott), and on symptoms. Subsequent research by Granados and coworkers (11) indicated that the Louisiana strain of the corn stunt pathogen was a mycoplasma-like body (MLB) and not a virus. Further work by Davis and Worley (8) demonstrated that the Rio Grande strain of corn stunt was a new type of plant pathogen, which they termed spiroplasma. The latter designation derives from the helical or spiral appearance of filaments of the pathogen. This pathogen is now referred to as the corn stunt spiroplasma (CSS). Among the several strains of the corn stunt pathogen described or found in the USA, spiroplasma has been associated with the Mississippi (O. E. Bradfute, D. C. Robertson, and L. R. Nault, *personal communication*) and Rio Grande strains (8) and MLB with the Louisiana strain (11). The CSS was detected by phase contrast microscopy and the MLB by electron microscopy of ultra-thin tissue sections. Differences in techniques for visualizing the agents may account for apparent differences in morphology.

At the time that the corn stunt epidemic was first observed in the southern USA, a major maize disease with symptoms similar to corn stunt was observed in southern Ohio by Janson and Ellett (13). The disease was initially thought to be corn stunt. However, the pathogen was not transmitted by *D. maidis* and a new, mechanically transmissible virus was isolated from diseased maize by Williams and Alexander (24). Thus, a new disease, named maize dwarf mosaic, was identified. The mechanically transmissible virus as-

sociated with the disease was named maize dwarf mosaic virus (MDMV). The virus was soon found in other states where it caused significant losses in maize (9). In several southwestern states, MDMV also caused an economically important disease of sorghum [*Sorghum bicolor* (L.) Moench] (9). Maize dwarf mosaic virus has been recovered from maize or sorghum in 27 states of the continental USA (Fig. 1). Early descriptions indicated that stunting and leaf reddening and yellowing as well as mosaic were symptoms of maize dwarf mosaic (24), but more recent studies have associated only mosaic with MDMV infection (10).

Maize dwarf mosaic virus has a filamentous particle and is aphid transmitted in a nonpersistent manner (2). It is related in host range, particle morphology and length, and serological properties to viruses in the sugarcane mosaic virus (SCMV) group (20). The A, C, D, E, and F strains of MDMV (15) infect Johnsongrass [*S. halepense* (L.) Pers.] which serves as the overwintering host for the virus (9). Other strains of SCMV and the B strain of MDMV do not readily infect Johnsongrass (23). Maize dwarf mosaic virus is also related to other maize, Johnsongrass, and sorghum viruses designated by various names, *viz.*, sorghum red stripe virus, Johnsongrass strain of SCMV, maize mosaic virus, and Johnsongrass mosaic virus (9).

From the mid-1960's to 1969 it appeared that corn stunt was important in the southern USA, and maize dwarf mosaic from the southern USA to the southern portion of the Corn Belt. However, there were several indications that CSS, MLB, or MDMV could not account for the entire stunting disease syndrome or occurrence. The principal difficulty was that these pathogens could not be consistently associated with field occurrences of the stunting disease (9). Several investigators speculated that another pathogen was involved in the disease syndrome (9).

Evidence for another pathogen was presented in 1969 when Rosenkranz (21) reported the occurrence of a new maize pathogen in southern Ohio. The pathogen was named the Ohio corn stunt agent (CSA-OH). This agent was transmitted by *Graminella nigrifrons* (Forbes), also a vector of the Louisiana strain of corn stunt agent (12). The CSA-OH was not transmitted by *D. maidis* (21). Although the name implied a spiroplasma or MLB, the morphology of CSA-OH was not reported.

In 1972, another virus associated with plant stunting and yellowing or reddening of leaves of

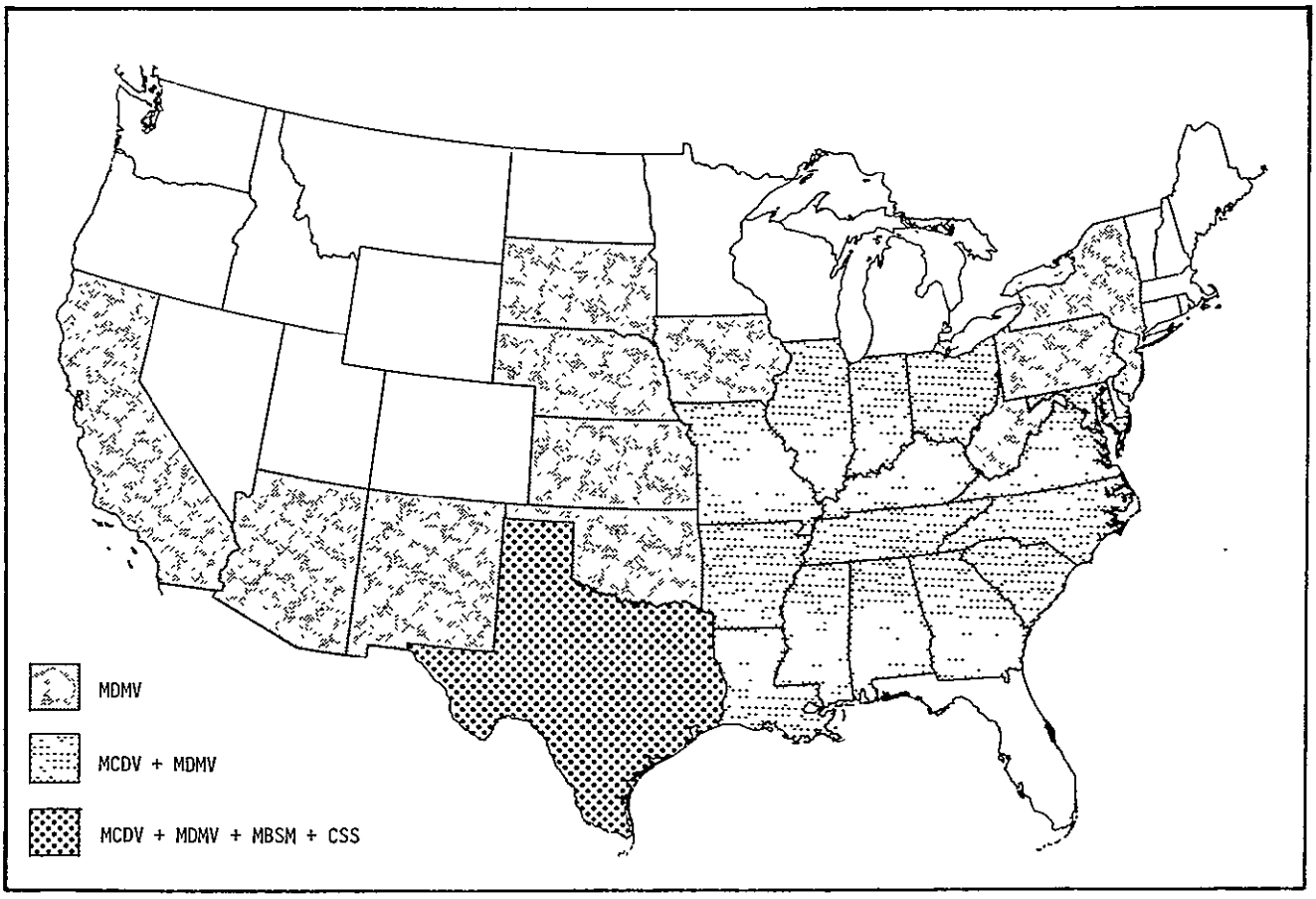

Fig. 1. Occurrences of maize dwarf mosaic virus (MDMV), maize chlorotic dwarf virus (MCDV), maize bushy stunt mycoplasma (MBSM), and corn stunt spirospasma (CSS) by state in the USA (10; O.E. Bradfute, L.R Nault, D.C. Robertson, and R.W. Toler, *personal communication*).

maize in southern Ohio was discovered by Bradfute and coworkers (4). This virus, named maize chlorotic dwarf virus (MCDV), was transmitted by *G. nigrifrons* in a semipersistent manner (19). Particles associated with MCDV were isometric and measured about 31 nm in diam. (3). Like MDMV, MCDV overwinters in Johnsongrass and infects sorghum (18). Chlorotic striping of tertiary veins on leaf lamina is diagnostic for MCDV infection of all hosts (16, 18).

Prior to 1972, several other viruses had been identified from diseased maize in the USA, but none was implicated in the stunting disease nor caused widespread damage (9). These viruses included SCMV, wheat streak mosaic virus (WSMV), maize leaf fleck virus, brome mosaic virus (BMV), and cucumber mosaic virus (CMV).

By the end of 1972, the etiology of the maize stunting disease or diseases found in the USA was unclear. Three strains of the corn stunt pathogen (Louisiana, Mississippi, and Ohio) (12, 22), MDMV (24), and MCDV (4) had been implicated in the disease(s). To resolve this problem, a cooperative investigation involving state, federal, and industry maize researchers was initiated (10). Leaf samples from maize in 23 states were field-collected and promptly shipped to Wooster where assays for virus and virus-like pathogens were made. Symptoms of each sample were recorded by the collector on a rating form which requested information on the presence of mosaic, chlorotic banding of secondary veins, chlorotic striping of tertiary veins, chlorosis, reddening, leaf tearing, twisting or rolling of whorl leaves, plant tillering, shoot proliferation, ears and their conditions, and stunting of plants. These symptoms had been associated previously with the stunting disease. In addition to samples shipped to Wooster, we made field trips to eight states to collect samples in collaboration with cooperators in each state. Five hundred and seventy-nine maize samples were assayed for MCDV and mechanically transmissible viruses during the 2 years of the study. Only a portion of these samples was assayed for other viruses, MLB, and CSS.

Assays performed were mechanical inoculations of indicator hosts, leafhopper transmission to maize, immune density-gradient centrifugation, and light and electron microscopy. Mechanical inoculations served to detect MDMV strains, WSMV, BMV, CMV, and other mechanically transmissible viruses. The leafhopper transmission assays were to detect *D. maidis*- and *G. nigrifrons*-persistently transmitted agents. The immune centrifugation and *G. nigrifrons*-transmission assays detected MCDV. Light microscopy detected spiroplasma, and electron microscopy new virus and virus-like pathogens. The performance of these assays involved close cooperation between personnel in electron microscopy, vector-virus, and virology laboratories at Wooster.

Results of the study indicated that MCDV was the most prevalent of the pathogens infecting survey samples of maize (10). It was identified from about 76% of the diseased samples from 16 states where the stunting disease is most prevalent. Maize dwarf mosaic virus was identified in 39% of diseased samples from 18 states in and outside of where the stunting disease occurs. Two samples (both from Texas) of the 168 tested were infected with a *D. maidis*-transmitted corn stunt spiroplasma. Mycoplasma-like bodies were observed by electron microscopy in one additional sample from Texas and one from Louisiana (5). Wheat streak mosaic virus was identified in two diseased samples. (10).

Of these pathogens, only MCDV was significantly associated with symptoms of plant stunting and yellowing or reddening of leaves (10). This association implicated MCDV as the principal incitant of the corn stunt-like disease in the USA. As added support that this widespread disease was maize chlorotic dwarf and not corn stunt, CSS or MLB were identified in only about 2% of samples with corn stunt-like symptoms. Maize chlorotic dwarf virus was also significantly associated with a chlorotic striping along tertiary veins. Maize dwarf mosaic virus infections were significantly associated with mosaic. None of the remaining symptoms listed on the rating forms occurred on more than 10% of diseased plants. The distribution of MCDV appeared generally limited to those states where both Johnsongrass and *G. nigrifrons* occurred together (10). Among these states, MCDV was not found in samples from Kansas and Oklahoma, states on the border of where the disease was known to occur and where Johnsongrass and *G. nigrifrons* occur together.

Four new viruses and a MLB were discovered to infect maize during the survey. The first was a rhabdovirus, possibly related to maize mosaic virus, that was identified in maize from Texas (6). This was the first report of a rhabdovirus infecting maize in the continental USA. An isometric virus particle transmissible by *D. maidis* was identified in several maize samples from Texas (O. E. Bradfute, D. C. Robertson, D. T. Gordon, L. R. Nault, and R.W. Toler, *unpublished*). This virus appears related to maize rayado fino virus. A mechanically transmissible virus with a long filamentous particle and a host range distinct from known maize viruses having similar particle size was discovered in several Texas samples (D.T. Gordon, O.E. Bradfute, D.C. Robertson and R.W. Toler, *unpublished*). A new mechanically transmissible virus with a short, filamentous particle was found in weed grasses in a maize field in Louisiana (O.E. Bradfute, D.T. Gordon, D.C. Robertson, R.W. Toler and K.S. Derrick, *unpublished*). This virus infects maize in experimental transmission tests. Finally, a MLB, named maize bushy stunt mycoplasma, was discovered in

several Texas maize samples (O.E. Bradfute, L.R. Nault, D.C. Robertson, and R.W. Toler, *personal communication*). This MLB is transmitted by *D. maidis* in a persistent manner. All of the new pathogens, except the rhabdovirus, were discovered in 1975 or 1976 and are presently being characterized further.

LITERATURE CITED

1. ALTSTATT, G.E. 1945. A new corn disease in the Rio Grande Valley. Plant Dis. Rep. 29:533-534.
2. BANCROFT, J.B., A.J. ULLSTRUP, M. MESSIEHA, C.E. BRACKER, and T.E. SNAZELLE. 1966. Some biological and physical properties of a Midwestern isolate of maize dwarf mosaic virus. Phytopathology 56:474-478.
3. BRADFUTE, O.E., R.E. GINGERY, D.T. GORDON, and L.R. NAULT. 1972. Tissue ultrastructure, sedimentation and leafhopper transmission of a virus associated with a maize dwarfing disease. J. Cell. Biol. 55 25a (Abstr.).
4. BRADFUTE, O.E., R. LOUIE, and J.K. KNOKE. 1972. Isometric viruslike particles in maize with stunt symptoms. Phytopathology 62:748 (Abstr.).
5. BRADFUTE, O.E., and D.C. ROBERTSON. 1974. Identification of the Mississippi corn stunt agent by electron microscopy. Proc. Am. Phytopathol. Soc. 1:35-36 (Abstr.).
6. BRADFUTE, O.E., D.C. ROBERTSON, and R.W. TOLER. 1974. Possible occurrence of maize mosaic virus in the continental United States. Proc. Am. Phytopathol. Soc. 1:36 (Abstr.).
7. CHOUDHURY, M.M., and E. ROSENKRANZ 1973. Differential transmission of Mississippi and Ohio corn stunt agents by Graminella nigrifrons. Phytopathology 63:127-133.
8. DAVIS R.E., and J.F. WORLEY 1973. Spiroplasma: Motile, helical microorganism associated with corn stunt disease. Phytopathology 63:403-408.
9. GORDON, D.T., W.R. FINDLEY, J.K. KNOKE, R. LOUIE, L. R. NAULT. O.E. BRADFUTE, E.J. DOLLINGER, and R.E. GINGERY. 1974. Distinguishing symptoms and latest research findings on corn virus diseases in the United States. Pages 153-173 in Proc. Annu. Corn Sorghum Res. Conf. Publ. 29. Am. Seed Trade Assoc., Washington, D.C. 214 p.
10. GORDON, D.T., and L.R. NAULT. 1977. Involvement of maize chlorotic dwarf virus and other agents in stunting diseases of Zea mays in the United States. Phytopathology 67:27-36.
11. GRANADOS, R.R. 1969. Electron microscopy of plants and insect vectors infected with corn stunt disease agent. Contrib. Boyce Thompson Inst. 24:173-187.
12. GRANADOS, R.R , K MARAMOROSCH, T. EVERETT, and T.P. PIRONE. 1966. Transmission of corn stunt virus by a new leafhopper vector, Graminella nigrifrons (Forbes). Contrib. Boyce Thompson Inst. 23:275-280.
13. JANSON, B.F., and C.W. ELLETT. 1963. A new corn disease in Ohio. Plant Dis. Rep. 47:1107-1108.
14. KUNKEL, L.O. 1946. Leafhopper transmission of corn stunt. Proc. Nat. Acad. Sci. USA 32:246-247.
15. LOUIE R., and J.K. KNOKE. 1975. Strains of maize dwarf mosaic virus. Plant Dis. Rep. 59:518-522.
16. LOUIE, R., J.K. KNOKE, and D.T. GORDON. 1974. Epiphytotics of maize dwarf mosaic and maize chlorotic dwarf diseases in Ohio. Phytopathology 64.1455-1459.
17. MARAMOROSCH, K. 1963. The occurrence in Arizona of corn stunt disease and of the leafhopper vector Dalbulus maidis. Plant Dis. Rep. 47:858.
18. NAULT, L.R., D.T. GORDON, D.C. ROBERTSON, and O.E. BRADFUTE. 1976 Host range of maize chlorotic dwarf virus. Plant. Dis. Rep. 60:374-377.
19. NAULT, L.R., W.E. STYER, J.K. KNOKE, and H.N. PITRE. 1973. Semipersistent transmission of leafhopper-borne maize chlorotic dwarf virus. J. Econ. Entomol. 66:1271-1273.
20. PIRONE, T.P. 1972. Sugarcane mosaic virus. No. 88 in Descriptions of plant viruses June 1972. Commonw. Mycol. Inst., Assoc. Appl. Biol. Kew, Surrey, England 4 p.
21. ROSENKRANZ, E. 1969. A new leafhopper transmissible corn stunt disease agent in Ohio. Phytopathology 59 1344-1346.
22. ROSENKRANZ, E.E. 1972 Present status of MDM, corn stunt, and other virus and virus like diseases of corn and sorghum. Pages 65-79 in Proc. Annu. Corn Sorghum Res. Conf. Publ. 27. Am Seed Trade Assoc., Washington, D.C. 224 p.
23. TOSIC, M., and R.E. FORD 1972. Grasses differentiating sugarcane mosaic and maize dwarf mosaic viruses. Phytopathology 62:1466-1470.
24. WILLIAMS, L.E., and L.J ALEXANDER, 1965. Maize dwarf mosaic, a new corn disease. Phytopathology 55:802-804.

DISCUSSION OF D.T. GORDON PAPER

L.M. Josephson: What was the number of samples that your percentages were based on?

D.T. Gordon: Five hundred and seventy-nine samples from 23 states These were assayed by infectivity and density gradient centrifugation. The numbers of samples received from different states varied For example, we had 61 samples from Tennessee, 51 from Mississippi, and 3 from California.

A.J. Ullstrup: Dr. Karl Maramorosch identified two types of corn stunt in Mexico, the Rio Grande and Mesa Central. What is the relationship of Mesa Central to Louisiana corn stunt?

O.E. Bradfute: I do not think the relationship is clear. Part of the problem is that the pathogens causing these diseases were only partially characterized. Symptoms were very well described in the laboratory, but the cultures are lost. We now look at these same problems with a little more sophistication that includes more means of characterization. But we cannot go back to those original isolates and make comparisons We cannot even isolate new cultures from plants in the regions where they originally occurred, because we cannot find these symptoms in the field.

S.P. Raychaudhuri: Your point is very relevant, and perhaps it would be useful to maintain different strains of viruses at a central place for comparison. Maybe the question of how this might be done could be discussed at the end of these meetings. Dr. Gordon reported the incidence of spiroplasmas as 1.2% and mycoplasmas, 1%. Is there any apparent difference in symptoms between those plants which showed spiroplasmas in thin section and those which showed mycoplasma-like bodies?

O.E. Bradfute: We would have difficulty distinguishing spiroplasma-infected from non-helical MLB-infected plants on the basis of symptoms in the field. Definitive symptoms of Rio Grande corn stunt and maize bushy stunt, as seen in experimentally-infected greenhouse plants, have not been consistently associated with these microorganisms when these symptoms were observed in the field. At the time when the data to which you refer were collected, we were unaware of maize bushy stunt and its association with a non-helical MLB. At this time, plants suspected of having Rio Grande corn stunt were examined by thin section electron microscopy (EM). Microorganisms identified by this

technique were called MLB, because thin-section EM is not a reliable method for detecting spiroplasmas. Recently, we have used Dr. Davis' dark field microscope technique to identify spiroplasmas. Therefore, 1.2% of the samples contained the helical filaments of spiroplasmas and an additional 1% contained MLB which could have been either helical or non-helical.

R.E. Davis: In *in vitro* clone cultures of the various spiroplasmas, we find the organism always produces the characteristic helical filaments. However, as the culture ages these organisms produce rounded bodies to which filaments are attached Just precisely what function these round bodies have in the development of these organisms, we do not yet know. When we look at sections of infected corn or citrus plants or insects containing certain other spiroplasmas, we find not only organisms with the characteristic helical filaments but also round bodies attached to helical filaments, such as seen in the *in vitro* cultures. Dr. Bradfute also has seen these in his preparations of spiroplasmas. It might become confusing if a single host plant should become infected with a spiroplasma and a mycoplasma. If you are looking at round bodies, you might not be able to determine with certainty which type of body you were looking at But where we do extensive sectioning of plants infected with spiroplasma, we never fail to find helical filaments. If we had a case in which we failed to find a spiroplasma and found only mycoplasma-like bodies, then we would say that it was probably not a spiroplasma disease

L.R. Nault: Based on what Dr. Maramorosch has published concerning Mesa Central, the symptoms we observe for maize bushy stunt are very similar to those described by him. These symptoms contrast with those associated with the Rio Grande strain.

D.T. Gordon: In Central and South America where corn stunt occurs, is it correct to say that this is a spiroplasma-caused disease? Do you also see symptoms of maize bushy stunt? If you do, what pathogen do you associate with those symptoms?

R. Gamez: It is true that in Central America we have the two types. What people refer to as Rio Grande is, I think, what has been clearly shown by Dr Davis to be caused by a spiroplasma. But, it is also true that there is a second type of stunt which is very virulent. Perhaps it is more prevalent than the Rio Grande type. It is referred to as corn stunt by all people

D.T. Gordon: I have been told that Dr. Carlos DeLeon did an experiment with maize plants showing symptoms resembling those of maize bushy stunt in which he moved these plants from a higher elevation, where presumably they were exposed to the Mesa Central strain of corn stunt, to a lower elevation At the lower elevation, these plants developed symptoms of Rio Grande corn stunt. I think that the reverse movement of plants was done also, and plants infected at the lower elevation showed symptoms of Mesa Central following movement to the higher elevation This implied that the symptomatology was a matter of environmental conditions or elevation. Is there any basis to this idea?

R. Gamez: I also have heard that account, but that is all I know.

D.T. Gordon: Has there been any experimental work on the influence of environment on corn stunt symptoms?

R. Gamez: Not that I know of.

A.J. Ullstrup: I saw some work of Dr. DeLeon in which he brought specimens of the Mesa Central from higher altitudes down to Veracruz and transmitted the pathogen to a particular maize genotype. He also transmitted the Rio Grande strain from the lower altitudes to this same genotype at the higher elevation. The symptoms were very much the same. This led him to doubt that there were two strains, as described by Dr. Maramorosch.

B. Tsotsis: Looking at the epidemiology of virus diseases in the U.S., maize dwarf mosaic has been around for more than a decade. Although it has been identified in 26 states, why do we not get additional spread, since there is susceptible corn grown contiguously to areas where the disease is endemic. Is there any explanation for the failure to find further spread of the disease?

L.M. Josephson: I think that there is a simple explanation to your question. I have never seen virus symptoms in areas other than where Johnsongrass occurs. The virus must have an overwintering host. There also has to be some sort of stress applied to plants. We may find a few infected corn plants adjacent to small clumps of Johnsongrass, but the spread is quite limited and most plants are healthy. Johnsongrass must be concentrated in an area before a large number of plants become infected.. You do not have Johnsongrass in Ohio other than in the bottomlands of the Ohio river and its tributaries. These are the principal areas of maize dwarf mosaic occurrence.

B. Tsotsis: There are no concentrated occurrences of Johnsongrass in Iowa, Indiana or Illinois, except in the case of the latter two states near the Ohio River Also, corn grows contiguously throughout these states and vectors are abundant Under these conditions, why is it that virus disease epidemics in the Ohio River valley do not spread northward? We have epidemics of leaf diseases caused by fungi and bacteria that spread fast over large areas away from the original source of the inoculum. Reports from Iowa in the 1960's failed to indicate any significant damage in corn caused by viruses.

V.D. Damsteegt: Reports by Dr. R.E. Ford link other perennial grasses as well as Johnsongrass to maize dwarf mosaic. Dubuque County in northeastern Iowa, which is separated by several counties from the occurrence of Johnsongrass, has had extensive outbreaks of maize dwarf mosaic. I think Dr. C.W. Boothroyd has mentioned a similar situation in New York where they had an extensive outbreak of maize dwarf mosaic without the occurrence of Johnsongrass within a reasonable distance. During summer 1976, Maryland had an outbreak of maize dwarf mosaic that caused tremendous loss. In all, some 5,000 acreas of corn were affected, and the incidence of maize dwarf mosaic was 60% or more. And yet we could not find Johnsongrass anywhere. I do not know how to explain this occurrence

R.W. Toler: What type of corn was this?

V.D. Damsteegt: It was sweet corn. In field corn, I did not see virus diseases in great amount.

R.W. Toler: What were the symptoms — just a mosaic?

V.D. Damsteegt: In the sweet corn, the disease was very severe. Symptoms included mosaic, a temporary wilting of plants, yellowing, stunting, and a reduction in yield. We have isolated only maize dwarf mosic virus from these plants

E.E. Rosenkranz: Perhaps we can explain isolated occurrences of maize dwarf mosaic by seed transmission. You only need one or two plants infected through the seed to start an epiphytotic within the field. Aphids transmit the virus from these plants, and it can spread quite rapidly. There are two reports, one from Ohio and one from California, of seed transmission of maize mosaic dwarf virus.

D.T. Gordon: There is also a third report from Iowa.

B.L. Renfro: Here at Wooster, we are only 6 to 8 hours away, by aphid flight, from the Ohio River valley Aphids get up into the wind and could travel here within this period. How long can aphids carry maize dwarf mosaic virus?

L.R. Nault: In laboratory tests, aphids lose the ability to transmit MDMV within about 25 or 30 minutes Maybe Dr. Knoke could extend this observation to a field situation.

J.K. Knoke: An important aspect in the field is that these aphids are not good fliers. They are moved around by winds. As Dr. Nault said, they lose the virus quickly, so they do not travel too many miles before they are no longer viruliferous. We see isolated instances of both A

and B strains of MDMV in northern Ohio counties. However, since these counties do not have a reservoir of Johnsongrass, the virus does not overwinter there Every year, it must move from the southern part of the state into these counties.

R.W. Toler: Would these aphids have to move the entire distance all in one day? Since you have sequential plantings of corn, sequential buildup of aphids and disease development could occur as the season develops northward.

J.K. Knoke: Yes, I think that is possible Outbreaks of the disease are found in the sourthern half of the state, and that is about as far north as they occur throughout the season

D.T. Gordon: Maize chlorotic dwarf virus is widespread and very damaging on corn, at least in the southern U.S. Based on information presented at this colloquium, MCDV does not appear to occur in other countries of the world. To me this is surprising, especially in the case of Central America. Have any of you from other countries seen plants with maize chlorotic dwarf symptoms? If so, have these been assayed sufficiently to detect MCDV? These symptoms do not appear typical of corn stunt as seen in Central or South America. But they certainly are striking and would not go unnoticed Also, do you have the vector of MCDV, *Graminella nigrifrons*, in Central America?

R.E. Gamez It is true that some of the symptomatology is, I think, rather similar to some symptoms that we observe and call corn stunt. It could be that we have this virus; I have not checked plants for MCDV. I assumed that they were corn stunt-infected plants. As to *Graminella*, I do not think it has been reported in Central America. However, I am not sure. I have searched for it, but have not found it I am under the impression that it has been reported in some places, but it is not a common insect; at least, it is not important in maize growing areas.

D.T. Gordon: *Deltocephalus sonorus* also has been demonstrated as a vector of MCDV. This leafhopper may occur in Central America more frequently than *G. nigrifrons* since it is a more tropical or sub-tropical species.

R.E. Gamez: We have searched for that leafhopper also and have not found it.

D.T. Gordon: That may be the answer to my question, namely, that vectors of MCDV do not occur in Central America. Is this true in South America as well?

R.J. Lastra: The only similar symptoms that I have found were those of corn stunt — the Rio Grande strain We cannot find the other type There are not too many plants with corn stunt symptoms In these plants, I always find the spiroplasma in the vascular system and no viruses As I mentioned, very few plants with these symptoms are found, even though *Dalbulus maidis* is a common insect in Venezuela. I think that the other insect you mentioned, *Deltocephalus sonorus*, has been reported also in South America. In the northern part of the country, they are common. We also find Johnsongrass (*Sorghum halepense*) in Central and South America

G. Martinez-Lopez: We also have seen some symptoms similar to corn stunt. The disease agent is transmitted by *Dalbulus maidis* I have been studying populations of leafhoppers in maize, and have not been able to find *G. nigrifrons*. We have some species of *Deltocephalus*.

J.K. Knoke: If *G. nigrifrons* has a choice, it will not feed on corn, but rather on other grasses that happen to be around the corn field. If you sample a small plot of corn, you essentially will find no *G. nigrifrons*. But in other grains and grasses around the plot, you will find many This may be part of the reason why MCDV does not spread well from the south to the north in Ohio.

A.J. Ullstrup: How many vectors have been reported for corn stunt?

L.R. Nault: *Dalbulus maidis*, *Dalbulus elimatus*, *Graminella nigrifrons*, *Deltocephalus sonorus*, and *Dalbulus tripsaci*. We have really talked in terms of three main vectors of maize viruses in the Americas; *G nigrifrons*, *D maidis*, and *Peregrinus maidis*. Of the viruses we have talked about in Central and South America, Africa, Asia, what are their potentials for entering this country? For instance, will rayado fino be much of a threat; will it spread beyond Texas?

R.E. Gamez: I think it all depends on movement of the leafhopper vector of rayado fino and its ability to survive the winter here. I am referring to *D maidis*. Do you have another leafhopper vector that could possibly transmit this virus, one that we might not have in Central America?

V.D. Damsteegt: This again points up the need for a central location where this sort of information could be correlated and where stocks of insects and pathogen cultures could be brought together for comparative studies. Our laboratory at Frederick, Maryland, which could do this kind of work, is very small. We would like to do these things, but we do not have enough room. Someone needs to do it somewhere.

R.E. Gamez: I was asked if I had seen your test plants, presumably infected with rayado fino virus collected in Texas. Symptoms on some are so similar to what I have observed that they could have been taken from my own greenhouse.

V.D. Damsteegt: I also have looked at these plants. The symptoms look like some shown in slides by several people. I think you have rayado fino. It is now in Texas, and I would not be surprised if it were found in other southern states. What do we know about the migration pattern of *D. maidis* in the United States? How far does it move northward? Could it be possible that it has long-range migrations at certain times of the year?

E.E. Rosenkranz: *Dalbulus maidis* migrates into the southern United States, but not every year. When it does migrate, this occurs very late in the season. The leafhopper moves from Texas into Louisiana, then into Mississippi and Alabama, but not before the end of July or early August I believe the northernmost states in which it has been found are the Carolinas For this reason, I do not think rayado fino would be an important disease in the U.S. *Dalbulus elimatus* has never been reported in southeastern United States. The only place it has been found is Arizona or New Mexico

I would like to question the validity of the etiology of the corn stunt disease in the U.S. When we transmit the agent from these stunted, discolored plants with *G nigrifrons* into experimental plants, we do not reproduce the field symptoms This makes me believe that there may be yet an unidentified pathogen involved in what we see in the field and that *G. nigrifrons* only transmits one component of the complex. When we look at a plant infected with experimentally transmitted MCDV, we find that the symptoms are extremely mild. We have to look very closely to detect symptoms. Only when we place a healthy plant of the same age next to the infected plant do we see the difference. These observations have troubled me for some years. Last winter, I tested 101 genotypes in a screenhouse facility where I released *G. nigrifrons* from viruliferous cultures. All other insects were excluded by a very fine screen; so MCDV was the only disease agent to which the plants were exposed. None of the 101 genotypes produced symptoms comparable to what we see in the field.

L.R. Nault: I will agree that the type isolate of MCDV produces relatively mild symptoms. It causes a yellow blotchiness in the whorl leaves of greenhouse-infected plants. However, we are talking about a fairly uniform environment in the greenhouse, whereas in the field we have a much greater fluctuation in temperature and rainfall

E.E. Rosenkranz: My facility was a screenhouse and should have had the same environment as the field, except for the screen.

L.R Nault: I have slides of MCDV-infected corn plants that show reddening, extreme stunting, and plant death. Other

plants in this experiment were not as severely affected and still others were nearly as large as uninoculated controls This experiment was done shortly after our initial isolation of MCDV from field-collected Johnsongrass. One thing that concerns me is that the type isolate has probably undergone in excess of 200 transfers in our laboratory. We select for good leaf symptoms which appear in a 7- to 14-day period. We do not select for symptoms that occur beyond 14 days. I am quite convinced that our type isolate is not representative of what occurs in the field. There may well be strains in the field that cause severe stunting. Do you use field isolates transferred directly from Johnsongrass or a strain which has undergone numerous transfers, such as ours?

E.E. Rosenkranz: Yes, our isolate has undergone transfers.

L.R. Nault: I think I need to go back to the field and obtain new isolates from Johnsongrass and then find out whether or not I can produce the severe stunting symptoms.

E.E. Rosenkranz: It is also possible that each plant that shows severe symptoms in the field becomes infected with what we would refer to as different isolates.

S. P. Raychaudhuri: We did some work in India on combined infections of nematodes and maize mosaic virus (a strain of sugarcane mosaic which resembles maize dwarf mosaic virus). When both were present in the plant, symptoms were very severe. But alone, the mosaic virus was not severe. It is possible that many other factors are involved in determining symptom severity.

E.E. Rosenkranz: I would like to see one agent or pathogen that would cause a great number of different maize genotypes to become colored differently in the field, i.e., some yellow, some orange, and some deep purple.

V.D. Damsteegt: This discussion points out some pitfalls of describing symptoms of a disease in the field and then routinely transmitting the virus by serial passage in greenhouse test plants in an attempt to maintain the isolate that produced the field symptoms. Some laboratories may be working with isolates that may not be typical of anything in the field.

Maize Streak and Other Maize Virus Diseases in West Africa

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We gratefully acknowledge the help of Mr. J. A. Medaiyedu, Entomologist, National Cereals Research Institute, Nigeria, in preparation of this manuscript.

ABSTRACT

Maize, *Zea mays* L., was introduced into West Africa about three and a half centuries ago. It is now the most widely grown cereal in the rain forest region and plays an important role in the farming system. Maize is utilized largely for human consumption. Despite its relatively long history in this area, maize improvement is just beginning to receive the much needed governmental and scientific attention. Much production is on small holdings, characterized by poor husbandry and lack of technological inputs. Pests and diseases such as stem borers, rusts, blights and maize streak constitute major constraints to high-level production.

The maize streak disease, transmitted by the *Cicadulina* leafhoppers, is the most important virus disease in West Africa. It is a potentially serious disease, varying in seasonal incidence and effects. Climatic conditions play a vital role in vector and disease outbreaks, but roles of the various epidemiological factors are incompletely understood. A brief account of the current research program on this disease is presented. Research efforts are being directed toward a thorough assessment of the problem and to the evaluation of suitable control methods, with much emphasis on disease-resistance breeding.

Maize stripe virus, which until recently was known to occur only in East Africa, has just been discovered in Nigeria. Its importance in West Africa is not determined.

WEST AFRICA — CLIMATE AND VEGETATION

The West African region is defined as including the lands south of the Sahara, that is, roughly south of latitude 18 N, north of the Guinea Coast and west of the boundary of Cameroon. Countries included are Cameroon, Nigeria, Ghana, Sierra Leone, the Gambia, Niger, Benin Republic (formerly Dahomey), Upper Volta, Ivory Coast, Mali, Senegal, Mauritania, Guinea Bissau (formerly Portuguese Guinea), and Liberia (Fig. 1).

West Africa may be considered a well-defined region, united not only by the river Niger but also by the fact that inhabitants have identical problems. It has a population density higher than the average of the African continent. Agricultural problems are commonly those associated with soil leaching and the resultant loss of fertility, especially in the areas of heavy rainfall in the south and with aridity and enormous rainfall variability on the Saharan fringe. Mean temperatures vary between 70 F and 95 F, a relatively small range. However, the temperature range increases from the coast inland.

The southern coastal lands receive the most rain and rainfall decreases northeastward. The wettest parts, with over 2,000 mm, are Guinea, southwest Sierra Leone, Liberia, and southeast Nigeria where rain-bearing winds cross obliquely. The 1,000 mm and less isohyets appear almost as parallel, straight lines running from west to east. Resulting also from seasonal alteration of dry northeast and moist southwest winds are increasing lengths of the wet season from north to south, from a 12-month duration in the Niger Delta and the foothills of the Cameroon Highlands to under 3 months north of St. Louis and Timbuktu.

West Africa has two major vegetation divisions: a) forests to the south, and b) savannah to

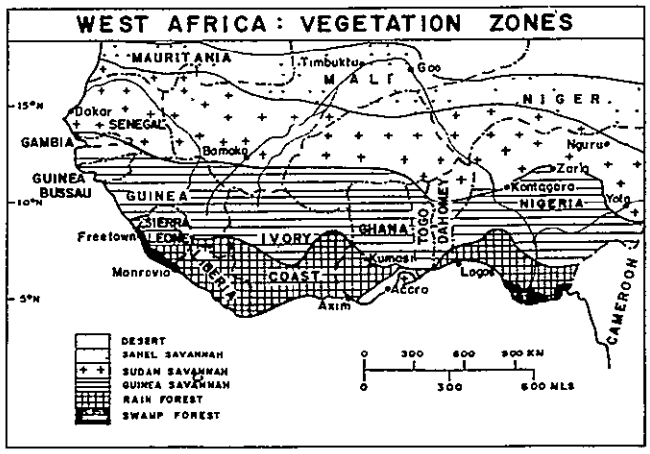

Fig. 1. Vegetation zones in West Africa.

the north. It is difficult to delimit where a forest becomes savannah due to incomplete mapping and differences of opinion and constant cutting of the edges of the forest for cultivation. Forests can be roughly subdivided into: a) swamp forests that occupy many parts of the coast, and b) the rain forest situated just north of the swamps in areas with over 1,100 mm annual rainfall. The savannah vegetation is delimited into three sub-types: 1) the Guinea savannah immediately north of the forests, 2) the Sudan savannah, and 3) the Sahel savannah north of latitude 17 N. Montane vegetation is found in the highland areas of the Cameroon and Plateau State of Nigeria.

HISTORICAL BACKGROUND OF MAIZE PRODUCTION AND USE

The history of maize, *Zea mays* L., in West Africa dates back to the 16th century when the Portuguese traders were reported (5, 7, 25) to have brought maize from the West Indies and Central and South America to the (then) Gold Coast (now Ghana) through Sao Tome. Historical evidence shows that the first maize types brought into West Africa were floury forms that are frequently found in the graves of the ancient Aztecs and Incas. But Porteres (18), on the other hand, suggested that maize came into West Africa from Arab countries. Most of the varieties in the northern parts of West Africa are of light-yellow flint types. Maize is now found throughout West Africa to the extent that it is difficult to convince the farmers that its origin is foreign.

Until the second half of this century, maize cultivation was primarily to satisfy the curiosity of the growers and, at best, for the subsistence of their families and a few neighbors. Thus, for the first three centuries after its introduction, nothing concrete was done to improve the crop and its management. This was true not only for maize but also for other food crops, because emphasis during the colonial era was on the production of so-called "cash crops" like cacao, rubber, coffee, cotton, and groundnut for export purposes. Food crops were to a large extent neglected until the years after World War II.

The advent of the maize rust (*Puccinia polysora* Underw.) in epiphytotic proportions in 1949 created awareness of the importance of maize as a food crop because of initial total crop failures and, in later years, severe reductions in yield (25). The gravity of problems posed by this disease led to the establishment in 1950 of the West African Maize Rust Research Unit (WAMRRU). In an attempt to obtain rust-resistant materials, WAMRRU imported maize cultivars from Central and South America, the Caribbean, the United States, and elsewhere. These served, as it were, as a germplasm pool from which maize breeders from countries in the West African region obtained breeding materials (1, 25). Thus, the rust epidemic served as an instrument for setting up organized maize improvement research efforts in West Africa, with the bulk of attention being devoted to disease-resistance breeding.

Problems (technical, social and economic) associated with the handling of improved maize led to an early realization that hybrid maize production was not yet practicable in West Africa. Improvement programs were, therefore, directed into the formation of synthetics. Examples of such are NS1 and NS2 for Nigeria and GS1, GS2, and GS3 for Ghana, with an average yield of 3,000 kg/ha compared to less than 1,000 kg/ha for local varieties. But efforts of the 1950's and 1960's in the development of high-yielding varieties were dampened by the lack of progress in cultural improvement and the lack of acceptability of improved varieties by peasant farmers. Thus, yields still stand at less than 1,000 kg/ha — about one-third of the world average and one-fifth of the yield an average Ohio farmer realizes (Table 1).

TABLE 1. Maize production (grain) in West Africa, Africa, U.S., Ohio, and the world, 1974

	Area (1,000 ha)	Production (1,000 metric tons)	Yield (kg/ha)
World	116,709	292,290	2,510
West Africa	3,047	2,753	862
Africa	18,726	26,766	1,429
U.S.	26,383	118,144	4,478
Ohio	1,435	8,194	5,710

Source: Food and Agricultural Organization of the United Nations (FAO) Production Yearbook, 1974.

Maize is planted both as a sole crop as well as intercropped with yam, cassava, beans, rice, etc. In Nigeria and most West African countries, only about 25% of the total maize production is under sole cropping (17).

Presently in West Africa, maize is used largely for human consumption. It is utilized in many ways in the preparation of various dishes (7, 13, 15, 25). Some dishes are light and hence serve as breakfast but others are more solid and are frequently made more palatable by admixtures of palm oil, fruits, vegetables, and meat and serve as main meals. Preparations are made from fresh maize or from mature grains. Weaning babies are usually fed on a thin maize porridge (prepared from soaked dry grains) to supplement breast-feeding until they are old enough to feed on solids. Quite a substantial portion of nutrients is removed during this preparation process that involves removal of the seedcoat, often inclusive

of aleurone layer and maize germ. In most cases, the end product is nothing but pure starch.

In southern areas where most maize is produced and accounts for more than 75% of the total cereals consumed, white floury maize is strictly adhered to. In the northern limit where maize is largely planted in home gardens, it is consumed as a vegetable, roasted or boiled. In this northern portion, yellow flinty types are preferred. Generally, yellow is preferred when maize is consumed green.

On a region-wide basis, maize is the third major cereal grown, following sorghum and millet. However, in the rainforest zone, which envelops the bulk of maize production, maize is the most widely grown cereal. Over 90% of the maize is produced by peasant farmers operating on small holdings of an average of 1 ha.

In spite of the relatively large hectarage under maize production in West Africa and the large number of people involved, the total production is ridiculously low because of the use of an unimproved production package of unimproved local varieties and traditional husbandry. This is clearly demonstrated in Table 1, showing that the U.S. produces about 40 times as much maize as the whole of West Africa, using only eight times the land area. This indicates a production efficiency of more than five times in favor of the USA over West Africa.

CURRENT STATUS AND FUTURE EXPECTATIONS OF MAIZE PRODUCTION AND USE

Presently, the tremendous potential of the West African region for maize production is recognized. This potential is largely due to: 1) the possibility of raising two crops per year with rainfed agriculture and three crops under irrigation, and 2) the possibility of extending the area of production beyond the rain forest zone into the Guinea savannah zone — an area that has been demonstrated to support higher maize yields than the traditional maize belt (Table 2).

The lack of adoption of improved production technology by farmers is the main constraint to raising the level of efficiency and, hence, increasing maize production in West Africa.

In most of the West African countries, notably Ghana and Nigeria, their governments have "stepped" into raising the production levels of food crops with "military dispatch." For example, in Nigeria, the government early this year launched a production campaign christened "Operation Feed the Nation." This campaign is backed up by: 1) massive propaganda to create awareness among the people for the need to farm, and also create awareness among agricultural scientists for the need to make more meaningful impact on the farmers, and 2) the provision of

TABLE 2. Grain yield (kg/ha at 12% moisture) of six maize varieties in three ecological zones in Nigeria

Variety	Description[a]	Ibadan (moist forest lowland)	Samaru (inter-savanna lowland)	Riyom (inter-savanna highland)
NCA	M.D. Medium	3,073	6,919	3,394
096EP6	Y.D. Late	3,701	6,542	3,767
TZA x T2B	W.D. Medium	3,177	6,856	4,072
S 1,2,3	Y.D. Medium	2,634	6,597	3,444
NCBRb0_2U	W.F. Early	2,856	5,543	2,969
Mean		3,123	6,394	3,456

[a] W=white, Y=yellow, and M=mixed grain color; D=dent and F=flint; early, medium and late=less than 50, 50-59, and 60 and above days, respectively, from planting to anthesis.

highly subsidized seed, fertilizer, and other production inputs. Maize, a crop relatively easy to grow and especially one whose produce can be easily "exhibited" and utilized, has attracted more attention in this campaign than any other crop. Awareness has been created and it is hoped that the interaction between farmers and maize improvement experts will lead to a sustained high-production level.

Higher yielding maize varieties are continuously being developed in individual national programs and at the International Institute of Tropical Agriculture (IITA), located at Ibadan, Nigeria, mandated to serve the humid tropics. Most of the varieties are being formed through the approach of population development. Composites with a wide genetic base are formed from cultivars of diverse origin, using the comprehensive breeding system designed by Eberhart *et al.* (6). High-yielding varieties are obtained from these composites using a recurrent selection technique, which depends on the number of growing seasons obtainable and manpower available. Also, individual countries benefit from each other through such cooperative trials as West African Uniform Maize Trial originated by USAID under the aegis of OAU/STRC (Organization of African Unity/Scientific and Research Commission): through the activities of IITA; at regional conferences; and on personal contacts and communications between scientists. There is still room for further cooperative ventures.

Efforts are being made to evolve solutions to the already identified management problems. These include: 1) the optimum plant population of maize as a sole crop and as an intercrop, using the most economic fertilizer levels, and 2) optimum timing and methods for all production operations, including planting; fertilization; disease, insect, and weed control; and harvesting

and storage. In disease and pest control, emphasis is on the development of resistant varieties. Common diseases include Puccinia rusts, Bipolaris (Helminthosporium) leaf blights, Curvularia leaf spot, streak, smut, Physoderma brown spot, and various fungal and bacterial stalk rots. Stem borers of the genera *Sesamia*, *Coniesta*, and *Buseola* represent the most important insect pests.

With the present drive for increased maize production coupled with the setting up of organized seed services in many West African countries, the next few years and most certainly the 1980's will witness the development and use of hybrid seeds. Maize will be increasingly used in the manufacture of livestock feeds and, hopefully, that of cornflakes, vegetable oil, etc.

PAST, PRESENT AND FUTURE OF MAIZE VIRUS RESEARCH

Until about 5 years ago, rust and blight were considered the only maize diseases of economic importance in West Africa. Maize streak virus disease was most certainly overlooked, not by design but because of the shortage of trained personnel to estimate its importance. Furthermore, it was then believed to be a late season (August - October) problem, although a recent investigation (10) indicates a gradual inroad of streak incidence in the early season (March - June) crop also. The disease is now known to be widespread throughout West Africa.

Following the recent awareness of the importance of maize streak virus disease in Nigeria, research was initiated to determine the incidence and extent of infection and the several factors connected with disease outbreaks. Areas include the identification and biology of vectors, their seasonal fluctuation in relation to streak incidence, the effects of climate on vector and disease incidence, and non-maize hosts of vectors and virus.

Some observations have been made on streak incidence in relation to the prevailing cultural practices.

Planting date. — Experience has shown that delayed planting of maize results in reduced grain yield. The unfavorable growing conditions as the season progresses arise from increased intensity of rains and attendant soil-impoverishing effects and the increased severity of diseases and numbers of pests. Data showed increased incidence of streak in plantings made later than the recommended date (Tables 3 and 4).

TABLE 3. Maize streak incidence in variety 096 planted on three dates in early season 1976 at Moor Plantation, Ibadan, Nigeria

Planting date	Streak incidence (%)
April 22	2.0
May 11	7.8
June 18	21.3

TABLE 4. Maize streak incidence and yield loss in variety 096 planted during regular season 1972 at Samaru, Nigeria

Planting date	Streak incidence (%)	Estimated yield loss (%)
June 1	24.3	9.7
July 1	86.7	50.2

Intercropping. — Among the advantages adduced for growing of two or more crops simultaneously on the same land in different but adjacent stands are the reduced incidence and severity of diseases and pests of component crops. Preliminary data, however, failed to show reduced incidence of streak disease in either the maize-bean (cowpeas) or the maize-yam intercrop systems. Streak incidence was as high or higher in mixed than in sole maize cropping.

Soil fertility levels. — There is no clear relationship between streak incidence and different levels of nitrogen and phosphorus but, in preliminary results, higher (but non-significant, statistically) streak counts were encountered in fertile than in poor soils. Perhaps the lush, green plants encourage more streak development than the chlorotic nitrogen-deficient ones.

In the area of control, emphasis is given to the development of disease-resistant varieties and any socially adoptable cultural control measures. Several maize cultivars and breeding lines have been evaluated for resistance to streak under natural infection over the past 4 years. Some of these results are presented elsewhere in the text.

TABLE 5. Effect of maize streak on grain yields of plants infected at different growth stages. [Variety = (Mix 1x Colgpo) Eto. Tuxp. grown at Mangombo, Zaire, 1973]

Growth stage at infection	Yield range per plant (kg)	Average yield per plant (kg)	Expected yield per ha (metric tons)
25 cm	0.0 -0.132	0.015	0.5
75 cm	0.0 -0.184	0.072	2.5
10 days before tasseling	0.006-0.377	0.168	5.8
Tasseling	0.123-0.435	0.291	10.1
Brown silk	0.130-0.490	0.305	10.6
No infection	0.185-0.459	0.378	13.2

Source: 1972-73 Annual report of National Maize Program of the Department of Agriculture, Republic of Zaire.

YIELD LOSS AND ECONOMIC IMPORTANCE OF MAIZE STREAK

Maize streak has a very high yield-reducing potential. It causes stunting in early-infected plants. Those infected at seedling stage produce no ears, whereas those infected at later stages have small and poorly filled ones. Plants may die prematurely. In a date-of-planting trial at Samaru, northern Nigeria, referred to earlier (Table 4), yield reduction of 50.2% was estimated to be due to streak. In neighboring Zaire, grain yield was found to decrease from 13.2 to 1 metric ton or less when plants were infected by streak at 25-cm height or less (Table 5).

SYMPTOMATOLOGY, IDENTIFICATION AND POSSIBLE HOSTS OF THE MAIZE STREAK AND MAIZE STRIPE VIRUSES

Symptoms induced by the maize streak virus (MSV) in maize include broken, almost continuous, narrow, chlorotic streaks centered on secondary and tertiary leaf veins. These are distributed uniformly over the leaf surface. The parallel, chlorotic streaks may partially or almost completely fuse, leaving irregular green lines or islands centered between veinlets. The green stripes vary from a few mm to several cm in length and from 0.5 to 1 mm wide (Fig. 2). These symptoms resemble those described by Storey (22).

Four grasses, *Digitaria ciliaris* (Retz.) Koel., *Eleusine indica* (L.) Gaertn., *Setaria barbhata* (Lam.) Kunth, and *Eragrotis gangetica*, were observed in the Samaru area of northern Nigeria with typical maize streak symptoms (2). However, there was no experimental proof, either through vector transmission to susceptible hosts or by serological relatedness, that these grasses were infected by MSV. Similar symptoms were observed on *Brachiaria deflexa*, *Setaria* sp., *Rotthela* sp., and *Paspalum polystachyum* R. Br. on fields at Moor Plantation, Ibadan, but serological tests did not indicate infection by maize streak virus (S.A. Shoyinka, *unpublished*). The maize stripe virus (MSpV) was detected in maize plants, singly or mixed with maize streak virus. Under field conditions, maize plants infected by MSpV showed bright yellow or chlorotic bands, about 0.5 - 1 cm wide, one each on either side of the midrib. These bands originated from the base and extended to about two-thirds the length of leaves. There was no noticeable bending of the apical shoot, as reported by Kulkarni (14). In *B. deflexa* and *Hyparrhenia dissoluta*, symptoms were more conspicuous than in maize. Leaves of infected plants showed intense chlorosis or mottling, sometimes with more discontinuous streaking than in maize plants infected by both viruses.

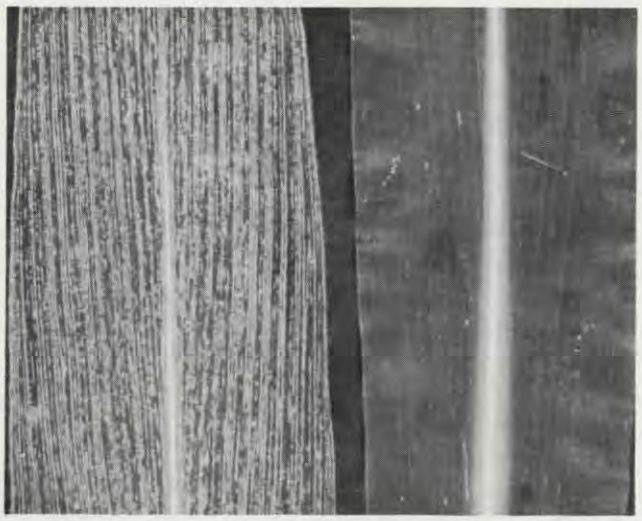

Fig. 2. Streak disease of maize: left, infected leaf, and right, leaf of a healthy plant.

Since the virus was not transferred, under experimental conditions, to healthy plants of either its maize or grass (weed) hosts, MSpV symptoms in any of them could not be determined for certain. However, individual plants of *Brachiaria* sp., *Setaria* sp., and *Hyparrhenia* sp. that showed either bright yellow chlorosis or severe mosaic symptoms gave positive reactions to MSpV antiserum in agar-gel diffusion tests.

Maize streak and maize stripe viruses can be identified on the basis of symptoms they induce in maize plants, vector specificity, serology, and particle morphology. Besides producing distinct symptoms in infected maize plants, maize streak virus is transmitted only by leafhoppers in the genus *Cicadulina* (Jassidae) that do not transmit maize stripe virus. Conversely, the vector of maize stripe virus, *Peregrinus maidis* (Ashm.) (Delphacidae), does not transmit maize streak virus.

The simple Oucterlony gel diffusion test offers the simplest method of identification of these viruses. Antiserum prepared against either of the two viruses will not react with the other's antigen.

CHARACTERISTICS OF MAIZE STREAK AND STRIPE VIRUSES

The morphological characteristics of Nigerian strains of maize streak and maize stripe viruses have not been determined, either with the electron microscope or with analytical ultracentrifugation. It is plausible, however, to suggest that these Nigerian viruses will not differ markedly from those reported by Bock (4) and Kulkarni (14) to which they are serologically related.

Although both viruses are isometric, their sizes differ considerably. The maize streak virus is 20 nm in diam and is usually paired (30 x 20 nm), with sedimentation coefficient ($S_{20,w}$) peaks

of 76S for paired and 54S for single particles. Also, its nucleic acid is single stranded.

MSpV, on the other hand, has particles 35-40 nm in diam with sedimentation peaks of 166 and 109 (14).

VECTORS OF THE MAIZE STREAK AND MAIZE STRIPE VIRUSES

Species of the known insect vector of MSV, *Cicadulina* sp., transmit the Nigerian isolates of the virus, under experimental conditions. Although a detailed study of vector transmission is still under way, adults collected from infected field plants and caged on healthy maize seedlings incited streak symptoms (Shoyinka, *unpublished*; Soto, *personal communication*). The virus is not transmissible by sap inoculation or by seed (10).

Due to difficulties in establishing colonies of *P. maidis*, the vector of MSpV (14), there is no information on the relationship between the virus and vector in Nigeria. However, the vector is known to occur in maize plots at Moor Plantation, Ibadan, where the virus was detected. It is possible that this insect also transmits the Nigerian isolate.

Of the five species of *Cicadulina* known to transmit the streak virus, only *C. mbila* (Naude) has been identified from Nigeria. It is interesting to note that *C. mbila* is a long-distance flier, compared to *C. storeyi* China (19), the best known vector of the virus. It can perhaps be proposed that this species plays a significant part in the inter-territorial dissemination of the virus.

A detailed survey is now in progress to identify major insect vectors of the maize streak virus in Nigeria. A study of life cycles is also thought to be a prerequisite for the very important investigation on the relationship between fluctuating vector population and seasonal variation in streak incidence. The investigation is designed to identify major flights in relation to weather changes. It may, therefore, be possible in the near future to relate the occurrence and size of vector flights to specific weather parameters and, thus, help in predicting the next season's streak incidence from previous season's weather records. For example, in Rhodesia the size of the spring flights of the vector has been found to be positively correlated to the amount of rainfall at the end of the wet season, since late rains produce grasses suitable for oviposition (19).

It is also hoped that this study will provide information as to the major non-maize hosts of both the vector and virus and give an idea as to the source of streak for newly planted maize. The idea that small grain crops, like wheat, are susceptible to streak (13) throws another significant dimension into the epidemiology, in that wheat under irrigation during seasons with cool night temperatures is now being encouraged in the northern parts of West Africa.

CONTROL OF THE STREAK DISEASE

The streak disease is widely distributed in West Africa. And, since the tropical environment is very protective in the sense no such harsh season as winter occurs, the disease might as well be considered as endemic. As conscious efforts are being made to intensify maize production, and under such cultural practices as irrigation, it is to be expected that the streak disease would also increase in importance and possibly reach epiphytotic proportions. Currently, a "blanket" investigation covering all possible methods of control has been initiated in Nigeria to provide information for packaged recommendations. Such studies include chemical and cultural control aspects and development of resistant cultivars.

Cultural control. — That time of planting has an effect on the incidence and, perhaps, severity of streak has been observed in all places where streak is a problem (2, 3, 10, 13). Planting early in the season allows the plants to escape the most vulnerable stage. With the accumulation of data relating vector-disease to climatic parameters, it should be possible to plant in line with the prediction of little streak or least vector activities. The removal of infected plants as a method of reducing disease spread may not solve the problem. Rose (19) has reported increased incidence of the disease as a result of vector disturbance during roguing. As more information is known about overwintering hosts, it should be possible to locate the focus of infection and source of spread and, hence, either plant away from or eradicate the source. Gorter (11) has found that a bare land strip of about 1 m wide around maize reduces streak incidence.

Chemical control. —There is, as yet, no chemical control for the streak disease once the plant has shown visible symptoms. But indirect, chemical control has been attempted by controlling the insect vector. Rose (20) found that aldicarb granules (10% a.i. at 38 g/12 m row) applied into the furrow before planting gave almost complete protection. Percent streak in insecticide-treated plots was only 0.5, compared with 56.1 in the check. Aldicarb is a systemic insecticide, but of high mammalian toxicity. It might be possible to find a safer, persistent and effective chemical. Also, (Soto, *personal communication*) found plants treated with carbofuran (to control stem borers) gave lower streak incidence. Vectors on grass weeds lying on bunds around the maize crop can be controlled by spraying with chemicals such as aldicarb, whereas crop hosts, like wheat, can be sprayed with contact insecticides like carbaryl.

Rose (20) also found that roguing infected plants may be practiced if it is followed by insecticide application. Roguing should be repeated after a few days, to allow earlier-infected plants to show symptoms. He has suggested that crops should be sprayed with a systemic insecticide such as 0.1% dimethoate, when 5% infection is found at an early stage of crop growth and one *Cicadulina* sp. is seen to about three plants.

Breeding for disease resistance. — The use of cultural and chemical control in lowering initial infection is not reliable for effective control under conditions extremely favorable to the disease, as found in West Africa. The only promising way of control is to breed resistant varieties. This is particularly true for an area like West Africa where over 80% of maize production is in the hands of peasants who are too poor and ill-equipped to cope with the financial and technical burdens of chemical control. In fact, breeding for resistance is our emphasis in maize disease control, and we have produced high-yielding varieties with resistance to rust and blight (8).

To develop a disease-resistant variety, a source of resistance has to be located and its mode of inheritance determined to enhance the achievement of the final phase—incorporation of the resistance into present high-yielding but susceptible varieties.

BREEDING FOR RESISTANCE TO STREAK IN AFRICA

Previous work in East and South Africa. — Gorter (12) reported that Fielding was the first to recognize that a South African maize variety — Peruvian Yellow (P) — possessed a high degree of resistance to maize streak virus infection. Rose (21) also found the variety Hickory (H) to be nearly as resistant as Peruvian. Selfs made within each of these two cultivars and the P x H cross showed appreciable resistance but were comparably low yielding. This resistance was then transferred through top-crossing into higher yielding flints and dents (12). Resistance in these P x H derivatives does not seem to conform to a simple Mendelian ratio and has been variously termed tolerance, field resistance, or mature plant resistance. Working with three lines derived from the P x H cross and controlled virus transmission, Storey and Howland (23) found that resistance was conditioned by one major gene modified by some minor ones. This resistance has been incorporated into some East African varieties by backcrossing (24).

Work in West Africa. — In Nigeria, emphasis is now on a search for resistant cultivars. Of the 95 cultivars screened under the natural epiphytotic of 1973, streak incidence was 90% and above on 73 cultivars. Some differences were observed in individual plant reactions. Plants with retarded development of streak symptoms were selfed. The absence of a repeat of the 1973 epiphytotic has not permitted a disease escape-free evaluation of the acquired germplasm. However, an evaluation trial of 1,500 cultivars obtained from CIMMYT and established at IITA in the 1975 early cropping

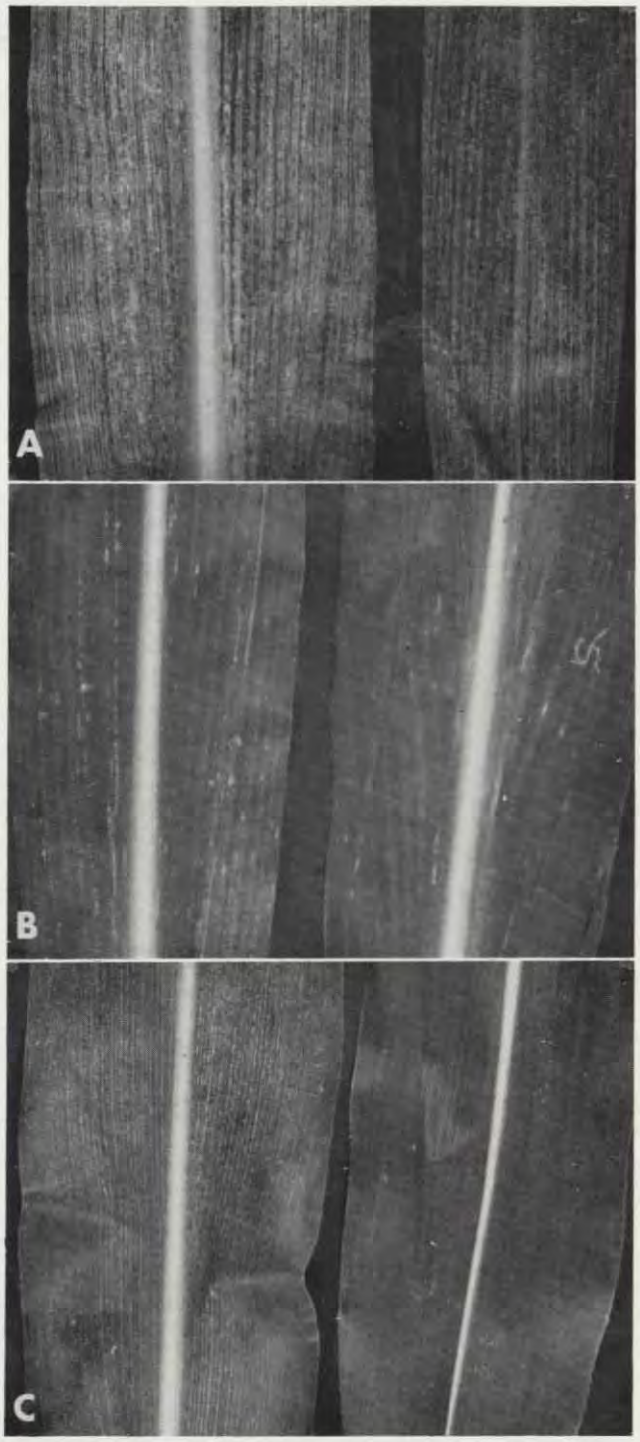

Fig. 3. Leaves from three maize selections: A) streak susceptible type, B and C) resistant types; on the left, most infected leaf on plant, and right, least infected.

season showed streak incidence ranging from 0 to 70% (9). Two cultivars from Nayarit and Yucata and one from the Bahamas had the highest incidence, but one Guatemalan and a Coahuila Mezcla cultivar showed very mild symptoms (a few chlorotic lines). Also, some plants among the recombinants of our population being developed for composite disease resistance showed very mild infection (Fig. 3). Streak symptoms were restricted to a few chlorotic lines on the leaves about midway up the plants but the upper leaves were clean. As these observations were made before tasseling, we were able to self these plants in an attempt to fix this reaction type. Plants showing this reaction type have been traced to the following cultivars: NCB (one of our widely-based composites), PMC 2 (Ex Peru), Tuxp. Cr. 1 Pl. Baja (Ex CIMMYT), Poey T 27 (Ex Mexico), and Pioneer X306A (Ex Jamaica). Slow streak developers were also found in TZ Yellow developed at IITA (Harrison, *personal communication*).

Selfed progenies of these plants with slow streak development and a large sample of TZ Yellow were tested in screenhouses with a high population of a mixed species of *Cicadulina* collected from the field. All broke down, except some plants of TZ Yellow. This breakdown could be due to the higher than required vector dosage or that resistance is not operative at the seedling stage. Attempts have been made (Soto, *personal communication*), to cut down on the number of hoppers per plant. It is, however, certain that this resitance is heritable because lines developed from plants with this reaction type in the 1973 epiphytotic gave the same type of reaction in the field in 1975 under natural infection.

Efforts are being made to determine the mode of inheritance of the mature plant resistance identified in these lines and the apparent seedling resistance found in the TZ Yellow population. Preliminary investigations indicate that the resistance in TZ Yellow is simply inherited.

Incorporation of resistance into our high-yielding but susceptible varieties will employ appropriate breeding techniques such as backcrossing for simply inherited types and recurrent selection if polygenically inherited. The possibility of raising three crops of maize a year will enhance a high rate of genetic progress and thus allow a fast rate of incorporation of resistance, while at the same time maintaining or improving the yield potential of the susceptible variety. Such multiple-season (per cycle) recurrent selection like S_1 testing or its S_2 modification is worth trying as we are doing for diseases as rust, blight, and Curvularia leaf spot. Selection is made using data collected at all three phases of each cycle, namely selfing, evaluation, and recombination.

STRAIN-RACES CONCEPT IN MAIZE STREAK AND STRIPE AND SIGNIFICANCE

McLean (16) has indicated that there exist several forms — supposedly strains — of the streak virus. But, Storey and Ryland (23) found it impossible to separate isolates collected from several localities in East Africa into symptomatologically distinguishable forms. More recently, however, Bock (4) claimed that there are strains of the streak virus that do not infect maize. Nevertheless, we should not underrate the ability of the pathogen to form strains/races, even though we do not yet understand the vehicle and mode of inheritance of disease traits in viruses. Mature plant resistance should not be discarded, in that seedling resistance (usually monogenic) is more vulnerable to a breakdown than the usually multigenically inherited mature-plant resistance. Ukuruguru Composite A, developed in East Africa and in which Storey's streak resistance has been incorporated, did not show resistance under field conditions in Nigeria. Either this composite is not pure for resistance or the resistance is not effective against the Nigerian isolates, even though they are serologically related to the East African isolate. Also, the variety Revolution developed in and found to be resistant to stripe in Reunion Island and to stripe and streak in the Republic of Benin (formerly Dahomey) is susceptible to streak infection in Nigeria. If there is no mistake as to what stripe and streak is, then this might be considered a case of non-stability of this monogenic, recessive resistance.

CONCLUSION

The rapid rate of spread of the maize streak disease in West Africa coupled with its high yield loss potential suggest a need for a thorough investigation of this disease. The areas of vector identification and biology as well as virus-vector environment interactions and the development of adequate control measures require immediate and thorough attention. The West African zone, due to its uniquely favorable environmental conditions for disease development and the limited financial and technical resources of the peasant maize growers, requires perhaps more than anything else the development of streak-resistant maize varieties.

LITERATURE CITED

1. AKPOSOE, M.K. 1970. Maize production and maize improvement in Ghana. Paper presented at an international seminar on Agricultural Research, Ibadan, Nigeria, April 27-May 1, 1970.
2. ANONYMOUS. 1972. Ninth annual report of AID-ARS. Project: Major cereals in West Africa (STRC J.P. 26).

3. ANONYMOUS. 1972-73. 1974-75. Annual reports of National Maize Program of the Dept. of Agric. Repub. of Zaire.
4. BOCK, K.R. 1974. Maize streak virus No. 133. *In* CML/AAB Descriptions of Plant Viruses. Commonwealth Mycological Institute, Kew, Surrey, England. 4 p.
5. BOSHOFF, S.P.E. 1926. Volk en Taal van Suid Afrika. J.H. de Bussy, Pretoria, S. Africa.
6. EBERHART, S.A., M.N. HARRISON, and F. OGADA. 1967. A comprehensive breeding system. Der Zuchter 37:169-174.
7. EBONG, U.U., and K.R. RAGHUNATHAN. 1970. Factors affecting acceptability of maize varieties in Nigeria. Afr. Soils 15:289-300.
8. FAJEMISIN, J.M. 1974. A resume of the programme for the breeding for resistance to maize diseases at the Fed. Dept. Agric. Res. Nigeria. *In* Proc. OAU/STRC Conf. Breeding for disease and insect resistance. IITA, July 1-5, 1974. Ibadan, Nigeria.
9. FAJEMISIN, J.M. 1976. Maize pathology. *In* 1975-76 Ann. Rep. of Fed. Dept. Agric. Res., Nigeria.
10. FAJEMISIN, J.M., G.E. COOK, FUNNSO OKUSANYA, and S.A. SHOYINKA. 1976. Maize streak epiphytotic in Nigeria. Plant Dis. Rep. 60:413-447.
11. GORTER, G.J.M.A. 1953. Studies on the spread and control of the streak disease of maize. Sci. Bull. Dept. Agric. & For., Union of S. Afr. No. 314. 20 p.
12. GORTER, G.J.M.A. 1959. Breeding maize for resistance to streak. Euphytica 8:234-240.
13. JACQUOT, M. 1966. Maize improvement at the IRAT Station, Sefa, Casabance, Senegal. Afr. Soils 11:29.
14. KULKARNI, H.Y. 1973. Comparison and characterization of maize stripe and maize line viruses. Ann. Appl. Biol. 75:205-216.
15. LE CONTE, J. 1966. Breeding of white maize in Dahomey: Niaouli 7. Afr. Soils 11:255-260.
16. MC LEAN, A.P.D. 1947. Some forms of streak virus occurring in maize, sugarcane and wild grasses. Union of S. Afr. Sci. Bull. No. 265. 36 p.
17. OKIGBO, B.E. 1976. Cropping systems and rotations for maize. Presented at the National Maize Production Course, Moor Plantation, May 3 - Aug. 20, 1976, Ibadan, Nigeria.
18. PORTERES, R. 1955. L' introduction du mais en Afrique. Jour. Agric. Trop. Bot. Appl. 2:221-231.
19. ROSE, D.J.W. Management of Cicadulina leafhopper populations to reduce streak in maize crops in the highveld in Rhodesia. Rhodesia Agric. J. 70:63-64.
20. ROSE, D.J.W. 1974. The epidemiology of maize streak disease in relation to population densities of Cicadulina spp. Ann. Appl. Biol. 76:199-207.
21. ROSE, F.M. 1938. Rotation crops. Empire Cotton Growing Assoc. Prog. Rept. 1936-37:21-25.
22. STOREY, H.H. 1937. A new virus of maize transmitted by Cicadulina spp. Ann. Appl. Biol. 24:87-94.
23. STOREY, H.H., and A.K. HOWLAND. 1967. Inheritance of resistance to the virus disease in East Africa. Ann. Appl. Biol. 59:429-436.
24. STOREY, H.H., and A.K. HOWLAND. 1967. Transfer of resistance to the streak virus into East African maize. Agric. and For. J. 33:131-135.
25. VAN EIJNATTEN, C.L.M. 1965. Towards the improvement of maize in Nigeria. H. Veenman and Zonen N.V., Wageningen. 120 p.

DISCUSSION OF J. M. FAJEMISIN PAPER

L.R. Nault: I noticed the similarity in the descriptions of maize stripe and hoja blanca of maize. Their particle sizes are a little different, but could the differences in EM measurement techniques account for this? Dr. Lastra, are there other similarities that you see between Dr. Fajemisin's maize stripe and your hoja blanca in maize? Are we really talking about two different pathogens?

R. Lastra: No, I believe they are different diseases. We do not know too much about the hoja blanca disease as yet. We know the vector, the particle, and a little bit about the method of transmission, but nothing about resistance.

L.M. Josephson: Dr. Fajemisin, you did not show any white or bleached leaves in your slides. I recall 20 years ago seeing streak in southern Africa and plants were almost white. Of course there is the merging of streaks to give what you might think is bleached or white tissue. Do you think that this is another disease?

J.M. Fajemisin: No.

S.P. Raychaudhuri: Why was carbofuran used and in what form was it applied to control the vectors?

J.M. Fajemisin: Carbofuran was used primarily to control stem borers and the control of streak was a secondary result. It was applied in granular form.

V.D. Damsteegt: You said that Dr. D.J.W. Rose has shown that roguing disturbs vectors and, therefore, increases streak. Yet, one of your recommendations is to rogue, then use insecticides and rogue again. Why not just use insecticide and then rogue?

J.M. Fajemisin: Dr. Rose said that streak is an exponentially increasing disease and advised roguing to remove the observable infected plants then apply the insecticide. Then, after about 3 to 4 days, you again rogue infected plants because at the time of insecticide application there were plants infected without symptoms. So, it is a good idea to rogue these after you have applied the insecticide. But he said that you should do this when there is 5% incidence of streak or one *Cicadulina* to three plants. I have no data on this.

V.D. Damsteegt: I think Dr. Rose showed that you could have an epidemic when you have as many as one insect per three plants in the field. One thing about the 10-meter band sprayed around a field: Dr. Rose talks about two kinds of *Cicadulina*; one is a short distance flier and tends to be sedentary, the other is a long distance flier that may move long distances. Dr. Grogan may wish to comment on what he saw there, in terms of healthy fields adjacent to totally infected fields.

C.O. Grogan: In Africa in 1973-74, I saw one field in particular that was very severely infected and another just across a little bank that was disease free. One of the big problems that they have in the so-called high area of South Africa is the presence of wheat. Wheat is being grown more and more and serves as a host. Viruliferous vectors move from wheat to maize in adjoining fields.

B.L. Renfro: Was there a difference in date of planting in those two fields?

C.O. Grogan: Yes. The disease-free one was planted a little bit later. That does confuse the issue.

J.M. Fajemisin: That is right. Right now we are confused because we do not know the effects of environment on streak incidence. One of my colleagues is working on the vector relationships, and as soon as I get back home I will have him contact this group to get information on vector relationships. We need to know how vectors build up, their peak seasons, and influences of weather parameters. Then, perhaps, we can advise when and how to plant maize to reduce streak.

B.L. Renfro: How important is wind direction on vector movement?

C.O. Grogan: My observation is that many of these insects appear to only travel short distances. One of the problems may have been cultivation around the edges of maize

fields where the insects were located. Later, you could see the disease advancing through the entire maize field. I think that not only wind is important, but also vector disturbance.

E.E. Rosenkranz: I believe that wind direction has an important role in the movement of leafhoppers over long distances. For instance, *D. maidis* migrates from southwestern U.S. in a northeasterly direction with the prevailing winds. They seem to migrate from Texas; we first find them in Louisiana, then MIssissippi and, finally, Alabama.

G. Martinez-Lopez: I am interested in the mature plant resistance you talked about. Does it mean that the plant, even though it is infected early in the season, would be able to recover later or is there a type of resistance to late infection?

J.M. Fajemisin: What happens is that if the plant is infected in parts lower than the ear, you see symptoms only on leaves below the ear. Leaves that develop after infection are less affected. Eventually the plant will grow out of these symptoms. In the leaves very close to the tassel, you do not find any. That is what I call mature plant resistance. Have you seen that kind of plant reaction?

G. Martinez-Lopez: I only have development of symptoms in leaves that appear after infection, never in the leaves developed before nor in inoculated leaves. I was wondering if this kind of resistance is due to late infection, when the plantings are already 2 to 2½ months old. They do not develop symptoms because the infection was too late. Even if the infection took place early in the growing season, the plants were able to recover. You find symptoms in one or two leaves, and the new leaves produced do not develop symptoms.

J.M. Fajemisin: What happens is that infected plants with this type of resistance grew out of the infection. Some nearby susceptible plants that were infected at the same age showed typical symptoms. In this latter case, early leaves showed few symptoms, subsequent leaves showed more, and eventually classical symptoms occurred. In the resistant types, tiny white spots appear at the point of infection. The next leaf shows more symptoms, but subsequent ones show fewer and fewer symptoms until there are none.

Virus Diseases of Maize in East Africa

E.J. Guthrie

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In Dr. Guthrie's absence, his paper was presented by Dr. V.D. Damsteegt.

ABSTRACT

Maize is an introduced crop in East Africa but has become the staple food for most of the population. Five virus diseases have been identified, of which streak and sugarcane mosaic are the most important. Maize stripe and line viruses, similar but apparently unrelated, cause negligible losses. Information on the fifth, apparently rare, disease, maize tassel abortion, is sparse and appears to be caused by either a virus or a mycoplasma.

Maize is a comparatively recent arrival in East Africa. The first introductions were made by explorers and traders, probably during the 16th century, and were of the Caribbean flint type. Some of this material persists today, particularly along the coastal belt where purple- and yellow-grained lines are frequently seen and in the yellow maize grown for feed purposes in some highland areas. White maize is generally preferred, however, and is largely derived from southern dents introduced since the beginning of this century. Early introductions by settlers were mainly of types cultivated in southern Africa and were derived from a comparatively narrow range of germplasm. Selection, notably by farmers, resulted in a number of local strains or cultivars. More recent introductions by plant breeders cover a much wider genetic range but are basically of the Tuxpeno type. Some introductions of Cuzco material by missionaries have persisted at the highest altitudes in Kenya (6).

Cultivation in East Africa covers a wide range of altitudes and ecological zones; maize is grown almost wherever there is cultivation. Altitudes range from sea level to more than 3,000 m and rainfall from an unreliable 500 mm to more than 4,000. Although maize may give way to indigenous cereals such as sorghums and millets in the drier areas, and to bananas and plantains in Uganda, it is the staple food of the majority of the people of East Africa. Kenya has a well-developed trade in hybrid maize seed.

Until recently, the only major program of research on maize virus diseases in East Africa was that of Storey, starting at Amani (Tanzania) in the 1920's and continuing at Muguga, near Nairobi. Storey's major contribution in this field was his classic work on the virus vector relationships of maize streak (17, 18, 19, 20, 22, 23). He later studied the genetics of resistance to this disease (24).

In a paper describing the virus diseases of maize known in 1936, Storey (21) listed streak and stripe, but he failed to differentiate between line and stripe (8) although both were probably present in his material. He was also unaware of the presence of sugarcane mosaic virus (SCMV) in maize although it was known to be present in sugarcane in East Africa.

Storey did not attempt to characterize the viruses he was working with, but more recently, with the development of new techniques and availability of modern equipment, it has been possible to begin studies on the viruses themselves and to build on the excellent foundations that Storey laid.

In a survey of maize virus diseases during 1968-71, Kulkarni (8) established the existence of three additional virus diseases of maize. These were maize line, which he differentiated from maize stripe, SCMV and a condition which he called maize tassel abortion disease and which he suggested might be of viral etiology.

Recent work on maize viruses by Bock and Guthrie has been confined almost entirely to maize streak.

MAIZE STREAK VIRUS

Distribution. — Maize streak virus (MSV) occurs throughout Africa south of the Sahara, on some Indian Ocean islands, and in India (1). It does not occur in the New World and is probably a virus of African Gramineae that found maize a congenial host when the crop was introduced into Africa.

Within East Africa, MSV is widely distributed but tends to be more severe at lower altitudes. A proposed expansion of the maize improvement program for coastal areas may, therefore, encounter streak as a major problem. Severity of the disease varies widely from season to season, presumably as a result of variations in the prevalence of the vector, but may be as high as 100%.

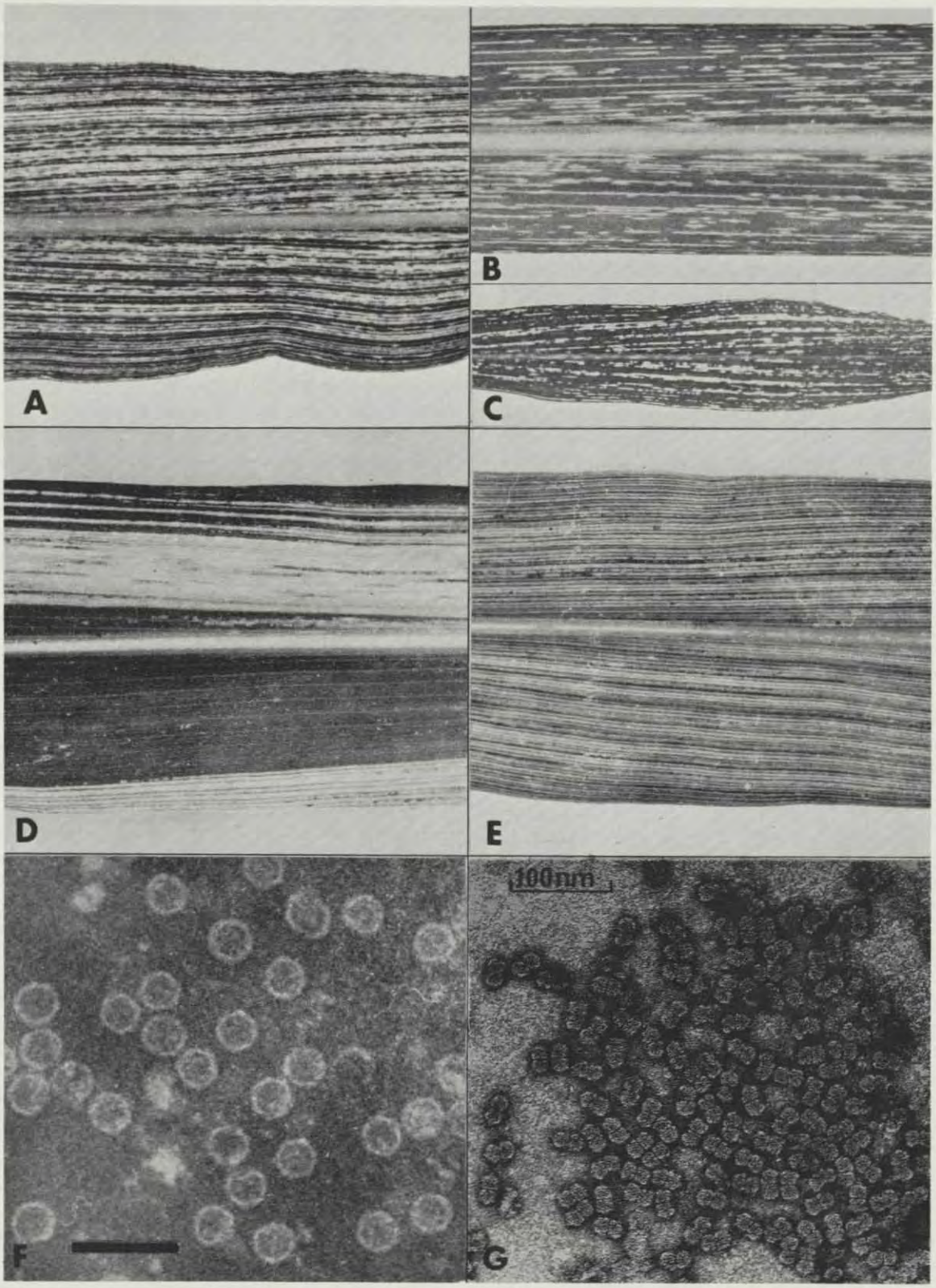

Fig. 1. Symptoms and particles of maize viruses in East Africa. A) maize streak virus symptoms in maize; B) maize streak virus symptoms in sugarcane (cv. Co678); C) maize streak virus symptoms in guinea grass; D) maize stripe virus symptoms in maize; E) maize line virus symptoms in maize; F) particles of maize stripe virus (bar represents 100 nm) (courtesy H.Y. Kulkarni); and G) particles of maize streak virus (bar represents 100 nm) (courtesy R.D. Woods).

Continuous maize cultivation, especially under irrigation, is an aggravating factor.

Symptoms. — MSV produces small, white-yellow spots at the base of the youngest leaves of maize, 4 to 7 days after infection. These spots elongate and coalesce into streaks several cm long and up to 2 mm wide (Fig. 1A). All leaves produced after infection show these symptoms. MSV is not transmissible mechanically but may be identified on the basis of symptoms, transmission by leafhoppers of the genus *Cicadulina* (Jassidae) or by serology.

Purification. — The virus was purified from infected maize leaf tissue by homogenizing in 0.01 M phosphate, 0.1% thioglycollic acid, pH 3.9 - 4.1 (2 ml/g tissue), clarifying with n-butanol (7 ml/100 ml extract), subjecting to 2 or 3 cycles of differential centrifugation, and resuspending in 0.01 M phosphate, pH 7.7.

MSV particles are isometric, 20 nm in diam, and normally occur in pairs measuring 20 x 30 nm. When joined in pairs the adjacent sides appear to be flattened (Fig. 1G). Small size of the single particle suggests that it may be inadequate to carry all the necessary genetic information. Particles may therefore be of two types, both being required for infection. Indications are that the nucleic acid is single-stranded RNA.

MSV is a member of a newly recognized group of viruses of similar morphology (2). Other members include bean golden mosaic (Galvez, *in press*), bean summer death (2), beet curly top (12) and cassava brown streak (Bock and Guthrie, *unpublished*).

Vectors and vector relationships. — MSV is transmitted by a number of species of jassid leafhoppers in the genus *Cicadulina*, including *C. mbila* (Naude), *C. storeyi* China, *C. bipunctella zea* (Mats.), *C. latens* and *C. parazeae* Ghauri.

The mode of transmission was studied by Storey in a classic series of experiments (17,18,19, 20, 22, 23). He found that the virus could be acquired in as little as 15 seconds; hoppers become infective within 12 hours and are then able to transmit the virus for the rest of their lives, which may be several months. There is circumstantial evidence that the virus may multiply within the vector but experimental proof is lacking. The virus is retained during moulting but is not transmitted through the egg.

Storey demonstrated the existence of active and non-active races within the vector. Activity was associated with permeability of the gut wall to the virus; a non-active race may be rendered active by puncturing the gut or by injection of virus into the haemocoel.

Host range and strain differences. — Host range of MSV is limited to the Gramineae, but a number of host-oriented strains have developed within the family. McLean (13) distinguished a number of strains on maize, sugarcane cv. Uba, *Eleusine*, *Sporobolus* and *Paspalum*. Streak has also been recorded in *Cymbopogon*, *Imperata*, *Rottboellia*, *Dactyloctenium*, *Diplachne*, *Eragrostis*, *Leptochloa*, *Setaria*, *Tragus*, *Euchlaena*, *Iriticum* and *Avena* (25). Bock, Guthrie, and Woods (3) showed that strains from maize, sugarcane (Fig. 1B), *Panicum* and *Eleusine* (Fig. 1C) although identical morphologically, could be distinguished on the basis of serology and host reaction. More recent work (Bock and Guthrie, *unpublished*) reinforced the conclusion that streak of wild grasses is not necessarily transmissible to maize. Streak in Nigeria reacts with East African streak antiserum.

It has recently been established (Bock and Guthrie, *unpublished*) that at least two MSV strains, on the basis of the reaction of streak-resistant lines of maize, are present in maize in East Africa. This finding is obviously of significance for maize breeding programs.

Control. — Maintenance of a cleared zone around maize plantings was found to be beneficial in controlling streak (5). Rose (15) suggested a combination of roguing and the use of a systemic insecticide. Roguing, alone, increased the incidence of streak in one trial, presumably by disturbing the vector.

There seems little doubt, however, that the method of control most likely to be effective under field conditions is the development of streak-resistant varieties of maize. This is particularly true in view of the vast areas of maize grown in small plots by relatively unsophisticated farmers. There have been numerous attempts to select for streak resistance in the field, but these have invariably encountered difficulties as a result of varying incidence of disease. A system of artificial infection using the insect vector, therefore, seems essential in screening for resistance.

Storey and Howland (24) described studies on the inheritance of streak resistance in maize, but their work was based on a recessive gene and, therefore, limited in its usefulness; programs to incorporate this gene into maize populations have not produced worthwhile results. Le Conte (11) reported the results of crosses made in Dahomey using a streak resistant line from the island of Reunion. This and other resistant lines are currently being studied at EAAFRO. It is intended to establish the mode of inheritance of resistance and then to make the lines available to plant breeders for incorporation into their programs.

Effect on yield. — The overall economic importance of maize streak is difficult to assess because of the variation in disease incidence from season to season. Measurement of the effect of streak on individual plants lends itself to greater precision. In recent experiments at EAAFRO, reductions in yield in three trials, which relied on natural infection with streak, were 33, 56, and 46%. A further trial, in which plants were infected

artificially at the two-leaf stage, showed a yield reduction of 61%. Attempts are now being made to correlate reduction in yield with growth stage at time of infection.

SUGARCANE MOSAIC VIRUS

SCMV was listed tentatively by Riley (14) for Tanzania, but the first positive identification in East Africa was made by Kulkarni (10) who recorded it from sites in all three East African countries. The disease has probably been generally distributed throughout East Africa for many years but escaped attention because of the unspectacular nature of its symptoms. Kulkarni's surveys showed SCMV to be present in every maize planting sampled, at an average level of infection of 20%. SCMV has been recovered from maize growing at an altitude of 2,600 m. Experimental assessment of yield loss due to SCMV infection was 25% for maize and 73% for sorghum.

Serological studies by Kulkarni showed that East African isolates of SCMV were related to American strains A, B and D, but not to others. No strain differences have been demonstrated within East Africa.

Attempts to select maize lines for resistance to SCMV by Kulkarni and more recently by Bock and Guthrie have been unsuccessful. It is possible that inoculation by aphid vectors rather than by mechanical means is a better screening technique. This is to be attempted.

MAIZE STRIPE VIRUS

This disease was first recorded by Storey (21) from the Amani district of Tanzania. Storey noted that symptoms were of two types and it now seems likely that both maize stripe and maize line viruses were present. Information presently available on maize stripe and maize line viruses is largely derived from the work of Kulkarni (10).

Information on the distribution of stripe is mainly based on observations made in Kenya, but it is probably present throughout East Africa and, possibly, elsewhere. There are records from Mauritius (16) and Reunion (4); but Storey (21) considered the former to be attributable to streak, whereas the latter may refer to maize line virus (MLV).

Symptoms. — Maize stripe virus produces narrow, closely spaced yellow stripes on maize leaves that later merge to give broad, yellow bands; in extreme cases the entire lamina turns yellow (Fig. 1D). A further symptom is a pronounced bending of the shoot apex.

Transmission. — Maize stripe virus is not transmissible mechanically but is by the Delphacid planthopper, *Peregrinus maidis* Ashm.

An acquisition feed of 5 days followed by a test feed of 6 days is apparently necessary for transmission, but *P. maidis* is an inefficient and unpredictable vector. There is some evidence that nymphs are more efficient vectors than adults. Barley and sorghum were also susceptible. Attempts to transmit the virus by feeding hoppers through membranes on partially purified preparations were unsuccessful.

Purification. — Partially purified preparations were prepared as follows: Fresh infected-leaf tissue was homogenized in 0.5 M tri-sodium citrate, 1% 2-mercaptoethanol (2 ml/g tissue), filtered through cloth and clarified with n-butanol (7 ml/100 ml extract) by stirring for 15 minutes, and incubated overnight at 15 C. After centrifugation at 20,000 g for 20 minutes, the supernatant was filtered and centrifuged at 100,000 g for 2 hours. Pellets were resuspended in 0.01 M sodium tetraborate, pH 8.5, and clarified by centrifugation (12,500 g for 5 minutes).

Particle morphology. — Preparations partially purified as above, negatively stained with potassium phosphotungstate or uranyl acetate and examined by electron microscopy, contained isometric particles 35 and 40 nm in diam (Fig. 1F).

Serology. — An antiserum was prepared which had an homologous titer of 1/128 when tested against the antigen in crude sap in agar gel diffusion tests. The antiserum did not react with MSV or MLV.

MAIZE LINE VIRUS

This virus was first described by Kulkarni (10) from two areas of Kenya. It occurs in Kenya coastal areas and is probably more widely, though sparsely, distributed in East Africa. It is also possible that the record of maize stripe virus in Reunion (4) refers to MLV. Infection levels observed in Kenya have invariably been low (> 2%) and losses negligible.

Symptoms. — Maize line virus produces continuous yellow lines along the veins of leaves; the yellowing spreads approximately 1 mm on either side of veins. Older leaves often show interveinal mottling (Fig. 1E).

Symptoms differ from those of maize streak virus in that the yellow lines are longer and more continuous and are on, rather than between, the veins. Unlike maize stripe, there is no apical bending. Tassel and cob formation are normal.

Transmission. — Like maize stripe virus, MLV is transmitted by *P. maidis* but not mechanically. On the limited information available, transmission is even less efficient than with maize stripe and long incubation periods are necessary.

Purification. — MLV was purified using the method described for maize stripe virus.

Particle morphology. — Particles of MLV are isometric and measure 28 and 34 nm in diam for "empty" and complete particles, respectively. They are smaller than those of maize stripe.

Serology. — An antiserum was prepared that had an homologous titer of 1/64 against crude sap in agar gel diffusion tests. The antiserum did not react with MSV or maize stripe.

MAIZE TASSEL ABORTION DISEASE

This disease was first described by Kulkarni (8) after his survey of maize virus diseases. Very little information is available on the disease, which is apparently of limited distribution and importance. The causal organism is probably a virus or mycoplasma. Attempts at purification were unsuccessful.

Symptoms. — Leaves of affected plants are chlorotic, much reduced in size, and borne horizontally on the stem. Male inflorescences of such plants are often trapped by terminal leaves and are without spikelets; cobs are either absent or deformed and poorly filled.

Transmission. — Kulkarni found that the disease was transmitted by the Delphacid planthopper *Malaxodes farinosus* Fennah, the vector of molasses dwarf disease (7). Infection was achieved using acquisition feeds of 24 hours followed by test feeds of 6 days; test feeds of 3 days were not effective. The disease was not transmissible mechanically.

Kulkarni noted that *Malaxodes* does not normally colonize maize and may not be the natural vector of the disease agent. The relationship between molasses dwarf disease and maize tassel abortion disease has not been fully investigated.

LITERATURE CITED

1. BOCK, K.R. 1974. Maize streak virus. No. 133 in CMI/AAB Descriptions of Plant Viruses. Kew, Surrey, England. 4 p.
2. BOCK, K.R. 1975. Maize streak, cassava latent and similar viruses. Abstracts, 3rd Int. Cong. for Virol. p. 93.
3. BOCK, K.R., E.J GUTHRIE, and R.D. WOODS. 1974. Purification of maize streak virus and its relationship to viruses associated with streak diseases of sugarcane and Panicum maximum. Ann Appl. Biol. 77·289-296.
4. ETIENNE, J., and B. RAT. 1973. Le stripe· Une maladie importante duc mais a la Reunion L'Agron. Trop. 28: 11-17.
5. GORTER, G J.M A. 1953. Studies on the spread and control of the streak disease of maize. Sci Bull. No. 341. Dept. of Agric. and For., Union S Afr. 20 p.
6. HARRISON, M.N. 1970. Maize improvement in East Africa: Crop improvement in East Africa. Pages 21-59 in C.L.A. Leakey, ed Technical Communication 19, Commonw. Bur. of Plant Breeding and Genet.
7. KULKARNI, H.Y. 1969. Transmission of the pathogen of molasses dwarf disease by Malaxodes farinosus. Phytopathology 59:1783-1786.
8. KULKARNI, H Y 1972. Survey of viruses affecting East African major food crops. Ph. D. Thesis, Univ. Nairobi, Kenya. 105 p.
9. KULKARNI, H.Y. 1973. Notes on East African plant virus diseases 5. Identification and economic importance of sugarcane mosaic virus in maize in East Africa. E. Afr. Agri. J. 39:158-164.
10. KULKARNI, H.Y. 1973. Comparison and characterization of maize stripe and maize line viruses. Ann. Appl. Biol 75:205-216.
11. LE CONTE, J. 1974. La virose du maize au Dahomey. L'Agro. Trop. 29:831-832.
12. MUMFORD, D.L. 1974 Purification of curly top virus. Phytopathology 64 136-139.
13. McLEAN, A.P.D. 1947. Some forms of streak virus occurring in maize, sugarcane and wild-grasses. Sci. Bull. No. 265. Dept. of Agric. and For., Union of S. Afr. 39 p.
14. RILEY, E.A. 1960 A revised list of plant diseases in Tanganyika Territory. Mycol. Paper No. 75, Commonw. Mycol. Inst., Kew, Surrey, England, 42 p.
15. ROSE, D.J.W. 1974. The epidemiology of maize streak disease in relation to population densities of Cicadulina spp. Ann Appl. Biol. 76:199-207.
16. SHEPHERD, E.F.S. 1929. Notes on chlorosis of maize and other Gramineae in Mauritius. Trop. Agric. 6 320.
17. STOREY, H H. 1925. The transmission of streak disease of maize by the leafhopper Balclutha mbila Naude. Ann. Appl. Biol. 12:422-439.
18. STOREY, H.H. 1928. Transmission studies of maize streak disease. Ann. Appl. Biol. 15:1-25
19. STOREY, H.H. 1932. The inheritance by an insect vector of the ability to transmit a plant virus. Proc. R. Soc. B. 112:46-60.
20. STOREY H.H. 1933. Investigations of the mechanism of the transmission of plant viruses by insect vectors. I. Proc. R. Soc. B., 113·463-485.
21. STOREY, H.H. 1936. Virus diseases of East African plants. IV. A survey of the viruses attacking the Gramineae. E. Afr. Agric. J. 1.333-337.
22. STOREY, H.H 1938. Investigations of the mechanism of the transmission of plant viruses by insect vectors. II. The part played by puncture in transmission. Proc. R. Soc. B. 125.455-477.
23. STOREY, H H. 1939. Investigation of the mechanism of the transmission of plant viruses by insect vectors. III. The insect's saliva. Proc. R. Soc. B. 127.526-543.
24. STOREY, H.H., and A.K. HOWLAND. 1967. Inheritance of resistance in maize to the virus of streak disease in East Africa Ann. Appl. Biol. 59:429-436.
25. STOREY, H.H , and A.P.D. McLEAN. 1930. The transmission of streak disease between maize, sugarcane and wild grasses. Ann. Appl. Biol. 17:691-719.

DISCUSSION OF E. J. GUTHRIE PAPER

V.D. Damsteegt: We only work with maize streak in the greenhouse on plants from seedling to maybe 2 months old. I do not see some of the symptoms that you showed on your slides, Dr. Fajemisin. Ours are more severe. In working with the disease in the greenhouse in Africa, do you find greenhouse symptoms more severe than those in the field?

J.M. Fajemisin: Yes, greenhouse-infected plants normally die. Leaves become white and they just wither away.

V.D. Damsteegt: The question was brought up about white, bleached leaves that Dr. Josephson saw in Africa. We see a lot of almost pure white symptoms, depending on the genotype. Some, however, are cream to buff-colored and others are entirely white with no color at all. In this latter case, essentially all the chlorophyll was destroyed. I would like to remind you of one other symptom with the

Cicadulina leafhoppers that is reported in the literature. Apparently, it is something that only is seen in greenhouse seedlings and not a field problem. *Cicadulina* is one of the hoppers that causes a toxic reaction in the greenhouse We get almost as much damage on seedlings with non-viruliferous leafhopper feeding alone as we do with the virus, at times. However, we get different levels of toxicity with different genotypes. Many insects may feed for weeks on a seedling and seem to have no effect on some. In other cases, one insect feeding on a seedling for one day causes gross swelling of veins, curling of leaves, and changes in growth pattern. If you inoculate these plants with the virus, they die. There are many variations and it is difficult working on this disease in the greenhouse.

G. Martinez-Lopez: Are the swellings resulting from feeding by *Cicadulina* at the feeding site or at a different site? It is reported to vary in the case of wallaby ear.

E.E. Rosenkranz: I believe that Dr N.E. Gryllis reported this.

V.D. Damsteegt: Dr. Gryllis reported two kinds of wallaby ear, one that is a systemic severe reaction and one that is mild. The mild reaction is about what our reaction with *Cicadulina* looks like. The swellings are on the undersurfaces of leaves, and, in some cases, where the insects fed. In other cases, each leaf that was present in the whorl will show swellings. The first leaf that shows swellings may be at the base, the next slightly farther up, etc In other cases, every leaf shows it and the reaction seems to be systemic, much like wallaby ear. It might simply be a matter of how much toxin the insect injects. I wish that we could identify the toxin.

G. Martinez-Lopez: I am concerned about *Cicadulina* species in Colombia, not only in maize, but in other crops where swellings occur. Many thought it was due to a toxin. We have observed that the swellings were not produced at the site of feeding. Probably these were systemic.

L R. Nault: Salivary phytotoxins may be either localized or systemic Many species here that feed on alfalfa and potato produce systemic effects. As soon as you remove the insects, the plants will outgrown the symptoms. These are probably the two most important criteria for establishing a phytotoxic effect; namely, that plants outgrow symptoms once the insect is removed, and no pathogen can be detected

V.D. Damsteegt: There is the possibility, however, that an undetected pathogen is involved. We discarded this idea because the *Cicadulinas* as well as *Nephotettix* species were reported to cause vein swellings. Members of that closely related family of leafhoppers have been known to cause swellings at the point of feeding It is transitory and plants usually grow out of it. It occurs on wild grasses much more severely than on maize.

G. Martinez-Lopez: Have you studied the effect of temperature on the development of these symptoms? With the virus, we have found that at high temperatures the symptoms just develop in a very small portion of the leaves. But if the plants are exposed to a lower temperature, symptoms develop in a larger number.

V.D. Damsteegt: I can only give an observation and no data. We get more of this reaction as we approach February and March, when the days become longer and the temperatures inside the cages are warmer. Whether the increased swellings are due to day length, light, temperature or combinations, I do not know.

D.T. Gordon: Since you have been working on maize streak for some time, I wonder what your estimate is for its potential spread from the area where it now occurs into the Western Hemisphere?

V.D. Damsteegt: I would say that in the United States streak is no problem for the immediate future. I would guess that for the whole of the Western Hemisphere, streak would most likely come in between latitudes 30 South and 30 North — the Caribbean, Venezuela, Colombia, Mexico, Florida, and southern Texas. This is where the vector can survive. The vector is said to be a long-distance flier, but I think there is probably more evidence that it tends to be sedentary and flies only at certain periods We do not have the vector here, as far as we know. The vector could come in but, if the virus was not present, it still would not cause a problem. However, if both vector and virus were introduced, the disease could become established quite easily in this area. I have tested 39 perennial grasses that are very common in the southern United States up to the Corn Belt, and of them, 13 are good symptom hosts. One of them is quackgrass, a potential overwintering host. Some are excellent oviposition and feeding hosts for *Cicadulina* on which they could live and thrive. We have tested eight or nine potential vectors so far—common species that are associated with grasses or Gramineae here, like *Dalbulus*, *Macrosteles*, etc We fed them on streak-infected plants, and injected them with infectious sap. But we got no transmission Only *Cicadulina mbila* transmitted streak in our laboratory. I do not think we have a real threat of a natural vector. I do not think streak is an immediate threat to corn in our area, but if we are talking in terms of the whole Americas, it could very well be.

A.J. Ullstrup: Have you tested very many maize genotypes for resistance to streak?

V.D. Damsteegt: There are 25 or 26 publicly generated inbreds that comprise the vast majority of all germplasm used in the U S Of those we studied about 23, and all were very susceptible. However, we tested them only in the seedling stage I have studied two corn lines from Africa that were reported to be resistant We tested them at seedling and 30 to 40-day-old stages. At the older stage, I would like to think we found mature plant resistance We also have studied about 140 inbreds and 110 to 140 more promising hybrids of some major corn companies; so far we have found no resistance This is like the experience in Africa — you screen a lot of lines and you do not find much. Until recently, there was only the Peruvian X Hickory cross that G.J.M.A. Gorter found back in the 1930's. That hybrid was the only source and its resistance was due to one recessive gene. This situation made a lot of people anxious because it does not take long for a virus to overcome one gene, and with that being the only source there was little protection against streak.

J.W. Lightfield: Based on what you have seen of symptoms on introduced grasses from Africa, what are our chances of picking up some of these viruses in the vegetative grasses or in corn from seed?

V.D. Damsteegt: If the grass did not show visible symptoms we were unable to recover the virus. If it was showing symptoms we could usually recover the virus, but not always With quackgrass, for example, we had symptoms but it took us 4 months of continuous bioassay to recover the virus by vectors. Not all grasses show classic streak symptoms Some show classic streak, some show more of a mosaic, and others show symptoms that you would never relate to streak. If you bring in clones of perennials or anything developing from rhizomes from Africa, they should be held and checked carefully Perhaps, one should also bioassay all of them, to detect symptomless carriers.

D.T. Gordon: What do you recommend as a good assay for streak?

V.D. Damsteegt: Transmissions with the vector. You might also do serology using the agar double diffusion test. The virus is very easy to purify and in a matter of 2 to 3 hours you could have it purified to the point where you could look at particles by EM.

I.W. Deep: You said that you do not feel that streak poses much of a threat to U.S. maize because both virus and vector would have to be introduced. But then I thought you indicated that even if both were introduced we still would not likely have a problem?

V.D. Damsteegt: From the standpoint of temperatures, *Cicadulina* can stand freezing but not for a prolonged period. It is also discontinuous in its habitat because it cannot survive droughts If it cannot withstand long periods of freezing and cold I do not think it could persist in the Corn Belt. Since it is not known to be a migratory species, like *Macrosteles* for example, it probably would not migrate across the country every year. It might on rare occasions.

I.W. Deep: You think that the vector would not become established because of temperature relationships, not necessarily because of the lack of suitable vegetation?

V.D. Damsteegt: I think that suitable hosts are around, but I do not think that in itself makes our area a suitable habitat. I base this on work done in Africa several years ago. Let me add, however, that I am talking about its persistence in the Corn Belt. Something like *Cicadulina* could possibly become established in the South — in southern Texas, southern Mississippi, southern Louisiana — for example. And with the right conditions, the vectors could move north fairly early up to the southern part of the Corn Belt — to southern Ohio, for example.

L.M. Josephson: How long will the virus remain viable in the vector?

V.D. Damsteegt: For the life of the vector.

L.M. Josephson: How long is that?

V.D. Damsteegt: One of the original leafhoppers that we got from South Africa as an adult, we used in transmission studies for about 80 days before we discontinued using it *Cicadulina* is a long-lived leafhopper.

R.W. Toler: Have you determined if St. Augustine grass is a host? It is quite common around the Gulf Coast in southern Texas

V.D. Damsteegt: No, I have not tested it. I should have because it is susceptible to other viruses.

L.M. Chilson: How susceptible is sugarcane?

V.D. Damsteegt: Uba cane has been shown to be susceptible. However, I do not believe that this strain is produced commercially anywhere in the world anymore.

Principal Maize Virus Diseases in India

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ABSTRACT

Maize is a major crop in India, used as food and fodder. It is grown extensively in Uttar Pradesh, Bihar, Rajasthan, Madhya Pradesh, Bombay, Andhra Pradesh, and Himachal Pradesh, as well as Kalimpong in West Bengal and in Sikkim. In spite of large acreages under cultivation, production is low compared to other countries. One of the important factors responsible for the low yield is incidence of various diseases. However, so far, no accurate assessment has been made of their damage. Maize is next to rice, wheat, jowar, and bajra as a food crop in India; in fact, it is the only staple cereal food for many people in the hilly tracts in northern and northeastern India. A mosaic disease of maize, transmitted by aphid vectors, resembles maize dwarf mosaic of USA and, perhaps, is a strain of sugarcane mosaic virus. It has been studied in detail since 1960 at the Indian Agricultural Research Institute, New Delhi. This disease can reduce yields to the extent of 31.81%. This virus is readily mechanically transmitted to maize but is not seed-borne. It also infects several other crops of agricultural importance.

Another virus disease of maize has been established in India. Disease symptoms first appear as minute chlorotic spots, later coalescing to form parallel, chlorotic lines running along the veins. This virus is transmitted by *Peregrinus maidis*; details are discussed.

Recently, the maize streak disease has been reported in India. It is characterized by production of chlorosis in affected leaves confined to stripes along the veins. The disease is transmitted by *Cicadulina mbila*, *C. zeae*, and *C. nicholsi*. *C. mbila* is the most efficient vector. The disease is of great concern because it also affects several other cultivated hosts, like wheat, barley, etc.

Vein enation of maize has been recently reported from Kalimpong in Darjeeling District. Severe swelling and numerous white, spindle-shaped galls develop on veins on the lower surface of leaf lamina. The disease is not sap or seed transmissible but is by *Cicadulina mbila*. The virus also infects wheat, rice, sugarcane, sorghum, oats, etc.

The history of maize cultivation is most fascinating and interesting. Columbus observed maize growing in the Western Hemisphere about 500 years ago. Many travelers in the ancient period carried it from its place of origin to other areas, particularly in both Americas where maize is now one of the most important crops. This particular crop can be adapted to varied climates, in comparison to other grains. It was largely grown in North America and Western Europe from 1800 to 1950. Today, this crop is grown on millions of acres in different parts of the world. According to CIMMYT Review Report of 1976, maize ranks third, after rice and wheat, as a food grain in developing countries. With the help of CIMMYT, India was very much benefited when the All India Coordinated Maize Improvement Program was initiated at the Indian Agricultural Research Institute nearly two decades ago in collaboration with the Rockefeller Foundation. Maize occupies a prominent position in developing countries in Asia, Africa, and Latin America. But only one-fourth of the world crop is produced in these countries, although they include half of the maize area planted. This is obviously due to low yields. According to the latest CIMMYT Report for North America and Europe, an average of 4,600 kg/ha was recorded there, but only 1,200 kg/ha for developing countries.

Maize is grown under several constraints like the difficult movement of new varieties into the tropics from temperate climates. For instance, plant height, which may be responsible for lodging, is traditionally about 4 m in the tropics but only 2.5 m in temperate areas. Another problem, which we are going to discuss at this Colloquium and Workshop, is that of losses caused by diseases and pests, especially viruses. Also, poor quality of maize protein is considered to be another factor encountered in maize improvement programs. Therefore, factors which hinder improvement of maize in developing countries are varied. New varieties developed must be more stable in yields; of good climatic adaptation; smaller in size; resistant to virus and other diseases and pests; and of better quality protein, etc.

Maize cultivation in India got a boost with the advent of the Rockefeller Foundation improvement program in the late 1950's. This program proved a great success and developed hybrids and com-

posites that tremendously increased yields. Maize growing became very popular in many areas in the country under the All India Coordinated Maize Improvement Project of the Indian Council of Agricultural Research. Maize is now extensively grown and occupies about 5 million ha of land. It is a staple food and fodder in many parts of India, like Uttar Pradesh, Bihar, Rajasthan, Madhya Pradesh, Maharashtra, Andhra Pradesh, Himachal Pradesh, Kalimpong (West Bengal), and Sikkim. It also supplies raw materials to some industries in the country. Under the above program, attention was mainly devoted to breeding high-yielding varieties resistant to fungal and bacterial diseases and borers. Early, very little, if any, work was done on diseases of viral or mycoplasmal origin. Hence, information on these diseases is not sufficient [Raychaudhuri (20)]. Most work has been done at a number of places outside the regular maize improvement program.

Maize streak is one of the earliest known virus diseases of maize and has long been recognized as an important disease in Africa [Fuller (12)]. In 1919, Brandes (5) demonstrated the transmission of sugarcane mosaic virus (SCMV) to maize and the latter is now considered to be a very important host. Recently, Thornberry (37) listed maize as a host of as many as two dozen viruses, some of which are now known to be caused by other agents, like mycoplasmas or spiroplasmas. The most important of these diseases are mosaic, streak, stunt, stripe, wallaby ear, and rough dwarf. Each of these is known to have the capacity to cause substantial losses to maize. Maize dwarf mosaic, which was first observed in Ohio, USA, in 1962 by Janson and Ellett (13), caused huge estimated losses. Similarly, pearl millet streak is becoming important in many cereal crops in India [Seth (24)] in recent years. No adequate survey of these diseases has been made in India so far.

MAIZE MOSAIC VIRUS IN INDIA

In 1952 a mosaic disease of maize was observed in the fields of the Indian Agricultural Research Institute, New Delhi, the first record of a virus disease in maize from India [Chona and Seth (10)]. Enough information on this disease is now available regarding etiology and epidemiology to determine control measures.

In a limited survey in 1962-1964 [Paliwal *et al.* (18)], it was found in every locality visited in the states of Delhi, Himachal Pradesh, Punjab, Rajasthan, West Bengal, and Andhra Pradesh. Mosaic incidence ranged from 2.2% to 10.6% in randomly-selected fields and was found to be correlated both with the seasonal differences in population levels of the vector *Rhopalosiphum maidis* (Fitch) and with the variety of maize grown. Incidence was higher in local maize varieties than in those containing exotic germplasm. Disease incidence at experiment stations was found to be low, perhaps due to the fact that their breeding materials contained a high percentage of exotic germplasm shown to be resistant.

Symptoms of disease begin as chlorotic specks, usually on one side of the midrib and in rows parallel to it. By elongation and coalescing of specks, chlorotic then blotchy areas develop. During subsequent growth, the pale areas predominate and infected plants can be recognized from a distance. Stunting and poorly formed and filled ears are other important symptoms.

The virus was easily sap tranmissible and inoculated plants developed symptoms in about 5 days at Delhi, during the month of July when average daily maximum temperature was around 43 C and minimum was 27 C. The longest incubation period was found to be 22 days in the month of January when the average daily maximum temperature was 23.5 C and minimum was 11.2 C.

The virus had a thermal death point of 50-55 C; a dilution end point of 1:50 to 1:100; and longevity *in vitro* at room temperature (28-32 C) 16 hours and at 7 C, a week [Chona and Seth (10)].

Five aphid vectors of the virus were identified: *R. maidis* (Fitch), *Aphis gossypii* Glover, *Macrosiphum granarium* Kirby [Chona and Seth (10)], *Schizaphis graminum* (Rondandi) [Seth and Raychaudhuri (26)], and *Myzus persicae* (Sulz) [Bhargava and Shukla (4)].

Seth and Raychaudhuri (26) determined the effect of hydrogen-ion concentration of sap on virus infectivity. The maize mosaic virus (MMV) was immediately inactivated at pH 4.0, and below, but at pH 4.4 it remained infectious even after 24 hours when stored at 7-10 C. Under similar conditions at pH 9.0, the virus remained infectious for 4 hours, but then was quickly inactivated. Optimum pH lies between 5.6 and 7.2, where maxmium infection was obtained.

Seth and Raychaudhuri (26) have also shown the effect of chemicals, including protein-precipitating and denaturing agents as well as organic solvents on the virus in extracted sap. Chemicals studied were acetone, ethyl alcohol, chloroform, commercial nicotine sulphate, glycerine, lysol, formalin, copper sulphate, and potassium permanganate. The virus was sensitive, even to low dosages.

Paliwal *et al.* (18) reported the presence of three strains of the virus and concluded that the type strain, reported by Chona and Seth (10), is very widespread in India and produces the most severe symptoms. Strains were differentiated on host reaction, physical properties, and cross-protection tests.

Two kinds of intracellular inclusions, one oval and the other elongated, were observed in epidermal cells of infected maize leaves [Paliwal and Raychaudhuri (15)]. The virus was purified and

rod-shaped particles were found 339-964 nm in length (avg. 544 nm) and 27-39 nm diam. [Paliwal and Raychaudhuri (17)].

The virus was found to be restricted to the family Gramineae and 27 hosts among these plants were found. The most important were maize, sorghum, ragi, and other minor millets. The virus, however, did not infect sugarcane.

Many maize varieties were found to be susceptible to MMV, but 24 exotic inbred lines of maize showed varying degrees of resistance. Four of these were considered highly resistant. Studies also showed that plants developed resistance with age. Some inbreds developed almost complete resistance after 32 days of growth but others only after 50 days [Paliwal and Raychaudhuri (16)]. Some work on inheritance of resistance was done by Agarwal *et al.* (1). Two inbred lines, CM103 and CM104, were found to be almost immune to the virus. Sap from these inhibited the virus *in vitro* [Raychaudhuri *et al.* (22)].

Our studies in 1967-1968 demonstrated that reaction to MMV in maize was governed by a single gene pair and resistance inherited in a partially or incompletely dominant manner Agarwal *et al.* (1)]. This was determined by developing appropriate crosses for genetic analyses from seven inbred lines and inoculating 7-day-old seedlings with the type strain. Two parents were immune, one highly resistant, and three susceptible. Four infection classes were evident 20 days after inoculation: immune, resistant, moderately susceptible (intermediate), and susceptible. F_1 plants of resistant x susceptible crosses, including reciprocals, showed an intermediate type of reaction (moderately susceptible). F_2 plants segregated in a ratio of 1 resistant: 2 intermediate: 1 susceptible in all resistant x susceptible crosses. F_1 plants backcrossed to resistant parents gave a ratio of 1 resistant: 1 susceptible, while with susceptible parent all plants were susceptible. No segregation was observed in resistant x resistant or susceptible x susceptible crosses.

A loss of about 32% in grain yield in a 100% infected crop, compared to a 100% healthy crop, was recorded by Raychaudhuri *et al.* (21). Application of different levels of potassium helped vegetative growth of both healthy and mosaic-infected maize plants. Nitrogen fertility had little effect on symptom expression in the local variety, but 8% more plants of Ganga 3 hybrid showed symptoms under high nitrogen application (120 kg/ha) as compared to those where no nitrogen was applied. However, 18% higher yield was obtained in virus-infected plants at high nitrogen than in healthy plants grown with no nitrogen applied. No difference was noted between no and 80 kg/ha phosphorus (P_2O_5) or potassium (K_2O).

No evidence of seed transmission of the virus has been obtained. Observations on MMV in northern India revealed that the virus probably survives during winter on infected stubbles of certain grasses, sorghum, etc. Aphid vectors colonize these overwintering plants and carry the virus to spring-sown maize crops.

By cross-protection tests, it was determined that the virus was related to SCMV. Perhaps it is a strain of this virus, although it does not infect sugarcane. This relationship is also amply supported by common aphid vectors, and similar physical properties, host range, and particle shape and size [Seth *et al.* (25)].

OTHER SAP-TRANSMISSIBLE VIRUSES IN INDIA

Other similar sap-transmissible viruses shown to infect maize in India are: sugarcane mosaic virus [Seth (23)]; ragi mosaic virus [Batra *et al.* (3)] reported from Delhi; eleusine mosaic reported from Deccan [Rao *et al.* (19)]; ragi mosaic observed in Mysore (Karnataka) [Subbayya and Raychaudhuri (33)]; bajra mosaic from Delhi [Seth *et al.* (28)] (Fig. 1); and from Uttar Pradesh by Chaudhary (8). Bajra mosaic virus also infects rice and is the first instance that a sap transmissible virus infects rice and has an active aphid vector. All of these viruses appear to be flexuous rods, have many common hosts and aphid vectors,

Fig. 1. Maize leaves infected with Bajra (pearl millet) mosaic virus.

and are very similar in properties. They are, therefore, considered to be members of sugarcane mosaic virus group.

A mottle disease of canna [Datta Gupta and Raychaudhuri (11)], caused by a sap-transmissible virus, has been recently reported. This virus also infects maize, experimentally, and produces prominent long streaks of light- and dark-green color parallel to the midrib of leaves. Four aphid species, *Myzus persicae*, *R. maidis*, *A. gossyppi*, and *Aphis fabae* Scop. are vectors. The virus had a thermal death point of 65-75 C, a dilution end point of 1:7,500 to 1:8,000, and remained infective for 35 days at 8 C and 14 days at room temperature (28-32 C). Optimum pH for viral infectivity was around 7.0. Virus particles were spherical and 25-29 nm in diam.

VECTORED VIRUSES OF MAIZE IN INDIA

At least two viruses infect maize in India that are not sap inoculable but are transmitted by the delphacid, *Peregrinus maidis* Ashm. One was reported by Chatterjee (7) from Maharashtra and designated as maize stripe virus. This virus causes chlorotic stripes on the infected maize plants. It has a limited host range but is transmissible to maize, sugarcane var. 419, and thick napier grass. It could not be transmitted to sorghum, pearl millet, wheat, rice and some minor millets tested.

Chlorosis of sorghum is the other virus disease reported from Maharashtra and Mysore (Karnataka) by Capoor *et al.* (6) that also affects maize. Early symptoms of the disease appear as broken, longitudinal chlorotic bands on leaves. The virus induces early flowering in sorghum and stimulates prolific growth of nodal buds and tillers near the ground level that remain dwarf, chlorotic, and sterile. Earheads are usually malformed and seeds germinate poorly.

The virus was not mechanically transmissible but was transmitted efficiently by *P. maidis*. The virus appears to belong to the propagative group of plant viruses. The vector is able to acquire and transmit the virus in a minimum access feeding period of an hour and a transmission feeding period of 15 minutes. Viruliferous vectors remain infective for life. After acquisition feeding there is a long delay before transmissibility, and the incubation period in the vector ranges from 18-22 days. No transovarial transmission of the virus has been observed. The virus infects *Canna orientalis*, *Cynodon dactylon* L., *Dichanthum annulatum* (Forsk.), *Dinebra retroflex*, *Zea mays mexicana* (Schrad.) Iltis, *Pennisetum typhoides* (Burm), *Zea mays* L. var. Golden Bantam, and *Saccharum officinarum* L. In sugarcane, the symptoms tend to get marked. Cherian and Kylasam (9) described a similar disease on sorghum in Coimbatore called "freckled yellows" that was transmitted by *P. maidis*.

Maize streak is another important virus disease occurring in India. The disease was first recognized at the end of the last century as a factor limiting maize production in Natal. Since then, the disease has been recorded from many parts of Africa, as far north as Egypt [Smith (32)]. Systematic work on this disease was first initiated in East Africa by Storey (34). Later, the work of Storey and McLean (36) revealed the possibility of existence of more than one streak virus affecting maize. These viruses are, however, very closely related and may be considered as strains. They seem to be specialized for particular hosts. Seth *et al.* (27, 29) described a Pennisetum strain of the virus from India, since it was first recorded on pearl millet. It is commonly found in nature on many cereals, including maize, millets, and grasses. More work is needed to establish the occurrence in India of African strains of the virus.

Streak disease symptoms in inoculated pearl millet appear after 5-7 days, as light-colored circular to elongated spots on the lowest exposed portion of the youngest unfolding leaf. These spots are of varying sizes and generally form on one side of and parallel to the midrib. They subsequently become larger and fuse and cover larger leaf areas, resulting in chlorotic bands running almost the entire length of the leaf. These bands are sometimes interrupted, intermittently, by green tissues. Later, chlorotic bands develop on both sides of the midrib. Subsequently, new emerging leaves both on the main shoot and on tillers show only well-defined chlorotic strips running the entire length of leaves. These strips may be of variable width. Strips are also present on leaf sheaths, but are not as well defined. Some variations in composition and formation of strips have been noted in certain host plants as a result of individual variation or varietal reaction. Diseased plants develop normal, but poorly formed and filled ears. Plants in the field have been observed to be affected at all stages of growth. Early infected plants remained very much stunted and produced almost empty heads, resulting in almost total loss.

In 1970, 10% disease incidence was observed in breeding trials at the Indian Agricultural Research Institute, New Delhi. In the off-season nursery, containing about 1,000 lines of pearl millet material sown in the same field in March 1971, up to 80% of the plants became infected in some lines, including male sterile ones. Overall infection in most lines ranged between 25-50%. The disease has also been observed in the states of Uttar Pradesh, Haryana, Rajasthan, Gujarat, and Delhi. Surveys in other states have not been undertaken to ascertain its importance. Similar disease symptoms, as observed on pearl millet, have also been

observed in nature on maize, wheat, sorghum, barley, oats, finger millet (*Eleusine coracana* L.), *Setaria italica* (L.) Beauv. and many other grasses and minor millets, etc.

Etiology of the disease was established experimentally by Seth *et al.* (27,29). The virus was not sap transmissible, but was transmitted by the jassids *Cicadulina mbila* (Naude), *C. zeae* China, and *C. nicholsi*, the first one being the most efficient vector. Earlier, five species of *Cicadulina*, *C. mbila*, *C. storeyi* China, *C. latens*, and *C. parazeae* Ghauri were reported as vectors.

Host range of the virus, tested experimentally, is given in Table 1. Except in wheat, barley and oats (where the initial reaction was slightly different), symptoms in most of the hosts, namely sorghum, finger millet, *Panicum miliaceum* L., *S. italica*, and *S. verticellata* (L.) Beauv. followed the same pattern as described for pearl millet. However, a slight variation in initial symptom development in wheat and barley was observed. Symptoms in all these hosts persisted until maturity. In maize, however, a tendency to recover a few days after symptom appearance was observed during summer months. In winter, symptoms persisted for longer periods and, sometimes, for the life of maize plants.

TABLE 1. Transmission of bajra (pearl millet) streak virus

Host	No. plants inoculated	No. plants infected	Infection %
Pearl millet			
cv. HB 1	55	24	44
HB 3	30	11	37
HB 4	70	42	60
Maize			
cv. Jawahar	45	10	22
Meerut local	37	7	19
Sorghum bicolor			
cv. Sowarna	42	14	33
CHS-2	32	9	28
Finger millet			
cv. UAS-1	50	21	42
I.E. 980	30	12	40
I.E. 996	35	10	29
I.E. 1022	25	7	28
Wheat			
cv. Kalyan sona	80	36	45
NP 824	40	10	25
NP 830	42	8	19
NP 890	50	12	24
Barley			
cv. K 24	70	39	56
Oat			
cv. Local	45	6	13
Panicum miliaceum	30	9	30
Setaria italica	35	9	26
S. verticillata	25	8	32
Sugarcane			
cv. CO 527	33	0	0
Surka Saharanpuri	40	0	0

In virus-vector relationship studies, it was observed that both nymphs and adults of *C. mbila* were capable of transmitting the virus. A single viruliferous adult or nymph transmits virus. The minimum incubation period of virus in the vector was about 3 days in summer and 6-8 weeks in winter. Similarly, the incubation period of the virus in the plant was about a week in summer but extended to many weeks in winter. The leafhopper can acquire the virus in 15-30 minutes. An increase in percentage of insects transmitting the virus was observed when *C. mbila* acquired the virus as nymphs, compared with acquisition by adults. Active and inactive individuals or races, as described by Storey (35), are suspected to be in India. Certain groups of insects never transmitted the virus and they apparently belong to an inactive group or race.

A similar virus transmitted by *C. mbila* has been observed to cause considerable damage to wheat in Delhi [Seth *et al.* (30)] and in Simla hills, under the name of eastern wheat striate [Nagaich and Sinha (14)]. In 1962, they observed infection of wheat as high as 35%, in certain fields in Simla hills. An estimated loss of 22% in wheat yields was reported. Electron microscopic examination of ultra-thin sections of diseased leaves revealed the presence of virus-like particles 40 nm in diam scattered in the cytoplasm of phloem cells [Nagaich and Sinha (14)].

Recently, Seth and Singh (31) at New Delhi studied the epidemiology of streak disease and found that the disease and the vector persisted on pearl millet, sorghum, maize, finger millet, and some grasses during the "kharif" or summer season, whereas in the "rabi" or winter season they persisted on wheat, barley, oats, rye, and some winter grasses (Figs. 2,3,4). In this way the disease is perpetuated from season to season. The virus was not transmitted in seed but affected their germination to a great extent. A large number of wheat and pearl millet varieties were tested but none was resistant except a few M=A=C=S lines of wheat that showed some tolerance. In the wheat variety 'Hira', root length, plant height, ear size, and number of tillers were much reduced, resulting in considerable yield reduction. Among the insecticides they tested (Phorate, Disulphotan, Dursban, and Carbofuran in granular forms) for control of this disease in pearl millets, Phorate showed some promise.

A new virus disease of maize called vein enation, has been recently reported from Kalimpong, Eastern Himalayas, in India by Ahlawat and

Raychaudhuri (2). Symptoms of vein swelling and vein enation or galls on maize leaves (Fig. 5) were observed in the field. These symptoms have been observed since 1972 in Darjeeling hills. Plants infected in the field were stunted and showed leaf chlorosis and root systems much reduced and rotted. Diseased plants developed partially sterile tassels that usually did not emerge due to the curling or twisting of leaves. Infected plants developed abnormal cobs and grain yield was reduced. Some infected plants died prematurely. Disease incidence in fields ranged from 1 to 15%. The disease was not sap transmissible, nor by seed or by aphids, but was by *C. mbila*. Inoculated plants in the greenhouse produced very similar symptoms as those observed in the field. Initially, symptoms appeared in 3 to 5 days as spots on leaf veins. Veins on the lower surface of the leaf blade showed severe swelling and numerous white spindle-shaped galls or enations that developed in 3-5 days.

The virus infected many hosts in the family Gramineae, including *Zea mays* L., *Triticum aestivum*, L. *Sorghum bicolor* L., (Moench), *Oryza sativa* L. (Fig. 6), *Avena sativa*, L. *Saccharum officinarum* L., *Eleusine coracana* L. (Fig. 7), *Setaria glauca* (L.) Beauv., *E. indica* (L.), *Paspalum sanguinale*, and *Coix lachryman* Jobi L.

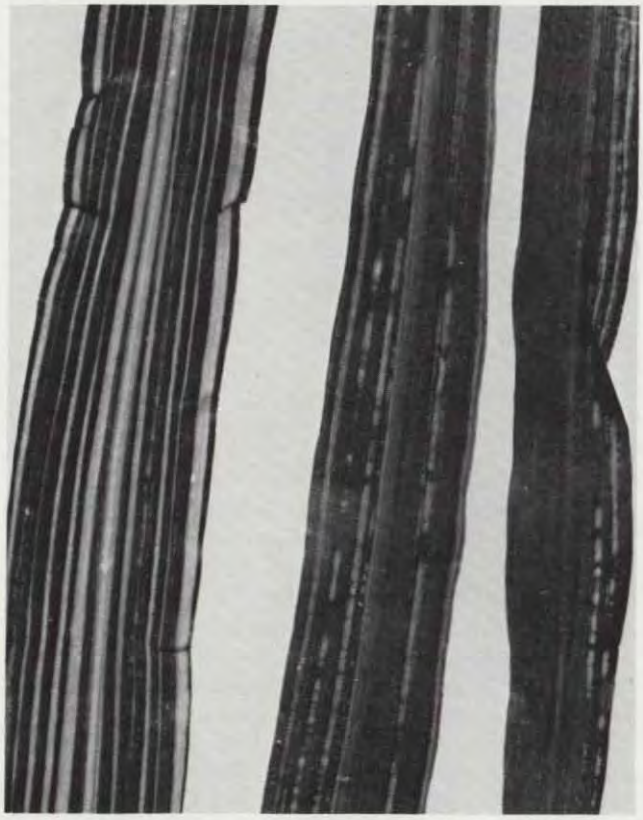

Fig. 2. Streak on pearl millet leaves showing sequence of symptom development.

Fig. 3. Bajra (pearl millet) streak on maize.

Fig. 4. Field infection by Bajra (pearl millet) streak virus on wheat.

Symptoms produced on all these hosts were similar to those of maize, except in sugarcane where only faint swelling of the leaf veins was observed.

Both nymphs and adults of *C. mbila* were capable of transmitting the virus. The insect could acquire the virus in 15-20 minutes and transmit it in about 20 hours. Once vectors became viruliferous they remained so for life.

The disease resembles maize rough dwarf, Fiji disease of sugarcane, rice dwarf, maize wallaby ear, and rice and corn leaf gall. However, its vector is different and some differences in host range have also been noted. Work is needed to establish the correct identity of this disease.

A virus disease of maize causing minute chlorotic spots, later coalescing to form parallel, chlorotic lines along leaf veins, was found in the midwest areas of India in 1971 [Varma *et al.* (38)]. The virus was transmitted by *P. maidis* with an incubation period of about a week in the vector. The virus was studied in thin sections of diseased leaves. The virus did not uniformly infect all cells. At the beginning of cellular infection, virions formed microcrystals irregularly distributed in the cytoplasm. Each microcrystal was surrounded by a 10-12 nm thick membrane. Orientation of particles in these crystals was always side-to-side, thus forming monolayered, hexagonally-packed microcrystals. Some microcrystals were also observed in mitochondria. No virion was detected in the nucleus. Once the cells are completely packed with microcrystals, their membranes disintegrate. Virions were of two types — bacilliform and bullet shaped. Those in the microcrystals were mostly bullet shaped (280 nm) and those free in the cytoplasm were bacilliform (300 nm). Width of virions was 55-58 nm. Each virion had a dense inner core of 10-11 nm diam, a middle layer 8 nm thick, and an outer layer (envelope) of 10 nm. Electron-opaque regions about 14 nm wide were between the layers. In the middle layer, 12 sub-units per turn were resolved. The virus was identified as maize mosaic virus I.

CONCLUSIONS

Ten viruses that infect maize occur in India. These viruses also have potential vectors that breed on various crop plants and grasses in this country and virus diseases can be a limiting fac-

Fig. 5. Maize vein enation on maize.

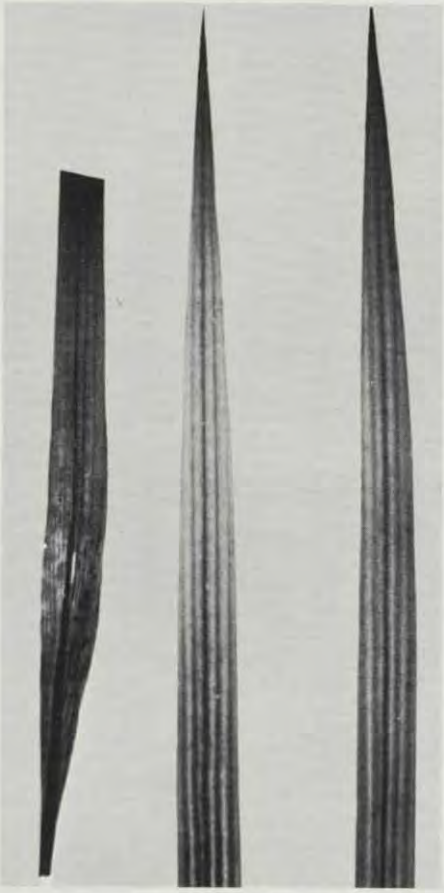

Fig. 6. Maize vein enation on rice.

Fig. 7. Maize vein enation on *Eleucine coracana*.

tor for maize cultivation if proper check is not kept on them. If methodical surveys were made there is a likelihood of discovering other maize diseases of viral and mycoplasmal origin that have not been reported from India.

I hope that I have been able to place before this group of international scientists, actively engaged in maize virus work, the problems concerning such diseases in India. I shall look forward to collaboration with organizations like OARDC and other centers so that we may have a proper understanding of such problems with a view to their control. The object is to protect whatever we produce and, therefore, it is most important that plant protection measures especially for controlling virus diseases should be intensified in India and other developing countries without any loss of time.

LITERATURE CITED

1. AGARWAL, K.C., B.L. RENFRO, and S.P. RAYCHAUDHURI. 1969. Inheritance of resistance in maize mosaic in India. Phytopathology 59:1015 (Abstr.).
2. AHLAWAT, Y.S., and S.P. RAYCHAUDHURI. 1976. Vein enation: a new virus disease of maize in India. Curr. Sci. 45:273-274.
3. BATRA, S.K., L.M. JOSHI, and S.P. RAYCHAUDHURI. 1970. The mosaic disease of ragi (Eleusine coracana). Pages 412-414 *in* Proc. 1st Int. Symp. on Plant Pathology (S.P. Raychaudhuri et al. eds.) New Delhi (IPS), 1966. (Abstr.).
4. BHARGAVA, K.S., and K.C. SHUKLA. 1966. Occurrence of maize mosaic in Uttar Pradesh. Page 428 *in* Proc. 53rd Session Indian Sci. Cong. III.
5. BRANDES, E.W. 1919. The mosaic diseases of sugarcane and other grass. U.S. Dept. Agric. Tech. Bull. 829. U.S. Dept. Agric., Washington, D.C. 26 p.
6. CAPOOR, S.P., D.G. RAO, and P.M. VARMA. 1968. Chlorosis of sorghum. Indian J. Agric. Sci. 38:198-207.
7. CHATTERJEE, S.N. 1971. Occurrence of stripe disease of maize in Maharashtra. Page 725 *in* Proc. 58th Session Indian Sci. Cong. III.
8. CHAUDHARY, A.K. SINGH. 1976. Occurrence of Bajra mosaic in Uttar Pradesh (India), with Myzus persicae Sulz. as an additional vector. Curr. Sci. (Bangalore) 45:76.
9. CHERIAN, M.C., and M.S. KYLASAM. 1937. Preliminary studies of the freckled yellow and stripe disease of Chalum. Proc. Assoc. Econ. Biol. Coimbatore (1936). 5:57-63. (Rev. Appl. Mycol. 17:169. 1938).
10. CHONA, B.L., and M.L. SETH. 1960. A mosaic disease of maize (Zea mays L.) in India. Indian J. Agric. Sci. 30:25-32.
11. DATTA GUPTA, M., and S.P. RAYCHAUDHURI. 1975. Studies on the mottle disease of Canna indica L. Indian J. Hortic. 32:106-109.
12. FULLER, C. 1901. Mealie variegation. Inst. Rept. of the Gov. Entomol., Natal, 1899-1900:17-19.
13. JANSON, B.F., and C.W. ELLETT. 1963. A new corn disease in Ohio. Plant Dis. Rep. 47:1107-1108.
14. NAGAICH, B.B., and R.C. SINHA. 1974. Eastern wheat striate — a new viral disease. Plant Dis. Rep. 58:968-970.
15. PALIWAL, Y.C., and S.P. RAYCHAUDHURI. 1965. Intracellular inclusions associated with maize mosaic infection. Experientia 21:695.
16. PALIWAL, Y.C., and S.P. RAYCHAUDHURI. 1965. Studies on maize mosaic disease. Pages 146-150 *in* Proc. All India Maize Improvement Conf., 1965.
17. PALIWAL, Y.C. and S.P. RAYCHAUDHURI. 1966. Electron microscopic study of maize mosaic virus. Indian J. Microbiol. 5:69-70.
18. PALIWAL, Y.C., S.P. RAYCHAUDHURI, and B.L. RENFRO. 1968. Some properties and behavior of maize mosaic virus in India. Phytopathology 58:1682-1684.
19. RAO, D.G., P.M. VARMA, and S.P. CAPOOR. 1965. Studies on mosaic disease of Eleusine in Deccan. Indian Phytopath. 18:139-151.
20. RAYCHAUDHURI, S.P. 1962. A brief review of maize diseases occurring in India. Pages 91-93 *in* Proc. of the Maize Seed Producers' Conf., ICAR, Pt. I.
21. RAYCHAUDHURI, S.P., R. IYER, and M.L. SETH. 1966. Influence of maize mosaic on host development under varying levels of potassium fertility and its infectivity to sorghum. Bull. Indian Pytopath. Soc. 3:27-31.
22. RAYCHAUDHURI, S.P., M.L. SETH, B.L. RENFRO, and K.C. AGARWAL. 1969. Research on maize mosaic in India. Page 5 *in* CIMMYT News 4.
23. SETH, M.L. 1967. Studies on a strain of sugarcane mosaic virus causing severe mosaic diseases on Co. 527 cane at Nellikuppam. Indian Phytopath. 20:54-56.
24. SETH, M.L. 1974. Maize and millet viruses. Pages 61-65 *in* Current Trends in Plant Pathology. (S.P. Raychaudhuri and S.P. Verma, eds.). Lucknow Univ. Bot. Dept. 341 p.
25. SETH, M.L., Y.C. PALIWAL, ROHINI IYER, and S.P. RAYCHAUDHURI. 1970. Studies on maize mosaic virus from India. Pages 373-377 *in* Plant Disease Problems, Indian Phytopathol. Soc., New Delhi.
26. SETH, M.L., and S.P. RAYCHAUDHURI. 1967. Further studies on mosaic disease of maize (Zea mays L.). Biologia Pl. 9:372-376.
27. SETH, M.L., S.P. RAYCHAUDHURI, and D.V. SINGH. 1971. A streak disease of Bajra (Pennisetum typhoides Burm. f. Stapf and Hubb.) in India. Curr. Sci. (Bangalore) 40:272-273.
28. SETH, M.L., S.P. RAYCHAUDHURI, and D.V. SINGH. 1972. A mosaic disease of pearl-millet (Pennisetum typhoides Burm. f. Stapf and C. E. Hubb., in India. Indian J. Agric. Sci. 42:322-325.
29. SETH, M.L., S.P. RAYCHAUDHURI, and D.V. SINGH. 1972. Bajra (pearl millet) streak: A leafhopper-borne cereal virus in India. Plant Dis. Rep. 56:424-428.
30. SETH, M.L., S.P. RAYCHAUDHURI, and D.V. SINGH. 1972. Occurrence of maize streak virus on wheat in India. Curr. Sci. (Bangalore) 41:684.
31. SETH, M.L., and S. SINGH. 1975. Maize streak in India. Indian Phytopathol. 28:144-145. (Abstr.).
32. SMITH, K.M. 1957. A textbook of plant virus diseases. 2nd ed., Little Brown and Co., Boston. 652 p.
33. SUBBAYYA, J., and S.P. RAYCHAUDHURI. 1970. A note on a mosaic disease of ragi (Eleusine coracana) from Mysore, India. Indian Phytopath. 23:144-148.
34. STOREY, H.H. 1925. The transmission of streak disease of maize by the leafhopper Balclutha mbila Naude. Ann. Appl. Biol. 12:422-439.
35. STOREY, H.H. 1931. The inheritance by a leafhopper of the ability to transmit a plant virus. Nature 127:928.
36. STOREY, H.H., and A.P.D. McLEAN. 1930. The transmission of streak disease between maize, sugarcane and wild grasses. Ann. Appl. Biol. 17:691-719.
37. THORNBERRY, H.H. 1966. Plant pests of importance to North American agriculture. Index of Plant Virus Diseases. Agricultural Handbook No. 307. Agric. Res. Serv., U.S. Dept. Agric., Washington, D.C. 446 p.
38. VARMA, A., S.N. CHATTERJEE, S.P. RAYCHAUDHURI, S. SINGH, and N. PRAKASH. 1975. Maize mosaic virus. Page 158 *in* Proc. 2nd International Virology Congress., Madrid, Spain.

DISCUSSION OF S. P. RAYCHAUDHURI PAPER

V.D. Damsteegt: The slides you showed on vein enation, that was transmitted by *C. mbila*, were of interest to me. They are exactly the symptoms we get in my lab when non-viruliferous *C. mbila* are fed on corn. They fit the description of vein enation.

G. Martinez-Lopez: What is the main difference between these symptoms and the symptoms of maize wallaby ear?

S.P. Raychaudhuri: They are very close and it could be wallaby ear. The Fiji disease has similar symptoms and so does rough dwarf of maize. All are very close in symptomatology.

V.D. Damsteegt: Is vein enation only transmitted by *C. mbila* or also by other *Cicadulina* species?

S.P. Raychaudhuri: There may be other vectors, but so far we have transmitted it only by *C. mbila*.

V.D. Damsteegt: Have you thought about comparing it with maize wallaby ear, as a possibility that it is the same pathogen?

S.P. Raychaudhuri: Yes, it could be but we are not sure. It occurs in a very characteristic area at 4,000 to 5,000 feet and at this elevation you do not always find vectors. Whiteflies are very rare and mostly aphids are present up in those hills. This needs further investigation.

E.E. Rosenkranz: Rough dwarf of maize is not transmitted by leafhoppers but rather by Delphacid planthoppers. On another point, is there any information on the relationship of the Indian maize mosaic to sugarcane mosaic virus?

S.P. Raychaudhuri: Yes, they are serologically related. We have already described it as a strain of sugarcane mosaic virus.

E.E. Rosenkranz: In addition to the maize mosaic, do you have the regular sugarcane mosaic virus on corn?

S.P. Raychaudhuri: No. In sugarcane, mosaic is not of economic importance in India.

A Mosaic Virus Disease of Maize in Thailand

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ABSTRACT

A mosaic disease affecting maize, sorghum and other naturally occurring grass species is reported. Properties of the pathogen suggest that it is a strain of the sugarcane mosaic virus (SCMV). It is similar to SCMV in morphology, physical properties, transmissibility, and host range. Maize seedlings infected with the mosaic virus were more susceptible to sorghum downy mildew incited by *Sclerospora sorghi* (Kulk.) Weston & Uppal than were virus-free plants.

The development of maize (corn) production in Thailand within the past 20 years has been phenomenal. Production increased from a meager 0.1 million ton/year in the early 1950's to 3.0 million tons in 1975 (Fig. 1). The export value of $300 million for maize in 1975 was second only to rice. The great expansion is attributed to many factors, the most important of which are the availability of new land from cleared forest, the development of a highway system, and an introduction of new maize varieties in 1952. The most prominent variety was 'Tiquisate Golden Flint' or the Guatemala variety. By the early 1960's it was estimated that 60 to 80% of the total maize production was the Guatemala variety. The area seeded to this variety continued to expand into the early 1970's.

In 1965, the Thailand National Corn and Sorghum Improvement Program was organized as a cooperative effort between Kasetsart University and the Ministry of Agriculture and Cooperatives with assistance from the Rockefeller Foundation. Its objectives were to provide rapid and efficient solutions to problems facing maize and sorghum production in Thailand. Improved cultivation techniques as well as new varieties obtained from this program have been instrumental in the current booming maize industry.

Most of the maize in Thailand is exported; only about 10% is used for animal feeding locally. The level of local consumption is not expected to change in the next few years.

Maize is grown almost entirely under natural rainfall conditions in Thailand. In 1972, drought contributed heavily to reduce maize production. Another important factor was diseases. Downy mildew caused by *Sclerospora sorghi*, first reported in 1968, became widely distributed by 1972 (1). This disease overshadowed the importance of all other diseases, including viruses. However, virus diseases had not gone unnoticed and investigations on them began in 1970. A mosaic virus, identified as a strain of sugarcane mosaic virus, has been the only virus found to date (4,7). This paper reports its characteristics as well as its influence on susceptiblility of maize to downy mildew.

MATERIALS AND METHODS

An isolate of the virus was obtained from naturally infected Guatemala maize at the National Corn and Sorghum Research Center in Nakornrajsima Province. It was maintained on Guatemala maize in an insect-proof greenhouse during the study. All mechanical inoculations were made by rubbing buffered homogenates of infected tissue on carborundum-dusted leaves of test plants.

Host range and symptomatology. — Twelve plants of each species or variety were inoculated and held for several weeks for symptom development; six uninoculated plants served as controls. Symptomless test plants were assayed for virus by mechanical inoculation to maize seedlings.

Physical properties. — Thermal inactivation point (TIP) was determined by heating inoculum for 10 minutes at 55, 60 and 65 C and dilution end point (DEP) by serially diluting infective sap with 0.05 M phosphate buffer (pH 7.2). Longevity *in vitro* (LIV) was determined by keeping infectious sap at room temperature (about 25 C) and inoculating test plants at 1, 3 and 5 days. Each test was assayed on 10 seedlings of 2-week-old maize and repeated three times. Assay data were recorded 2 weeks after inoculation.

Aphid transmission. — Aphid transmission tests were made with the corn leaf aphid *Rhopalosiphum maidis* (Fitch). After 1-hour preacquisition fast, aphids were placed on young, infected maize leaves for a virus acquisition access period of 1 minute. They were then transferred, at a rate of five insects/plant, for an inoculation feeding exposure time of 1 hour. Thirty-six plants were inoculated with viruliferous aphids and virus-free aphids were placed on 18 control plants.

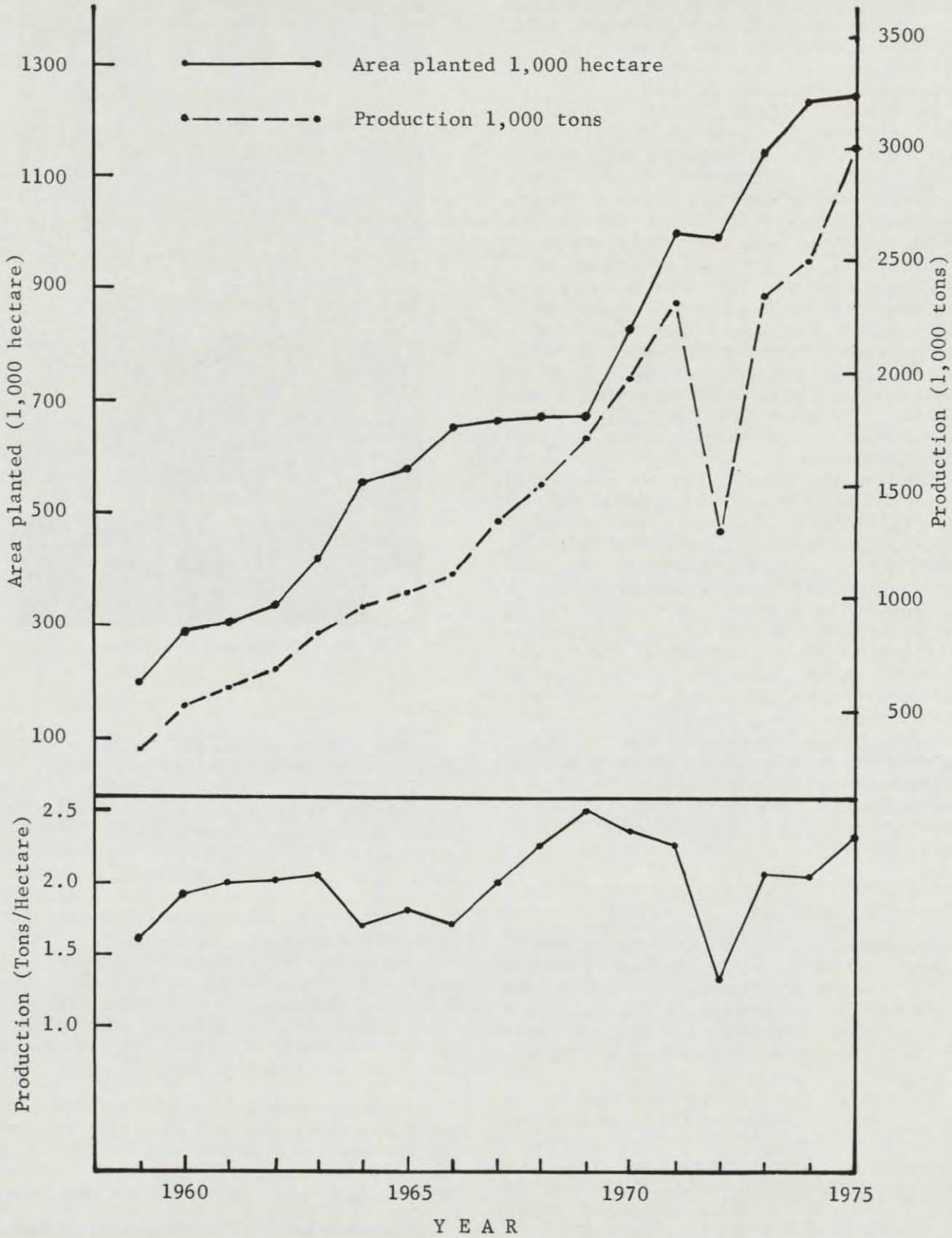

Fig. 1. Area planted, total production, and average yield per hectare of maize in Thailand from 1959 to 1975.

Morphology. — The Brandes' dip method was used to determine virus morphology. Fresh cuts of infected maize leaves were dipped in a distilled water droplet on a collodion-coated grid. Grids were shadowcasted with 80% platinum and 20% palladium and observed under an electron microscope, Hitachi Model HU 11-C.

Purification. — Purification was done by the method described by Shepherd (6). Guatemala maize plants infected for 2-3 weeks were used as source material. Infected leaves were homogenized in 0.5 M sodium citrate containing 0.5% mercaptoethanol (2 ml/g of tissue). The filtered homogenate was clarified by adding an equal volume of chloroform and emulsifying for 1-2 minutes in a blender. The emulsion was broken by a low-speed centrifugation for 15 minutes at 8,000 rpm in a Sorvall GSA rotor. The aqueous phase was centrifuged for 90 minutes at 30,000 rpm. The pellet was resuspended in 0.005 M borate, pH 8.2. The same differential cycle was repeated 2-3 times. The partially purified preparations were stored with a crystal of thymol as the preservative.

Effect of the mosaic virus on reaction of maize to downy mildew. — Two varieties of maize were used in this experiment; Guatemala, downy mildew susceptible, and Suwan 1 (Thai Composite #1 DMR BC_2) resistant. Seven-day-old maize seedlings were mechanically inoculated with a virus homogenate prepared by grinding 1 g of infected maize leaf in 2 ml of 0.05 M phosphate buffer, pH 7.2. Plants were kept at 18-22 C and 90-95% R.H. After 1 week, 200 infected plants of each variety were inoculated with a downy mildew spore suspension at a concentration of 20 spores/low power microscopic field. An equal number of non-virus inoculated plants were given the same treatment. Each treatment consisted of three replications. Data on the number of downy mildew infected plants were recorded 10 days after inoculation.

RESULTS

Symptoms. — The first symptoms induced by the virus were small chlorotic spots on leaf bases. As the disease progressed, broken, chlorotic streaks were formed interveinally (Fig. 2). In most cases, symptoms disappeared when plants were more than 1 month old. No marked stunting was observed.

Host range.—The following Gramineae species were systemically infected: *Chloris barbata* Sw.; *Dactyloctenium aegyptium* (L.) Beauv.; *Digitaria adscendens* (L.) Beauv.; *Panicum maximum* Jacq.; *Pennisetum glaucum* (L.) R. Br.; *P. pedicellatum* Trin.; *P. polystachyon* Schult.; *Sorghum vulgare* (L.) Moench.; and *Zea mays* L. 'Guatemala.'

No symptoms were observed and no virus was recovered from the following species: (Gramineae) *Cenchrus echinatus* L.; *Sorghum halepense* (L.) Pers.; (other herbaceous species) *Amaranthus caudatus* L.; *A. spinosus* L.; *Beta vulgaris* L.; *Brassica chinensis* L.; *B. oleracea* L.; *Capsicum annuum* L.; *C. frutescens* L.; *Cassia occidentalis* L.; *Chenopodium amaranticolor* Coste & Reyn.; *Citrullus vulgaris* Schrad.; *Cucumis melo* L.; *C. sativus* L.; *Datura stranomium* L.; *Euphobia geniculata* Orteg.; *Gomphrena celosioides* Mart.; *G. globosa* L.; *Helianthus giganteus* L.; *Lycopersicon esculentum* Mill.; *Nicotiana glutinosa* L.; *N. tabacum* L.; *Phaseolus aureus* Roxb.; *P. vulgaris* L.; *Pisum sativum* L.; *Phyllanthus niruri* L.; *Ruellia tuberosa* L.; *Solanum melongena* L.; *Tridax procumbens* L.; *Vigna sinensis* (Torner) Savi.; and *Zinnia elegans* Jacq.

Fig. 2. Mosaic symptoms on maize.

Physical properties. — Thermal inactivation point was between 60 and 65 C. The virus was slightly infectious at a dilution of 10^3 but not 10^4. Sap infectivity diminished after 1 day and was still detectable after 3 days, but not after 5 days.

Transmission. — The virus was transmitted by aphids in a stylet-borne manner. *R. maidis* transmitted the virus to 9 of 36 plants tested.

Fig. 3. Electron micrograph of virus particles from maize showing mosaic symptoms (X 33,000).

Morphology. — Flexuous rod particles 600-800 nm long were observed (Fig. 3). The mean length of 123 particles was 687.71 ±20.76 nm.

Purification — Virus preparations obtained were opalescent and highly infectious.

Effect of the mosaic virus on reaction of maize to downy mildew. — Virus-inoculated and non-inoculated Guatemala maize plants (downy mildew susceptible) developed systemic downy mildew infection levels of 77.41 and 63.64%, respectively. However, mosaic virus predisposed the Suwan 1 plants (downy mildew resistant) to downy mildew infection to a much greater degree, from 27.39 to 51.20% (Table 1).

TABLE 1. Effect of mosaic virus infection on reaction of two maize varieties to sorghum downy mildew (DM)

Treatment	Downy Mildew Infection (%) Rep. 1	Rep. 2	Rep. 3	Avg.	DM infection response (%)
Suwan 1					
Virus inoculated	52.09	68.18	33.33	51.20	186.86
Non-inoculated	29.41	27.77	25.00	27.39	100
Guatemala PB 9					
Virus inoculated	64.70	84.21	83.32	77.41	121.63
Non-inoculated	68.42	62.50	60.00	63.64	100

LSD for varieties: 0.05 = 24.22, 0.01 = 55.87; C.V. (%) = 17.0.

LSD for virus inoculated and non-inoculated: 0.05 = 17.17, 0.01 = 29.37; C.V. (%) = 20.0.

LSD for treatment combinations of virus inoculated and varieties: 0.05 = 25.06, 0.01 = 41.57.

DISCUSSION

Characteristics of the maize mosaic virus in Thailand are similar to those of the maize dwarf mosaic virus in the USA (2, 7) and maize mosaic virus of Israel (5) and elsewhere. It should be considered a strain of sugarcane mosaic virus (6). However, the mosaic virus in Thailand is presumably a rather mild strain, since stunting is not very pronounced and symptoms generally disappear by the tasseling stage. Downy mildew has been so devastating during the past several years that the mosaic disease was considered secondary and yield loss has not been assessed.

The synergistic effect of the virus on downy mildew infection is interesting. Plans have been made to test this effect in the field where its effect on maize productivity can be assessed. If laboratory results are confirmed in the field, resistant germplasm for this virus will need to be sought and a cooperative research program with plant breeders established.

Since the maize mosaic virus in Thailand proved to be mild and no other major maize viruses have been found, maize viruses have received little attention at the national level. However, since the maize mosaic virus, vector and wild grass hosts are prevalent in growing areas, we are concerned with preventing it from becoming more important. A more virulent strain of the virus either by mutation or introduction would certainly be a real hazard.

LITERATURE CITED

1. AMPHOL SENANARONG. 1972. Incident of corn-downy mildew disease in Thailand. Asst. Newsletter 5:159-170. (In Thai).
2. BANCROFT, J.B., A.J. ULLSTRUP, MIMI MESSIEHA, C.E. BRACKER, and T.E. SNAZELLE. 1966. Some biological and physical properties of a Midwestern isolate of maize dwarf mosaic virus. Phytopathology 56:474-478.
3. DIVISION OF AGRICULTURAL ECONOMICS, MINISTRY OF AGRICULTURE AND COOPERATIVES. 1975. Corn Production and Marketing. Problems of Corn Farmer and Government Aid, 1974-Present. Agric. Econ. Rept. No. 95. 76 p. (In Thai).
4. KLEIN, M., I. HARPAZ, A. GREENBERGER, and I. SELA. 1973. A mosaic virus disease of maize and sorghum in Israel. Plant Dis. Rep. 57:125-128.

5. KORNKAMHAENG, P. 1972. Some properties of a virus causing mosaic symptom on corn in Thailand. M.S. Thesis. Kasetsart University, Bangkok. (In Thai).
6. PIRONE, T.P. 1972. Sugarcane mosaic virus. No. 88 *in* Descriptions of plant viruses. June, 1972. Commonw. Mycol. Inst., Assoc. Appl. Biol., Kew, Surrey, England 4 p.
7. SHEPHERD, R.J. 1965. Properties of a mosaic virus of corn and Johnson grass and its relation to the sugarcane mosaic virus. Phytopathology 55:1250-1256.
8. SIRITHORN, P. 1976. Comparison on mosaic virus isolated from maize, sorghum and sugarcane and its effect on resistance to downy mildew. M.S. Thesis. Kasetsart University, Bangkok. (In Thai).

DISCUSSION OF T. SUTABUTRA PAPER

G. Martinez-Lopez: Do you think the reason that maize dwarf mosaic virus is the only maize virus reported from Thailand is because there are no studies on other maize viruses at present? Do you think that this is the only one you do or will have?

T. Sutabutra: One problem is that downy mildew is so common and so devastating it may obsure other symptoms. It also has occupied a great deal of our time. However, Dr. Renfro and I have toured quite a bit of the country and we have never seen corn stunt or virus symptoms.

L.R. Nault: Are *Peregrinus maidis* or *Cicadulina* species present in Thailand? It seems that wherever these insects occur they transmit viruses. Perhaps an approach would be to collect and study these vectors.

T. Sutabutra: I do not know if they occur. We have not looked for them.

L.R. Nault: They are in India and it is hard to believe they would not be in Thailand.

B.L. Renfro: There are quite a few entomologists working there on cereals and other crops. We will search for them.

V.D. Damsteegt: There are no reports of *Cicadulina* being in the Indochina area. But there is no reason why *Cicadulina* should not be. They have the wet savannah land that is ideal for it. It may be that no one has collected enough to detect *Cicadulina*. Perhaps the barrier between that area and Africa or India is so great that it is not able to move into Indochina. Or, predators and parasites may have prevented its establishment.

S.P. Raychaudhuri: When downy mildew symptoms first appear, from a distance they look like maize streak. Unless you collect spores (early in the morning or at midnight), and prove that downy mildew is involved, it could be streak or some other disease.

B.L. Renfro: We do have trouble reading early symptoms of downy mildew. To illustrate, one of our staff collected 100 plants suspected of having mildew and all of them failed to develop mildew symptoms. Apparently, they were infected with sugarcane mosaic virus. This virus is very common throughout Asia, Afghanistan, and the Pacific Islands.

E.E. Rosenkranz: Perhaps one reason why you do not have more virus diseases in Thailand is that you have not grown corn for a long enough time. Perhaps the situation is analogous to that in the United States 20 years ago when we had no virus problems.

T. Sutabutra: I am not sure about the answer to your question. The major production of maize in Thailand is recent, but we have had maize for a long time. It is not a new crop to Thailand, but new varieties have been introduced to increase yields.

Viral Diseases of Maize and National Programs of Maize Production in the Philippines

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ABSTRACT

Mosaic and stripe are two viral diseases of maize recognized in the Philippines. Leaf gall has also been noted and suspected to be caused by a virus. The diagnostic symptoms of these diseases, and transmission and suscept range of mosaic and stripe viruses are discussed. National programs to boost maize production are described.

Corn (*Zea mays* L.) is one of the major cereal crops in the Philippines. It is the staple food of 20% of 42 million Filipinos; in addition, it serves as a supplement to rice, the staple food of the majority, during poor rice harvest. It ranks second to rice in the utilization of agricultural resources.

In the 1975-1976 crop year (1 July 1975 - 30 June 1976), the Philippines produced 2,568,380 tons of shelled corn from 3,062,450 ha—an average yield of 0.83 ton/ha (Dept. Agr., Bur. Agr. Econ. Rept., 1975). During the same period, the Philippines utilized 2,608,379 tons of corn. About 39,999 tons or 1.5% of the total amount utilized was imported from the USA and Thailand (Dept. Agr., Natl. Food Agr. Council Rept., 1975). Of the 2,608,379 tons of corn utilized in 1975-1976 (Table 1), about 64.06% was used for human consumption, 12.33% for livestock/poultry feeds, 9.12% for mixed poultry/livestock feeds, 9.70% for manufacture of corn starch, 0.32% for other industrial uses, 1.64% for seeds and 2.83% wastage (Dept. Agr., Natl. Food Agr. Council Rept., 1975).

TABLE 1. Amount of shelled corn utilized in the Philippines in 1974-1975

Item	Consumed (metric tons)	%
Food	1,670,927.59	64.06
Feeds of livestock/poultry	321,613.13	12.33
Mixed poultry/livestock feeds	237,884.16	9.12
Manufacture of corn starch	253,012.76	9.70
Other industrial uses	8,346.81	0.32
Seeds	43,777.42	1.64
Wastage	73,816.13	2.83
Total	2,608,379.00	100.00

The poor average yield of corn may be attributable to low yield potential of traditional peasant farmers' varieties; occurrence of downy mildew; insect infestation, particularly by stem borers and corn weevil; lodging; and poor tillage and cultural practices.

Unlike the corn downy mildew, viral diseases of maize are, fortunately, of minor importance in the Philippines. No comprehensive record of their distribution and effects on yields is available. However, the economic significance of these diseases, particularly corn mosaic, is reflected in terms of the capacity of the corn mosaic virus to infect other economically important crops, such as sugarcane (*Saccharum officinarum* L.), abaca (*Musa textiles* Nee), and even cultivated sorghum (*Sorghum bicolor* (L.) Moench) (2, 4, 8, 11, 12). Another economic significance of corn mosaic is that corn is a preferred host plant of *Rhopalosiphum maidis* (Fitch), the vector of sugarcane mosaic, abaca mosaic, and red leaf disease of grain sorghum (4, 8, 12).

Fig. 1. Corn mosaic virus infected corn plant showing the dots or elongate to spindle-shaped pale areas on basal part of the leaf (upper left).

MOSAIC DISEASES OF MAIZE

Symptoms. — Early symptoms of the disease appear as dots or elongate to spindle-shaped pale areas on the lower part of the leaf (Fig. 1). The large, spindle-shaped areas are not always solidly chlorotic; a green portion, circular to elliptical in shape, may be present in the center. The spindle-shaped and elongate pale areas, whose long axis is parallel to the leaf veins, are arranged closely in rows on the leaf blade on one or both sides of the midrib (Fig. 2). Soon the pale areas coalesce

Fig. 2. Corn mosaic virus infected corn plant showing the spindle-shaped and elongate pale areas with long axis parallel to leaf veins.

and the effect is a somewhat diffuse blotching of green tissue upon a paler-green background (Fig. 3). Succeeding leaves that emerge may show either a distinct or a rather vague mottling. In general, young leaves are more chlorotic than older ones because they tend to regain their normal color as they mature. Often, toward the base of these leaves, particularly before they recover the normal green color, alternating broad lines of green and pale green are produced.

As the disease progresses, there is a considerable reduction in the size of infected plants (Fig. 4). Also, plants that become infected at a later stage of development produce barren or nubbin ears (Fig. 5).

Corn plants infected by abaca mosaic virus (Fig. 6) show irregular light areas, or mottled appearance, at the base of young, expanded leaves (Fig. 7). As these plants grow older, the light-yellowish streaks become less distinct. As a whole, leaves appear paler or much lighter green than those of healthy plants. Infected plants are stunted and produce abnormally small-sized ears.

Fig. 3. Portion of corn mosaic virus infected corn leaf showing the coalesced, pale areas and diffused blotching of green tissues upon a paler green background. (Photo courtesy of D.A. Benigno.)

Fig. 4. A stunted corn mosaic virus infected corn plant (right) and a healthy corn plant (center).

Transmission. — Corn mosaic virus has been reported to be transmissible to corn by *R. maidis* and *Aphis gossypii* Glover. No transmission by *Peregrinus maidis* Ashm. or *Proutista moesta* Westwood from corn to corn has been recorded (8). *A. gossypii* has been reported to acquire the virus after 1-2 hours feeding on infected plants. Three aphids were used to transmit the virus to corn. Incubation period of virus in plants was 12-23 days. The virus has also been transmitted within 8-28 days, when healthy corn plants were abraded with carborundum and then swabbed with the freshly-crushed diseased tissue. Also, about 62-85% infected plants were produced within 5-17 days when pieces of sterile cotton soaked in freshly extracted juice of diseased plants were wrapped around the base of the young leaves of corn plants and then pricked with a pin into the midrib.

In addition to corn mosaic virus, a strain of abaca mosaic virus has also been transmitted to corn by *R. maidis* and *A. gossypii* (4). Vectors acquired the virus after 2-3 days feeding on infected abaca plants and transmitted it to corn plants; symptoms were observed after an incubation period of 5-13 days. The virus was reciprocally transmitted from corn to abaca with an incubation period in abaca of 11-22 days. Mechanical transmission of abaca mosaic virus to corn was also effected by pin-pricking and rubbing with sap. Incubation period of the virus in corn ranged from 19-42 days (1, 5, 6, 7).

Virus properties. — Fresh extracts from corn mosaic virus-infected leaves heated for 10 minutes at 40, 45, 48, 52, 54, 55, and 56 C, showed that the virus was inactivated at 54, 55, and 56 C (8).

Undiluted and diluted (1:10) diseased leaf extracts inoculated on corn plants resulted in infection. No infection was obtained beyond the 1:10 dilution (8).

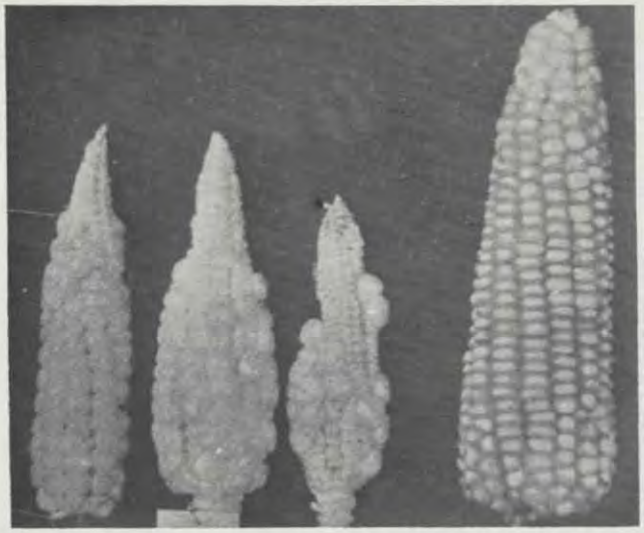

Fig. 5. Malformed corn ears (three at left) obtained from corn mosaic virus infected plants, and normal ear (right). [O. M. Lawas and W.L. Fernandez (8)].

Fig. 6. Leaves of abaca mosaic virus infected abaca plants showing the characteristic mottling pattern.

Fig. 7. Abaca mosaic virus infected corn seedling leaves showing long, more or less broad, irregular stripes from the base to the tip (left), and small, irregular mottling characteristic of grass mosaic (center) compared with healthy leaf (right). [M.S. Celino and G.O. Ocfemia (4)].

Host range. — In addition to corn plants, the following grasses, weeds, and crop species have been recorded as suscepts of the corn mosaic virus: *Eragrostis amabilis* (L.) W. & A. ex Nees, kulape (*Paspalum conjugatum* Berg.), *Digitaria corymobosa* [*Roxb.*] *Merr.*, *D. sanguinales* (L.) Scrop., *Eleusine indica* (L.) Pers., *Echinocloa colonum* (L.) Link, *Sorghum bicolor* (L.) Moench, *Saccharum officinarum* L., and *Musa textiles* Nee (2, 8, 12).

CORN STRIPE

Corn stripe has been a common malady of maize in the Philippines from 1944 until 1950 (13). An affected field is conspicuous in that there is yellowing, poor vigor, irregular height and narrow leaves of plants. Vigor is appreciably reduced and ears formed are small and distorted (Fig. 8) and

Fig. 8. Misshapen corn ears of glutinous corn from plants severely affected by stripe virus. Note reduced size of ears, number of kernels, and shriveled grains. [G. M. Reyes (8)].

tassels are partially or completely sterile. Yield loss has been estimated at 43%.

Symptoms. — The initial symptom of the disease is the presence of small, yellowish-white streaks visible on both sides of the youngest unfurled leaf. Stripes, in general, are greenish-yellow and irregular in length and breadth and run parallel with the veins. Stripes may occupy a distinct area from the base to the tip, although at times they are disconnected; they also involve the midrib in rare instances. Striping may also occur on the leaf sheath and occasionally on the exposed outer husks. Stripes vary in width from less than 0.5 to 5 mm or more and are not equidistant. This alternate arrangement of greenish-yellow and

Fig. 9. Portions of leaves of 35-day-old glutinous corn showing characteristic yellow stripe symptoms (three leaves at right) compared with a healthy leaf (left) [G.M. Reyes (13)].

almost normal leaf green is the most diagnostic symptom (Fig. 9). Some yellow stripes coalesce and form wider ones. In a few cases, only half of the blade is severely affected and shows more yellowing, whereas in others only the basal portion to about half is yellowed. In several instances during the early stage of growth of the plant, nearly the entire lamina turns yellow.

Stripe symptoms persist until maturity, but as leaves expand the colors become diffuse somewhat or change and are not as contrasting as during the early stage when leaves show a very conspicuous yellow stripe pattern. In rare cases, however, a few of the persisting stripes turn pale greenish-yellow to yellowish-white.

Transmission. — In 1948, corn stripe virus was transmitted to corn by *P. maidis* (M. B. Capito, *unpublished*). No transmission has been obtained from corn to corn by *R. maidis*.

All nymphal instars of *P. maidis*, except the first, acquired the virus after 24-hour feeding on diseased plants, and 10 individual nymphs were used after a 24-hour inoculation access period to transmit the virus to corn. The incubation period in plants was 15 days. Adults acquired the virus after 12-48 hours feeding on infected plants and 10 individual adults transmitted the virus to corn after 6-12 hours feeding on healthy plants, with an incubation period of 10-15 days in the plant.

Mechanical transmission of the virus was not affected by pin-pricking or sap transmission with the aid of carborundum.

Host range. — The corn stripe virus also infects *Rottboellia exaltata* L. and *Sorghum bicolor*.

MAIZE LEAF GALL

This disease was first reported in September 1929 in a field planted to Yellow Flint in which 90% of the plants were affected (10). Since then, the disease has not been observed.

Symptoms. — The early symptom is characterized by the appearance of somewhat shiny streaks on the upper surface of leaves. These streaks coalesce both laterally and longitudinally over the entire width of the leaf. Leaves curl along the margins and tend to bend upwards in the form of short bows. In the region of shiny areas, veins become more transparent. Galls are formed on the main veins. At first, galls are small and elongate and vary from 1-3 mm x 0.33-0.5 mm. Galls coalesce and sometimes extend to the leaf sheaths. Old galls may become knot-like in appearance.

There is a gradual shortening of the leaves of infected plants and they become stiffer and shorter (Fig. 10). The most seriously infected plants are much stunted. Leaves are very short and the youngest ones pale green and almost aborted.

Transmission. — No positive transmission by artificial inoculation has been demonstrated so far. The writer believes that this disease may be identical to the wallaby-ear disease reported from Australia.

Fig. 10. Maize leaf gall infected (right) and healthy (left) plants of the same variety and age.

NATIONAL PROGRAMS ON MAIZE PRODUCTION

Intensified corn production program. — To promote and hasten farmer adoption of recommended packages of production technology, the Intensified Corn Production Program, which is monitored at the University of the Philippines at Los Banos (UPLB), cooperates with the Bureau of Plant Industry and the Bureau of Agricultural Extension in the following activities:

1. *Applied research* — Variety and fertilizer trials are conducted in farmers' fields through applied research kits. Adaptability of recommendations are demonstrated on farmers' fields by production kits.

2. *Seed production* — Recommended varieties are made available locally by encouraging farmer cooperators to produce recommended varieties for their own use and for neighboring farmers. Farmers producing seed are supervised by corn production technicians and seed inspectors.

3. *Training* — Corn production technicians are trained at UPLB and in regional training centers on the latest and improved cultural practices. These technicians then become corn production supervisors in their area and train corn farmers.

4. *Extension and information drive* — Bulletins, brochures, newsletters, and posters are continuously prepared and updated and made available to technicians and leading farmers. Informational materials are broadcast regularly from 23 radio stations in the corn priority provinces.

5. *Credit and financing* — Production loans are made available to corn farmers by the Philippine National Bank, Rural Bank, and Agricultural Credit Administration, through their agricultural credit supervisor. Loans are extended to farmers after they present the Farm Plan and Budget, prepared in conjunction with corn production technicians. A production loan of 765 pesos/ha is extended; 510 pesos for production inputs and 255 pesos for labor.

6. *Price support marketing* — The government fixed the floor price of shelled corn at 0.90 pesos/kilo with 15% moisture content or at 0.45 pesos on cob at harvest (9).

"Masaganang maisan". — The increasing demand for this staple crop by the corn-eating populace, its expanding industrial uses, and the fast developing poultry and livestock industries require a well-directed program for developing the white corn and feed grain industries. Unfortunately, the domestic supply of feed grains has never been sufficient to meet local demands.

In response to well-recognized production and marketing constraints that jeopardize self sufficiency goals plus the worldwide shortage of grain and its upward price trends, the Philippine government launched a massive white corn and feed grains program called "Masaganang

Maisan" at the UPLB in March 1975. This is a major production scheme whose principal goal is to increase yields of corn and other feed grains.

Aims of the program are to: satisfy the increasing demand for white corn for human consumption; produce sufficient yellow corn, sorghum, and soybeans to meet the feed requirements of the poultry and livestock industries; produce enough corn for export in the form of corn starch and other by-products; and study and establish joint ventures or multi-lateral feed grains projects with foreign investors.

Three sectoral production models are conceived, viz., the small farmers, commercial and/or integrated-plantation-type concept, and development of large-scale production involving hundreds of thousands of idle government or private lands into feed grain plantations.

In a nutshell, strategies of implementation are: 1) concentrating production in major corn-producing and consuming areas and in geographic compact areas composed of 42 priority provinces; 2) arranging and providing credit facilities to the farmers under the supervised credit scheme (provisions of credit without collateral are arranged with the Rural Bank, Philippine National Bank, Agricultural Credit Administration, and Development Bank of the Philippines). Credit needs of large-scale commerical plantations are handled by the Development Bank of the Philippines and Philippine National Bank; 3) providing and facilitating supply and distribution of fertilizer, chemicals, and other inputs to programmed provinces; 4) intensifying information campaign and extension work; 5) involving local officials in the provincial action committees and municipal action teams; 6) distributing seed production at the provincial level and developing seed production centers; 7) creating the national management committee; 8) formulating marketing programs and establishing price supports for white corn and feed grains; 9) increasing manpower employment and training unskilled technicians to improve their managerial skills to attain the increased targets; and 10) facilitating the provision of credit facilities for grain dryers, threshers, and other farm equipment and machineries through the Development Bank of the Philippines (Dept. Agric., Natl. Food Agr. Council Rept. 1975).

LITERATURE CITED

1. BENIGNO, D.A., and S.E. DEL ROSARIO. 1965. Mechanical transmission of the abaca mosaic virus and some of its physical properties. Philipp. Agric. 49:197-210.
2. BENIGNO, D.A., and A.D. KARGANILLA. 1973. The first virus disease of sorghum in the Philippines. Philipp. Phytopathol. 9:1 (Abstr.).
3. CASTILLO, B.A., J.A. AGATI, and F.Q. OTANES. 1954. Studies on the rate of abaca mosaic spread under different conditions. Philipp. J. Agric. 19:257-272.
4. CELINO, M.S., and G.O. OCFEMIA. 1941. Two additional insect vectors of mosaic of abaca or Manila hemp plant, and transmission of its virus to corn. Philipp. Agric. 30:70-78.
5. CELINO, M.S., and A.L. MARTINEZ. 1955. Mechanical transmission of a mosaic virus from abaca to corn. Philipp. Agric. 39:377-392.
6. CELINO, M.S., and A.L. MARTINEZ. 1956. Mechanical transmission of the abaca mosaic virus. Philipp. Agric. 40:120-128.
7. ELOJA, A.L., L.G. VELASCO, and J.A. AGATI. 1962. Studies on the abaca mosaic disease: I. Sap transmission of the abaca mosaic virus. Philipp. J. Agric. 27:75-84.
8. LAWAS, O.M., and W.L. FERNANDEZ. 1949. A study of the transmission of corn mosaic and some of the physical properties of its virus. Philipp. Agric. 32:231-238.
9. MERCADO, A.C., V.R. CARANGAL, and B.A. ADAY. 1973. Maize germplasm utilization at the farm levels in the Philippines. Pages 215-223 *in* Proc. Tenth Inter-Asian Corn Improvement Workshop, 10-12 December, 1973, Malaysia.
10. OCFEMIA, G.O. 1931. Notes on some economic plant diseases new in the Philippine Islands. Philipp. Agric. 19:581-589.
11. OCFEMIA, G.O. 1949. The rapid dissemination of the mosaic disease of abaca, or Manila hemp (Musa textiles Nee) in the field. Philipp. Agric. 33:142-144.
12. PAULSEN, A.Q., and A.D. KARGANILLA. 1973. Note: Red leaf disease of grain sorghum in the Philippines. Philipp. Agric. 57:264-268.
13. REYES, G.M. 1950. Progress of investigation on the yellow stripe disease of corn. Philipp. J. Agric. 15:105-118.

DISCUSSION OF O. R. EXCONDE PAPER

V.D. Damsteegt: The last disease you mentioned was maize leaf gall. Do you have any evidence of *Cicadulina* being involved in these swellings? Is there a report of this genus in the Philippines?

O.R. Exconde: I am not sure about published reports of its occurrence, but we do have *Cicadulina* in the Philippines.

S.P. Raychaudhuri: The presence of *Cicadulina* in the Philippines has been reported.

R.E. Ford: Do you have data indicating that abaca mosaic virus is serologically related to sugarcane mosiac virus?

O.R. Exconde: Yes. One of our virologists did a serological study that indicated a relationship.

B.L. Renfro: From Asia, we have heard the reports of 10 virus diseases of maize in India, one in Thailand, and three in the Philippines. None of these at this time are reported to be highly damaging. But I think that researchers in Asia are quite concerned about possibilities of introductions of new vectors and viruses. They feel that maize is quite vulnerable, since their maize varieties are products of their national programs and, by and large, developed without testing against other important diseases like streak, stunt, maize chlorotic dwarf and highly virulent strains of MDMV.

Maize Virus Disease Identification by Symptomatology

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Approved for publication as Journal Article No. 189-76 of the Ohio Agricultural Research and Development Center, Wooster. Based on cooperative investigations of the Agricultural Research Service, U.S. Department of Agriculture, and Ohio Agricultural Research and Development Center, Wooster.

ABSTRACT

Disease diagnosis by symptoms is fast, easy, and inexpensive. Strengths of disease identification by symptoms depend on the experience of the researcher and constant association of a pathogen with a particular symptom. Symptomatology is also useful for studying virus strains and host variability.

We routinely diagnose familiar plant diseases by observing symptoms of abnormal growth because symptoms are generally reliable. However, different plant diseases may have similar symptoms and these similarities may prevent us from identifying new or unfamiliar ones. In cases of viral diseases, accurate identifications of new or unfamiliar diseases often depend on increasing a researcher's ability to associate particular symptoms to specific viral infections. Demonstrating this association may involve complex procedures of isolating, purifying, characterizing, and maintaining the virus. Furthermore, techniques from different disciplines (e.g. biochemistry, electron microscopy, entomology, and plant pathology) may be required to determine the virus and its symptom association and for fulfilling Koch's postulates.

Slide presentation. — The audience was asked to diagnose the maize disease shown in each of 16 Kodachrome slides, by symptoms alone. The slides emphasized the limitations of disease identification solely by symptoms and demonstrated the need to include additional diagnostic methods. The slide presentation was not intended to detract from the use of symptoms in disease identification.

Disease diagnosis by symptoms has been useful because it is faster, easier, and cheaper than any other method. It has been particularly useful in screening for host resistance. Sometimes diagnosis by symptoms is the only diagnostic method available; for example, there is no other way to test infectivity or pathogenicity except by host response. The usefulness of symptoms for disease diagnosis is enhanced when the virus has been isolated and sufficiently characterized by electron microscopy, sedimentation, serology, and vector relationships to permit its association with a particular host symptom.

Isolation — A first step in symptomatology. — The difficult and complex problems associated with isolation of an obligately vectored virus or virus-like organism have been discussed (1, 2, 3, 4, 7). Isolation of mechanically transmissible viruses is simpler. In the case of the mechanically transmissible maize dwarf mosaic virus (MDMV), isolation was also facilitated by the recovery of virus from local lesions on some maize inbreds (6). To increase the probability of obtaining a pure culture of MDMV, an isolate from a single local lesion on a rub-inoculated leaf of maize inbred Ill. A or Pa32 was serially transferred eight times through each individual inbred. Furthermore, inbred N20 was also rub-inoculated during each transfer to check the consistency of symptoms produced by the subcultured isolate. This method permitted us to select four additional strains of MDMV from southern Ohio.

Virus strains and virus population. — In addition to disease identification, symptomatology is also useful for studying virus strains. The isolation and identification of MDMV strains resulted in finding strain-specific resistance in different maize inbreds (6). Specificity of strain-specific resistance should allow us to identify the genes for resistance to each strain. Occurrence of strain-specific resistance also suggests that breeding for general resistance to a complex virus population under field conditions would obscure this type of resistance.

Strains of MDMV may exist as one of several virus populations under field conditions. Changes in predominance among strains or viruses from year to year and location to location can confuse the analysis of inheritance of resistance. These

changes may explain the inconsistency of reactions of inbreds tested under field conditions in different years and locations. For example, maize inbreds Oh509A and Oh513 have had a fair degree of resistance to MDMV under Ohio field conditions in past years; but recently this resistance appears to be decreasing. The effect of location variation on inbred performance is best exemplified by inbred Ab28A. Under natural disease conditions, Ab28A performs well in Mississippi but poorly in Ohio (W.R. Findley, *personal communication*). In addition to the influence of virus and location variability on the reaction of an inbred to virus infection, we have some evidence suggesting a lack of genetic homogeneity in inbreds (5) that may also influence the variability in host reactions to virus infection.

To monitor changes in virus-strain populations, host differentials and antiserum are needed for easy virus identification. Their use will permit assessment of the importance of these changes in different years and locations. To determine the importance of host variability on virus resistance in the field, the parameters for the expression of virus resistance need to be established. Standardized methods in establishing these parameters will permit us to objectively judge the relative resistance of an inbred.

In conclusion, we need not only to identify viruses by means of symptoms, but also determine the variability of the virus population. Symptomatology is the first and the simplest tool available. The only prerequisite for use of this powerful tool is some basic knowledge about symptomatology and a discerning eye, tempered with experience.

LITERATURE CITED

1. BRADFUTE, O.E. 1976. Electron microscopy as a means for discovery of new maize viruses and virus-like pathogens. Pages 103-107 in Proc. Maize Virus Dis. Colloq. and Workshop. 16-19 Aug , 1976. Ohio Agric. Res and Dev. Center, Wooster. 145 p.
2. DAVIS, R.E. 1977. Spiroplasma: Role in the diagnosis of corn stunt disease. Pages 92-98 in Proc Maize Virus Dis. Colloq. and Workshop. 16-19 Aug , 1976. Ohio Agric. Res. and Dev. Center, Wooster, 145 p.
3. GINGERY, R.E. 1977. Dectection and identification of maize viruses by rate-zonal density gradient centrifugation. Pages 108-110 in Proc. Maize Virus Dis. Colloq. and Workshop. 16-19 Aug., 1976 Ohio Agric Res. and Dev. Center, Wooster. 145 p.
4. GORDON, D T. 1977 Routine serological assays for diagnosis of maize virus diseases. Pages 99-102 in Proc. Maize Virus Dis. Colloq. and Workshop. 16-19 Aug , 1976 Ohio Agric. Res and Dev Center, Wooster. 145 p
5. LOUIE, R., W.R. FINDLEY, and J.K KNOKE. 1976. Variation in resistance within corn inbred lines to infection by maize mosaic virus. Plant Dis. Rep 60:838-842.
6. LOUIE, R., and J.K KNOKE. Strains of maize dwarf mosaic virus Plant Dis. Rep 59 518-522
7. NAULT, L.R. 1977. Vectors of maize viruses Pages 111-116 in Proc Maize Virus Dis. Colloq. and Workshop 16-19 Aug., 1976. Ohio Agric. Res. and Dev Center, Wooster. 145 p.

DISCUSSION OF R. LOUIE PAPER

V.D. Damsteegt: If you go out into the field and make 10 collections at 10 different locations for MDMV, do you ever pick up one strain by itself or are there always two or more together?

R. Louie: We have found plants doubly infected with strains of MDMV. This is common with strains A and B We also found strain E along with strain D in southeastern Ohio. It is probably more difficult to find plants infected with only one strain. We were able to pick up strain C midpoint in the disease epiphytotic at Portsmouth only because we had large biweekly plantings of a susceptible line. Previously, because we had only one planting, these minor strains often became masked or otherwise obscured by more prevalent strains Another way we picked out strains was by exposing trap plants in the field. Since trap plants are exposed only for a short period of time, we increase our chances of finding other strains. Dr. Knoke will talk more about this later.

G. E. Scott: Do you commonly get three or four strains in one plant?

R. Louie: Perhaps we would if we had the techniques: Strains that we have the most tools for diagnosing are A and B. Quite often we find these together. We have assayed these strains by serology and mechanical transmission and found these combinations.

R.W. Toler: Do you have maize resistant to the different strains of MDMV?

R. Louie: We have differential resistance to them. Some are differentially resistant only to one strain. We will show you some differentially-resistant plants in our plots.

R W. Toler: Do you have a set of differential varieties or differential accessions that we could use? For example, would I plant a set of differentials, inoculate them, and determine whether I had A, B, C, D, or F?

R. Louie: Not yet, but we are working on a host range differential

R.W. Toler: Do you have differences in virulence among the strains?

R. Louie: Yes Strains E and B are probably more virulent than strains A, D, or F, although they do not induce as severe a leaf symptom.

R.W. Toler: In other words, the effect on overall yield is greater than the effect demonstrated by the severity of leaf symptoms

B. Tsotsis: Are you envisioning in your breeding program for resistance a need to have eight different genes, five different genes, or one gene, depending on the number of strains? Are maize lines identified that are able to confer resistance to more than one strain?

W.R. Findley: Right now we do not have good data on that. There may be one gene that controls resistance to all these strains, but we have inbred lines that react differently to different strains so there are apparently different genetic systems, depending on what inbred you use to test the reaction. I think we are going to have to test inbreds

for reaction to specific strains. Pa405 is one inbred that may be resistant to all strains of MDMV. We have made some tests with M14 X Pa405. We have studied both the F_2 and F_3's and backcrosses to resistant and susceptible parents. It looks as if a single gene is involved, but we would like to check further. We have rechecked some F_3's and the data still supports the original hypothesis. I think that we are going to find, as I indicated, a different genetic system for some strains.

B Tsotsis: Well, my question was operational rather than fundamental We have the option of trying to resolve the disease problem by either having sufficient variability in the genetic collection or getting to such a fine point that you have screened these things out and recombined them. In corn breeding, I think we should work toward a multiline approach. Of course, you have the opportunity of extending the genetic base by combining lines. I was interested as to whether we can cover most or a number of sources of resistance in a few corn genotypes instead of making crosses to every one of them.

W.R. Findley: Our approach is not working too well for us either. For example, Oh07 is a good source of tolerance to MDM, and yet we find more susceptibility in some of the recoveries involving Oh07. We think we can explain this on virus strain differences. We seem to have different strains that predominate in different seasons

E.E. Rosenkranz: Have you ever tried to find differential hosts rather than differential lines or genotype within corn? You know already that it is very easy to distinguish strains A and B on the basis of infection of hosts other than maize.

R. Louie: No, I have not done that. My purpose is to see if I can find the genetics behind the inheritance of resistance in maize.

R.W. Toler: Do you have any other methods you can use to separate these strains other than symptomatology and some host differentials in corn—such as as serology?

R. Louie: There are some differences in the ability of aphid vectors to transmit each of these strains.

R.W. Toler: Are there strain-specific vectors?

R. Louie: No. There are some 13 species that can transmit MDMV but no strain specificity Dr. Gordon could probably answer the question on serology.

D.T. Gordon: The only serological difference that we know so far is between strains A and B

Spiroplasma: Role in the Diagnosis of Corn Stunt Disease

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Research on virus and "virus-like" diseases of maize has flourished in recent years. We have witnessed the discovery, in maize, of new viruses as well as of the first recognized example of a new group of prokaryotes—the spiroplasmas. New geographic distributions of known disease agents have been charted, and much more has been learned about the role of insect vectors and weed plants in the survival and distribution of these pathogens. New information has continued to modify earlier, sometimes over-simplified concepts of maize virus diseases. The laboratory has provided the major setting for much of this progress through controlled studies on maize disease agents and their effects in a systematic approach. Yet, effective practical application of this progress must take place where the pathogens are encountered—in the field. There, one must confront a complexity characteristic of naturally occurring diseases in maize. The task of disease diagnosis under these circumstances can be particularly difficult in work on the condition known as "corn stunt" (CS).

Many of the pathogens we are concerned with in this Colloquium and Workshop induce stunting as a part of the virus or "virus-like" syndrome in maize. Dr. Gordon's presentation (14) touched on some of the confusion in disease nomenclature and resulting practical problems in disease control that can arise from insufficient information on corn stunting pathogens. The broad spectrum of other symptoms often associated with stunting disorders in maize underscores the need for developing reliable aids for correct diagnosis. It is our intent now to focus on this need for one specific corn stunting disorder termed "corn stunt disease." For this discussion, we restrict the term to the disorder induced by a spiroplasma and known as the "Rio Grande" corn stunt.

SPIROPLASMAS

Following the original report in 1971 (13) of a helical, cell wall-free prokaryote in maize with Rio Grande corn stunt disease, research on this type of organism developed rapidly. The trivial term "spiroplasma"—originally proposed (8) for the motile, helical organism associated with corn stunt and for other, similar microorganisms—has been adopted (27) as the genus name for the citrus stubborn disease pathogen (*Spiroplasma citri*). A new family (Spiroplasmataceae) was recently proposed (29) to accomodate the spiroplasmas within the class Mollicutes (Table 1), and it is not unreasonable to expect spiroplasmas to be recognized eventually as a new order within the class. Certainly, the importance of morphology in determining higher taxa in the class Schizomycetes is well established, and helical morphology clearly sets spiroplasmas apart from other members of the class Mollicutes.

TABLE 1. Classification of cell wall-free prokaryotes within the class Mollicutes[a]

Class	Mollicutes
Order	Mycoplasmatales
Family I	Mycoplasmataceae
Genus I	*Mycoplasma*
Family II	Acholeplasmataceae
Genus I	*Acholeplasma*
Family III	Spiroplasmataceae
Genus I	*Spiroplasma*
Genus of uncertain affiliation	*Thermoplasma*
	Anaeroplasma

[a]L forms are cell wall-deficient variants of bacteria and therefore are not considered members of the class Mollicutes. Thus, a criterion for consideration of a microorganism as a member of this class is the absence of evidence favoring its derivation from, or reversion to, a walled bacterial form.

Several other examples of this newly recognized type of microorganism have been reported. To date, we are aware of the association of spiroplasmas with eight diseases (Table 2). Of these spiroplasmas, all but one (the sex ratio spiroplasma in *Drosophila*) have reportedly been cultivated *in vitro*. Growth in liquid is typified by presence of helical cells during log phase increase. Helical cells are also abundant in colonies on agar. Colonies produced by spiroplasmas on solid agar media (Fig. 1) are often granular and surrounded by numerous "satellite" colonies. Spiroplasma colonies frequently do not exhibit the "fried egg" shape typical of mycoplasma colonies. A causal relationship with disease has so far been described in detail in three cases; those of citrus stubborn (22, 23, 26), corn stunt (2, 32), and the

honey bee disease recently discovered by Clark (3). Characterization of cloned cultures *in vitro* has been carried out for two spiroplasmas (10, 27), and detailed characterization continues in various laboratories for these and the remaining cultivable spiroplasmas. Thus far, only the citrus stubborn disease agent has been assigned a Latin name.

TABLE 2. Diseases of proven or suspected spiroplasma etiology

Disease	Spiroplasma reported (date)	Ref(s)
"Rio Grande" corn stunt	1971	12,13
Citrus stubborn	1973	27
Sex ratio condition in *Drosophila*[a]	1974	33
Suckling mouse cataract[b]	1975	30
Honeybee (unnamed fatal disease)	1976	3
Witches' broom of cactus (*Opuntia tuna*)	1976	18
Bermuda grass (yellow leaf)	1976[c]	
Rice (unknown disease)	1976[d]	

[a]The microorganism associated with this condition was first reported in 1961. It has been considered a spirochete (25,28,31), but its resemblance (4) to spiroplasmas was subsequently demonstrated by electron microscopy and serology (33).

[b]The agent (SMCA) associated with this condition was first isolated from specimens of the rabbit tick and was propagated in embryonated chicken eggs but was thought to be a virus and later suggested to be a mycoplasma (34). In 1968, a probable strain of SMCA, also originally from rabbit tick, was cultivated *in vitro* but was reported as a spirochete (24). A role of SMCA spiroplasma in naturally occurring disease is yet unknown.

[c]T.A. Chen & H.C. Su (*personal communication*).

[d]T.A. Chen & J.C. Chiu (*personal communication*).

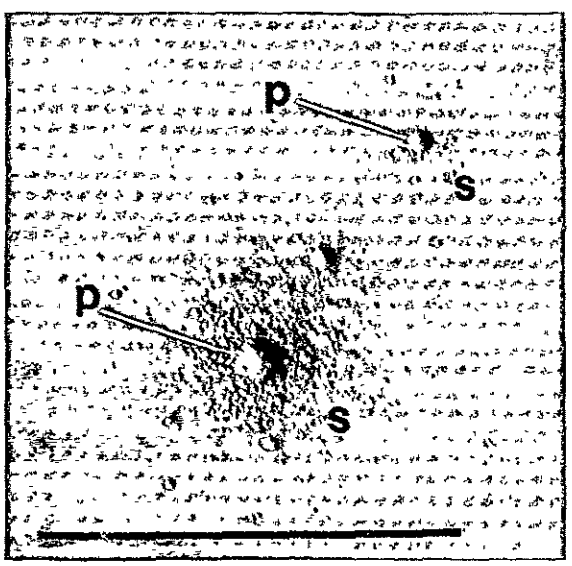

Fig. 1. Colonies produced by the honey bee spiroplasma (*Spiroplasma* sp. Strain AS 576) on solid agar (1%) medium incubated for 5 days at 33 C. Note principal colonies (P) surrounded by numerous satellite (S) colonies. Bar represents 1 mm.

In each of the cases listed in Table 2, the spiroplasmas were first recognized by their size and distinctive helical morphology. Helical morphology and characteristic motility (8, 9, 11) are constant features of all the spiroplasmas studied in detail during their active growth. In the case of corn stunt disease, these features provide the basis for an approach to disease diagnosis by optical microscopy.

DIAGNOSIS OF CORN STUNT DISEASE

Diagnosis of naturally occurring virus and "virus-like" diseases of maize must be performed amid an often confusing array of abnormalities induced by a variety of causal factors in the field. Depending upon circumstance, one may draw upon a single or several diagnostic aids. Chief among these is the study of symptoms. Symptomatology provides the first signal of disease and forms a basis for preliminary diagnosis. In practice, the symptom syndrome is often the only basis for diagnosis. However, symptoms caused by a given pathogen may vary greatly with host plant genotype, strain of pathogen, and environment. Multiple infections by several agents in an individual plant may be common in the field. Moreover, vastly different causal factors may induce superficially similar symptoms. Difficulties of diagnosing disease based on symptoms in open-pollinated varieties of maize can be particularly perplexing. Although the use of symptomatology can be highly practical for disease diagnosis in the field and provide valuable information for preliminary diagnosis, the method can be aggravatingly deceptive—as Dr. Louie has aptly demonstrated at this Colloquium (19). Nevertheless, it is often the only approach available or practical. Where possible, however, it is augmented by other diagnostic aids.

Thus, tests on mode of transmission of the suspected causal agent(s) may provide supporting evidence for preliminary diagnosis, and electron microscopy can provide a means for detecting known disease agents and lead to a discovery of new pathogens (1). However, these methods are most valuable for the important fundamental information they provide and are generally unsuitable for large-scale field diagnoses. Attempts to cultivate pathogenic spiroplasmas likewise can provide important basic information but are not suitable for large-scale application. Serological methods may eventually provide one of the most useful aids to corn stunt diagnosis, but this method has yet to be developed. In the case of corn stunt disease, direct observation of the living pathogen by optical microscopy provides a highly

useful, reliable aid to diagnosis and is the method I wish to consider in some detail.

Optical Microscopy. — The development of optical microscopy as an aid for corn stunt disease diagnosis grew out of fundamental laboratory studies on the nature of its causal agent. Previous work (16, 17, 19, 20) had demonstrated the presence of a mycoplasma-like organism in tissues of infected plants. Subsequently, this microorganism was reported (12, 13) to be a motile, helical, cell wall-free prokaryote—the first recognized example of a new taxon of pathogens, spiroplasmas (8). The constant association of the spiroplasma with corn stunt disease, both in plants and in insect vectors, was determined by phase contrast or dark field microscopy of plant juice or hemolymph and abdominal smears from leafhopper vectors. These and other data supporting the conclusion that the observed spiroplasma is the causal agent of corn stunt disease are summarized in several reports (4, 5, 6, 7, 12, 13). Detection of the spiroplasma by optical microscopy yielded highly reliable diagnoses of corn stunt in greenhouse-grown plants.

Laboratory studies thus presented a rather smooth, coherent picture of corn stunt disease; a disease with "Rio Grande strain" symptoms induced by a relatively easily detected motile, helical, cell wall-free microorganism—a spiroplasma. However, application of spiroplasma detection to field samples (diagnosed as corn stunt disease based on symptomatology) revealed a complex situation.

Method. — In the initial work on diagnosis of corn stunt disease in plants, phase contrast optical microscopy was employed. Fresh unstained specimens were observed. Tissue samples were generally removed from tassels of infected plants or from the midrib at the base of a leaf with CS disease symptoms. Juice expressed from the tissue was observed immediately at a magnification of 1000X or 1250X. The unique shape and motility, and abundance of the spiroplasmas, made them readily detectable. However, for plants free of spiroplasmas, at least 20 random microscopic fields of three separately prepared slides were searched before absence of the spiroplasmas was recorded. In later work, we followed the same procedure for sampling but turned to the use of dark field optical microscopy for observations. Currently, we utilize a dark field condenser on a microscope equipped with a 100X oil immersion objective with iris diaphragm to observe specimens at a total magnification of about 100X. In our experience, spiroplasmas are more readily detected by dark field than by phase contrast optical microscopy.

NATURALLY OCCURRING DISEASE

During the early stages of research on the corn stunt spiroplasma, it seemed possible that this new microorganism could be associated with a laboratory isolate of the causal agent but bear no relation to naturally occurring disease in the field (6). Corn plants with disease induced by other "strains" or isolates of corn stunt were therefore examined. However, absence of detectable spiroplasmas in either infected corn or Johnsongrass originating in Ohio, Kentucky, or North Carolina suggested these latter disorders might not be induced by the corn stunt spiroplasma (4, 6). The association of maize chlorotic dwarf virus (MCDV) with these disorders has now been well established (15), as has been described by Dr. Gordon at this Colloquium and Workshop. Our subsequent work on diagnosis of naturally occurring corn stunt disease has been carried out in Mexico.

In the first of this work, carried out in collaboration with Dr. Carlos DeLeon (CIMMYT, Mexico), optical microscopy was found to be highly reliable as an aid to diagnosis of the Rio Grande "strain" of corn stunt (6). The method also revealed the presence of spiroplasmas in plants of maize with symptoms of the "Mesa Centrale strain," when these were limited to plants showing an effect of disease on ear development. However, plants with other syndromes—such as reddening of leaves and stunting, with no obvious effect on ears when present—often contained no detectable spiroplasmas. Interestingly, such plants are commonly referred to as typical of infection by the "Mesa Centrale strain" of the corn stunt disease agent.

In the summer of 1975, in collaboration with Dr. DeLeon and Dr. Jorge Galindo and Sr. Benigno Quintanilla Vascope (Colegio de Postgraduados, Escuela National de Agricultura, Chapingo, Mexico), we obtained similar findings. All plants with typical "Rio Grande" strain corn stunt symptoms contained spiroplasmas; such symptoms were prevalent at low and middle elevations but were not evident at higher elevations. At middle and high elevations, however, plants with red leaves and stunting were fairly common. As in the previous work, spiroplasmas could be detected in some but not all plants with this syndrome. At elevations of about 2000 m, spiroplasmas were detected in only 2 out of about 25 plants with the red leaf-stunting syndrome, although all would normally be termed corn stunt by custom. Thus, because leaf reddening accompanied by stunting is commonly referred to as typical of "Mesa Centrale" corn stunt infection, one must ask, "What is the nature of this Mesa Centrale corn stunt disease?"

The presence of an as yet undetected or perhaps unknown disease agent may provide one

possible explanation. Moreover, mixed infection by such a hypothetical disease agent and corn stunt spiroplasma could explain the occurrence of spiroplasmas in some stunted plants with red leaves and no symptoms of the "Rio Grande" strain of corn stunt disease. This hypothesis, of course, suggests that an undetected agent is responsible for the red leaf and stunting syndrome. The discovery of MCDV revealed that this type of syndrome in maize in the U.S. may often be associated with the presence of a virus (14). There is a striking resemblance between symptoms of MCDV in certain corn varieties and the red leaf-stunting symptoms often termed typical of a type of corn stunt in Central America. This circumstance encourages investigation of the possible presence of MCDV in Mexico and Central America. The propensity of certain corn varieties to develop reddening of leaves and stunting under a variety of stresses, however, suggests that the search for a causal agent should not be limited to MCDV.

PROBLEMS AND PERSPECTIVES

To place in perspective the utility of spiroplasma detection in the diagnosis of corn stunt disease, several factors must be considered. Certainly, detection by optical microscopy is simple and relatively rapid, but the reliability of the method could be seriously affected: (a) if certain strains of the corn stunt spiroplasma produce few or no helical filaments in some corn varieties or under some environmental conditions, or (b) if an unrelated spiroplasma could reside in and form helical filaments in maize. We must acknowledge these possibilities, even though the working hypothesis for our field work specified that helical forms must be found in a plant with symptoms primarily induced by the corn stunt spiroplasma. Laboratory experience encourages confidence in this working hypothesis. However, only one isolate of the corn stunt disease agent was utilized in the laboratory under conditions optimal for disease development.

Whether strains of corn stunt spiroplasma exist which form few or no helical filaments under certain conditions is unknown. In any case, their relationship to corn stunt spiroplasma would be in doubt. Indeed, at present there would be serious question whether such hypothetical strains could properly be termed spiroplasmas, unless helical forms could be demonstrated *in vitro*. However, by electron microscopy, it has been seen that cells of corn stunt-infected plants do often contain rounded mycoplasma-like bodies from which helical filaments may arise. Some cells in a given ultrathin section may contain many such rounded bodies and no apparent helical filaments. This could present a different cause for concern.

It is possible that morphology of corn stunt spiroplasma in an infected cell is influenced by stage of disease or age of cell infection in a manner that is analogous to loss of helicity and development of rounded bodies in aged culture *in vitro*. Thus, in some cases, there might be very few helical forms late in the season in a given plant with advanced spiroplasma infection, rendering corn stunt diagnosis difficult or impossible. Nevertheless, laboratory experience suggests that helical forms may readily be found even after tassel formation. Helical spiroplasmas are generally abundant in the tassels of infected plants. Our experience with field samples of plants with Rio Grande corn stunt disease generally confirms this finding. It must be emphasized, however, that certain plants infected by corn stunt spiroplasma could also possibly contain a nonhelical mycoplasma. The identity, in possibly double infected plants, of rounded mycoplasma-like bodies without attached helical filaments is therefore unresolved.

Finally, the possibility that spiroplasmas unrelated to corn stunt spiroplasma could reside in and form helical filaments in maize is a serious consideration. To date, no information suggests that maize could serve as a congenial host for such spiroplasmas. Yet, this possibility should be kept in mind, while in practice spiroplasmas found in maize are presumed to represent the corn stunt pathogen itself.

DETECTION AND DIAGNOSIS

The considerations that must be made in evaluating optical microscopy as an aid to corn stunt disease diagnosis reemphasize an observation made earlier (6). Detection of spiroplasma in corn plants indicates only the presence of the corn stunt disease agent. Our field experience thus suggests that spiroplasmas may be found in plants displaying symptoms primarily due to factors other than corn stunt infection. This feature underscores a well-appreciated distinction between detection of a pathogen and disease diagnosis. Detection of a pathogen is a physical finding; diagnosis is interpretation.

CONCLUSION

Development of new diagnostic aids contributes significantly to progress in understanding corn stunting disorders. Detection of spiroplasmas by optical microscopy can be one important aid to diagnosis. However, it is the combined use of all feasible diagnostic aids that permits the difficult judgment in final diagnosis of naturally occurring disease.

LITERATURE CITED

1. BRADFUTE, O.E. and D.C. ROBERTSON. 1977. Electron microscopy as a means for discovery of new maize viruses and virus-like pathogens. Pages 103-107 in Proc. Maize Virus Dis. Colloq. and Workshop, 16-19 Aug., 1976 Ohio Agric. Res. and Dev. Center, Wooster. 145 p.
2. CHEN, T.A., and C. LIAO. 1975. Corn stunt spiroplasma: Isolation, cultivation, and proof of pathogenicity. Science 188:1015-1016.
3. CLARK, T.B. 1976. Spiroplasma sp., a new pathogen in honey bees. J. Invert. Pathol. 66. (In press).
4. DAVIS, R.E. 1974. New approaches to diagnosis and control of plant yellows diseases. Pages 289-302 in The Third International Symposium on Virus Diseases of Ornamental Plants, 11-15 Sept. 1972. College Park, Md. No. 36.
5. DAVIS, R.E. 1974. Sprioplasma in corn stunt-infected individuals of the vector leafhopper Dalbulus maidis. Plant Dis. Rep. 58:1109-1112.
6. DAVIS, R E. 1973. Occurrence of a spiroplasma in corn stunt-infected plants in Mexico. Plant Dis. Rep. 57:333-337.
7. DAVIS, R.E., R.F. WHITCOMB, T.A. CHEN, and R.R. GRANADOS. 1972. Current status of the etiology of corn stunt disease. Pages 205-225 in Pathogenic Mycoplasmas (CIBA Foundation Symposium, J. Birch, ed.). Elsevier and North-Holland, Amsterdam.
8. DAVIS, R.E., and J.F. WORLEY. 1973. Spiroplasma: Motile, helical microorganism associated with corn stunt disease. Phytopathology 63:403-408.
9. DAVIS, R.E., and J.F. WORLEY. 1972. Motility of helical filaments produced by a mycoplasma-like organism associated with corn stunt disease Phytopathology 62:752-753. (Abstr.).
10. DAVIS, R.E., J.F. WORLEY, T.B. CLARK, and M. MOSELEY. 1976. New spiroplasma in diseased honey bee (Apis mellifera L.): Isolation, pure culture, and partial characterization in vitro. Proc. Amer. Phytopathol. Soc. 3. (Abstr.) (In press).
11. DAVIS, R.E., J.F. WORLEY, and M. MOSELEY. 1975. Spiroplasmas. Primary isolation and cultivation in cystine-tryptone media, and translational locomotion in semi-solid versions. Proc. Amer. Phytopathol. Soc. 2:153. (Abstr.).
12. DAVIS, R.E., J.F. WORLEY, R.F. WHITCOMB, T. ISHIJIMA, and R.L. STEERE. 1972. Helical filaments produced by a mycoplasma-like organism associated with corn stunt disease. Science 176:521-523.
13. DAVIS, R.E., J.F. WORLEY, R.F. WHITCOMB, T. ISHIJIMA, and R.L. STEERE. 1972. Helical filaments associated with a mycoplasma-like organism in corn stunt-infected plants. Phytopathology 62:494. (Abstr.).
14. GORDON, D.T. 1976. Maize virus diseases in the United States. Pages 45-51 in Proc. Maize Virus Dis. Colloq. and Workshop, 16-19 Aug., 1976. Ohio Agric. Res. and Dev Center, Wooster. 145 p.
15. GORDON, D.T., and L.R. NAULT. 1977. Involvement of maize chlorotic dwarf virus and other agents in stunting diseases of Zea mays in the United States. Phytopathology 67:27:36.
16. GRANADOS, R.R. 1969. Electron microscopy of plants and insect vectors infected with corn stunt disease agent. Contrib. Boyce Thomp. Inst. 24:173-187.
17. GRANADOS, R.R., K. MARAMOROSCH, and E. SHIKATA. 1968. Mycoplasma: Suspected etiologic agent of corn stunt. Proc. Nat. Acad. Sci. U.S.A. 60:841-844.
18. KONDO, F., A.H. McINTOSH, S.B. PADHI, and K. MARAMOROSCH. 1976. Electron microscopy of a new plant-pathogenic spiroplasma isolated from Opuntia. Pages 56-57 in Proc. 34th Ann. Meet., Electron Microscopy Soc. Amer., G.W. Bailey, ed., Claitor's Pub. Div., Baton Rouge. 659 p.
19. LOUIE, R. 1976. Maize virus disease identification by symptomatology. Pages 89-91 in Proc. Maize Virus Dis. Colloq. and Workshop, 16-19 Aug., 1976. Ohio Agric. Res. and Dev. Center, Wooster. 145 p.
20. MARAMOROSCH, K., R.R. GRANADOS, and H. HIRUMI. 1970. Mycoplasma diseases of plants and insects. Advan. Virus Res. 16:135-193.
21. MARAMOROSCH, K., E. SHIKATA, and R.R. GRANADOS. 1968. Structures resembling mycoplasma in diseased plants and in insect vectors. Trans. N.Y. Acad. Sci. 30:841-855.
22. MARKHAM, P.G. and R. TOWNSEND. 1974. Transmission of Spiroplasma citri to plants. Coll. Inst. Nat. Sante. Resch. Med. 33:201-206.
23. MARKHAM, P.G., R. TOWNSEND, M. BAR-JOSEPH, M.J. DANIELS, A PLASKITT, and B.M. MEDDINGS. 1974. Spiroplasmas are the causal agents of citrus little-leaf disease. Ann. Appl. Biol. 78:49-57.
24. PICKENS, E.G., R.K. GERLOFF, and W. BURGDORFER. 1968. Spirochete from the rabbit tick, Haemaphysalis leporispalustris (Packard) J Bacteriol. 95:291-299.
25. POULSON, D.F., and B. SAKAGUCHI. 1961. Nature of "sex ratio" agent in Drosophila. Science 133:1489-1490.
26. RANA, G.L., G.H KALOOSTIAN, G.N. OLDFIELD, A.L. GRANETT, E.C. CALAVAN, H.D. PIERCE, I.M. LEE, and D.J. GUMPF. 1975. Acquisition of Spiroplasma citri through membranes by homopterous insects. Phytopathology 65:1143-1145.
27. SAGLIO, P., M. LHOSPITAL, D LAFLECHE, G. DUPONT, J.M. BOVE, J.G. TULLY, and E.A. FREUNDT. 1973. Sprioplasma citri gen. and sp n.: A mycoplasma-like organism associated with "stubborn" disease of citrus. Int. J. Syst. Bacteriol. 23:191-204.
28. SAKAGUCHI, B., and S. KOBAYASHI. 1965. Morphological observations of "sex ratio" agents of D. willistoni and D. nebulosa. Drosophila Inform. Ser. 40:54.
29. SKRIPAL, I.G. 1974. On improvement of taxonomy of the class Mollicutes and establishment in the order Mycoplasmatales of the new family Spiroplasmataceae fam. nova. Mikrobiol. Zh. Akad. Nauk. Ukr.SSR 36:462-467.
30. TULLY, J.G., R.F. WHITCOMB, D.L. WILLIAMSON, and H.F. CLARK. 1976. Suckling mouse cataract agent is a helical, wall-free prokaryote (Spiroplasma) pathogenic for vertebrates Nature 259:117-120.
31. WILLIAMSON, D.L., 1974. Unusual fibrils from the spirochete-like sex ratio organism. J. Bacteriol. 117:904-906.
32. WILLIAMSON, D.L., and R.F. WHITCOMB. 1975. Plant mycoplasmas: A cultivable spiroplasma causes corn stunt disease. Science 188:1018-1020.
33. WILLIAMSON, D.L., and R.F. WHITCOMB. 1974. Helical, wall-free prokaryotes in Drosophila, leafhoppers, and plants. I.N.S.E.R.M. 33:283-290.
34. ZEIGEL, R.F., and H.F. CLARK. 1974. Electron microscopy of the suckling mouse cataract agent: A noncultivable animal pathogen possibly related to mycoplasma. Infect. and Immun. 9.440-443.

DISCUSSION OF R.E. DAVIS PAPER

A.J. Ullstrup: What is the diameter of spiroplasma colonies?

R.E. Davis: Diameter of a colony produced by the honeybee spiroplasma growing on the surface of 1% agar can reach 1 cm. That is large for a spiroplasma. Colonies produced by *Spiroplasma citri* may reach about 2 mm in diam.

A.J. Ullstrup: How long do organisms remain alive in a colony?

R.E. Davis: It depends somewhat on the medium and the organism. A colony produced by *Spiroplasma citri*, for example, might contain viable organisms up to 3 weeks, on some media.

A.J. Ullstrup: When you sent a culture of the El Salvador corn stunt spiroplasma (derived from a sample provided by Dr. A. Diaz) to Dr. Nault for bioassay, inoculated plants did not develop the red leaf symptom. The plants developed "Rio Grande" symptoms Were the corn genotypes in Mexico the same as those used here and in El Salvador? Did you have the same environment? Does the red leaf symptom occur in Mexico?

R.E. Davis: I am not certain of genotypes of plants with red leaf symptoms in Mexico or El Salvador In Mexico, for example, samples were taken from fields of open-pollinated varieties. The red leaf stunting symptom does occur in Mexico, it is generally referred to as corn stunt, or, simply, stunt.

A.J. Ullstrup: Perhaps this sample from El Salvador was from a higher altitude where it might have been cooler.

R.E. Davis: It is possible that high elevation and cooler temperatures enhance development of red leaf symptoms in Rio Grande strain-diseased plants. However, the sample from El Salvador was collected at a low elevation, where broad chlorotic streaks and severe stunting are supposed to be characteristic of the Rio Grande strain.

A.J. Ullstrup: Is it conceivable that the environment during the bioassay might have been quite different from that in El Salvador?

R.E. Davis: That is quite possible; plants inoculated by Dr. Nault were maintained in a greenhouse at about 30 C.

I.W. Deep: It is interesting to note that the red leaf symptom resembles those symptoms mentioned by Dr. Josephson as well as symptoms in the first slide that Dr. Ray Louie showed and the picture of Dr. Lansing Williams holding up the plants from southern Ohio. These plants were red. This red is everywhere but plants do not develop this symptom when inoculated with maize dwarf mosaic virus or with maize chlorotic dwarf virus.

R. I. Brawn: The genotype of the corn plant determines, in the absence of virus diseases, whether or not it can manufacture anthocyanin. Is the presence of anthocyanin following virus infection related to the known genotype of the corn plant? For example, most sweet corns today are r^g. This gene blocks the production of much of the anthocyanin. If you are transferring a virus from a high anthocyanin producing plant to a low one, is the red symptom always related? Dr. Ullstrup, do you know?

A.J. Ullstrup: No, all that I know is that WF9 and all its relatives tend to produce this red pigment very frequently on injury, on maturation, and under cool nights.

R.I. Brawn: There are some sweet corns that are recessive at the A-locus of (ABP1 for plant color) and they have an absolutely total block on anthocyanin production.

L.R. Nault: Maybe I can clarify this further. The pure culture of corn stunt spiroplasma from El Salvador, that I received from Bob Davis, induced symptoms in Aristogold Evergreen Bantam Sweet Corn which were indistinguishable from the Rio Grande corn stunt. Some slight reddening was produced, but mostly I observed a strong chlorotic striping. This symptom is what we use to diagnose the Rio Grande corn stunt. This is in contrast to the maize bushy stunt mycoplasma that produces strong reddening in this variety.

W.R. Findley: Certain lines of corn will not turn red but others redden each year. The slide that Dr. Louie showed was, I believe, OH43 that yellows when infected with MDMV and reddens with MCDV I cannot make a valid statement as to why this happens.

L.R. Nault: OH43 also will not redden when inoculated with the maize bushy stunt mycoplasma. On the other hand, Aristogold becomes extremely red. In the greenhouse, OH43 will not produce anthocyanin, no matter what it is inoculated with.

T. Sutabutra: Will the spiroplasma infect other cereals?

R.E. Davis: No one has yet been able to transmit the corn stunt spiroplasma to plant hosts other than corn, teosinte, and *Zea perennis*.

R. Gamez: I would like to comment on corn stunt-infected samples from El Salvador. Both the red type and yellow (Rio Grande) types co-exist in fields. I am certain that I almost know precisely where Dr Diaz collected this material for Dr. Davis and there one can see both types — plants with reddening next to plants with yellow stripes. Both are referred to as stunt.

R.E. Davis: Precisely! This is really one of the points I was trying to make. One must raise the question, Is this second (red type) really a type of stunt? Certainly, it is possible that it is It is possible that it is induced by a spiroplasma that we could not culture. It is also possible that the plant was doubly infected and that we isolated and cultured only one. The red symptom might have been caused by the other pathogen(s) in the infection. What might be the nature of this hypothetical other pathogen? We are posing some provocative questions that need further study.

R. Gamez: There is another point that is also true It is possible to transmit both red and yellow types of corn stunt with *Dalbulus maidis*, both in Costa Rica and in El Salvador.

R.E. Davis: It is also possible that the insect vector does not always screen out components of a mixed infection. And, it is also possible that sometimes insects do not transmit a pure culture.

R.E. Gingery: I want to ask a fundamental question about the spiroplasma. Do you have any notions about how it maintains its helicity?

R.E. Davis: We have searched for some kind of internal ultrastructure that might account for helicity. Also, there must be some kind of structure to account for motility. We do see a kind of fibrillar structure in the spiroplasma (J.F. Worley, *unpublished*), but it is difficult to find reliably. I think it is a question of developing proper fixation procedures for this material.

J.K. Knoke: Dr. Davis, you mentioned that as the culture begins to die out, it forms round bodies. Is it possible to rejuvenate those round bodies in any way and get back to the spiral form?

R.E. Davis: I cannot give you a definite answer to that question. Round bodies form in the aging culture and accumulate during the death phase. Whether or not these are viable when first formed, we do not yet know. There is perhaps one other time that cultures under some circumstances can form round bodies. That is during the very early stage of culture development or in the initiation of the culture We are not certain whether these round bodies have a role in the growth of the spiroplasma. This has been noted by Dr. Calavan (Riverside, California) and colleagues.

G. Martinez-Lopez: I would like to ask Dr. Gamez about observations made in Costa Rica. Yesterday, I talked about the presence of corn stunt in Colombia. There, we just have the reddening of the leaves and the shortening of internodes, but we do not have the Mesa Central or the Rio

Grande types of symptoms. The agent(s) inducing this reddening of the leaves and the shortening of the nodes is transmitted by *Dalbulus*. What I have done, in a certain sense to keep things simple, is to define corn stunt as a specific disease caused by a spiroplasma. Now, I raise the question, are there other strains of this spiroplasma that could induce different symptoms like leaf reddening and which I would also consider genuine corn stunt? To call a number of diseases corn stunt is a little confusing and, perhaps, this has happened here in the U.S. and elsewhere. I wonder, at least from the very limited experience I had in Mexico, if some of the diseases that are referred to there as stunt, especially these stunted reddened plants, are really corn stunt? Are they strains of the spiroplasma, if you will allow me to limit the definition of corn stunt to strains of this organism? It is a possibility that is being looked into. Transmission by *Dalbulus* certainly does not prove that it is the pathogen of corn stunt, for this vector may be capable of transmitting other agents also. One example may be a different virus, or a different type of agent.

R. Gamez: We have shown that *Dalbulus maidis* can transmit, simultaneously, the spiroplasma and the rayado fino virus.

R.W. Toler: In the corn stunt disease, what happens to the concentration of spiroplasma after inoculation?

R.E. Davis: I have to admit that we have not done extensive studies on this question. One can detect spiroplasma a few days before symptoms appear. Concentrations become greatest at the basal portions of a given leaf with symptoms. Youngest leaves and the tassel contain high concentrations. However, in mature, drying plants, we seem to find fewer helical organisms. The concentration thus may decline, but we have never sampled a live Rio Grande corn stunt-infected plant that was so old we could not find some motile, helical bodies

R.W. Toler: So you can detect spiroplasma bodies in the plant right up to maturity, as long as it shows symptoms.

R.E. Davis: We can still find the spiroplasma at least through late tasseling stage. This is true not only for greenhouse-grown plants, but also for plants from the field where they may be under greater stress from various environmental conditions. All our diagnoses of naturally infected field-grown plants in Mexico were done late in the season when the plants were tasseling.

R.W. Toler: Can you detect a difference in concentration of the spiroplasma in resistant versus susceptible plants?

R.E. Davis: I do not know.

V.D. Damsteegt: Where in the plant do you find spiroplasmas?

R.E. Davis: To our knowledge, the spiroplasmas are present only in phloem tissue. In a given corn plant with symptoms, the highest concentration is found in the youngest leaves, usually at the base, which is also the youngest part. In very young plants that are not yet showing symptoms, we have found spiroplasmas first in the roots. Even several weeks after symptoms begin to appear, roots contain numerous spiroplasmas. As I have said, we also find high concentrations in the tassel. In fact, the highest counts of spiroplasmas we have ever observed by light microscopy were obtained in samples from infected tassels. These observations refer to greenhouse-grown material, and I presume that the relationship is similar in the field. In juice from tassels, we have observed up to 500 spiroplasmas in a single microscopic field.

A.J. Ullstrup: Did you look at pollen?

R.E. Davis: No.

S.P. Raychaudhuri: Do you think that these spiral forms and round bodies could be two different stages of the same pathogen? Can you work out the life cycle?

R.E. Davis: Well, we know that the round bodies can be a part of the pathogen because we have seen them attached to it Whether or not they have a role in growth or multiplication of the spiroplasma, either in the infected cell or in *vitro*, we do not know. As a matter of fact, we do not yet know how these organisms multiply. In a rapidly growing log phase culture we see the helical form It is in late log phase that we begin to see round bodies, so the spiroplasma appears to be multiplying without a large pool of these round bodies.

Routine Serological Assays for Diagnosis of Maize Virus Diseases

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This research was financially supported in part by Grant No. 316-15-55 and Cooperative Agreement No. 12-15-75-2-14 from Cooperative State Research Service, U. S. Department of Agriculture.

Approved for publication as Journal Article No. 179-76 of the Ohio Agricultural Research and Development Center, Wooster.

ABSTRACT

A micro-immune agar-gel double diffusion assay has been adapted for a combined assay of maize chlorotic dwarf and maize dwarf mosaic viruses. The assay is simple, relatively inexpensive, and should enable most workers to test their maize materials for these viruses. Serological methods for diagnosis of maize virus diseases have been especially valuable for obligately vectored viruses. They have permitted establishment of the incidence and distribution of maize chlorotic dwarf virus in the USA.

Louie (6) illustrated the limitations and advantages of disease diagnoses by symptomatology. He indicated that an association could be demonstrated between virus infection and presence of certain symptoms by employment of suitable assay methods. This paper deals with serological assays that demonstrate such associations and that permit rapid assay of maize chlorotic dwarf virus (MCDV) and maize dwarf mosaic virus (MDMV), the most commonly encountered maize viruses in the USA (5). Limitations of these methods also are discussed.

In the U.S. maize virus survey (4), the choice of an appropriate assay for each of the known U.S. maize viruses was a primary consideration. Maize dwarf mosaic virus and the minor-occurring U.S. maize viruses (sugarcane mosaic virus, wheat streak mosaic virus, brome mosaic virus, and cucumber mosaic virus) are mechanically transmissible and readily identified by infectivity and symptomatology on indicator hosts. Maize chlorotic dwarf virus, in contrast, is transmitted only by leafhopper vectors (9). An infectivity assay involving leafhopper transmission of MCDV is suitable for a small number of samples but not for the relatively large number involved in the survey. The assay chosen for MCDV was the immune density-gradient centrifugation assay. This assay, originally developed by Ball and Brakke (3), will be referred to as the immune centrifugation assay.

MATERIALS AND METHODS

The immune centrifugation assay of MCDV involved partial purification and concentration of virus from diseased plants (5). Materials were extracted from 6 g samples of leaf tissue. The extract was clarified with chloroform and the virus concentrated by either high speed centrifugation or precipitation with polyethylene glycol. Sedimented material was resuspended with buffer at 1/10 to 1/25 volume relative to tissue fresh weight. The suspension then was divided into equal portions. One portion was mixed with anti-MCDV serum and layered on a sucrose density gradient, the other was layered on a second gradient. Both were centrifuged with four additional gradients containing antiserum-treated and untreated suspensions from two additional samples. Thus, each centrifugation allowed assay of three samples. After high speed centrifugation, gradients were scanned and virus bands detected by ultraviolet absorption.

RESULTS AND DISCUSSION

Maize chlorotic dwarf virus was identified when a virus band was present at the appropriate location (7) in the centrifuged gradient containing the untreated suspension and absent in the gradient containing the treated suspension (5). When MDMV was similarly treated with anti-MCDV serum, no effect on the MDMV band was observed. This observation with MDMV illustrates the specificity of the immune centrifugation assay.

Principal virtues of the immune centrifugation assay were that it incorporated two independent means of MCDV identification and conserved antiserum. The two means of identification were banding position of the virus in the centrifuged gradient (7) and reaction with the antiserum. In the assay for MCDV, 0.21 ml of antiserum diluted 1:200 was routinely used per sample. Thus, each assay consumed 0.00105 ml of undiluted antiserum.

In the survey, assays frequently involved plants showing plant stunting and red or yellow discoloration of leaf blades. These symptoms had been previously attributed to corn stunt and were thought to be incited by a spiroplasma or mycoplasma-like body (MLB) (5). Other plants without symptoms or with symptoms of other virus diseases were selected as controls for an analysis of the association between MCDV infection and the above symptoms. Of 575 plants assayed, 290 had corn stunt-like symptoms. Of these, 91% were infected with MCDV, as determined by the immune centrifugation assay (5). For the 575 samples, MCDV infection was significantly associated with chlorotic striping along tertiary veins, leaf reddening, leaf yellowing, and stunting of plants (5). In comparison, MDMV infection of these samples, as assayed by infectivity and symptomatology on indicator hosts, was significantly associated only with mosaic symptoms. Thus, immune centrifugation assay results indicated that maize chlorotic dwarf and not corn stunt is the principal stunting disease of maize in the USA. In support of this conclusion, *Dalbulus maidis* (DeLong & Wolcott)-transmissible corn stunt spiroplasma or MLB was found in only a few plants with corn stunt-like symptoms (5).

One disadvantage of the immune centrifugation assay was that only 12 samples could be assayed/person/day. Also, the assay was rather expensive since an ultracentrifuge, rotor, and analytical equipment were required. Furthermore, a highly trained person was required.

So that MCDV could be more easily assayed, a simplified test was developed for researchers who lack the equipment and trained personnel to perform the immune centrifugation assay. Research on this objective was conducted by Dr. J. A. Foster while he was a post-doctoral associate in the maize virus laboratory at the Ohio Agricultural Research and Development Center. Foster (*personal communication*) adapted a micro-immune agar-gel double-diffusion assay that had been developed to assay potato virus X (8) and barley yellow dwarf virus (1). The micro-immune diffusion assay was similar to the Ouchterlony double diffusion assay (2), except that antiserum and antigen wells were considerably smaller. The wells in the micro-immune diffusion assay were cut with a 20-gauge hypodermic needle (1.0 mm inner diam). The Ouchterlongy wells were cut with no. 1 (4.0 mm diam) to no. 4 (8.5 mm diam) cork borers. The reduction in antiserum and antigen volumes was 16- to 64-fold. Approximately 6.25×10^{-5} ml of undiluted antiserum per sample was required for the micro-immune diffusion assay or 16.8-fold less antiserum than required for the immune centrifugation assay. Foster (*personal communication*) enhanced visualization of the precipitin bands by staining the agar with acriflavin.

Reliability of the micro-immune diffusion assay was determined by a comparison with the immune centrifugation assay (D.T. Gordon, *unpublished*). Concentrated, partially purified preparations from field-collected maize samples were used. Preparations were concentrated 50-fold based on tissue fresh weight for the micro-immune diffusion assay and 12-fold for the immune centrifugation assay. For the amount of tissue extracted, these were maximal concentrations that provided enough volume for the respective assays. Sensitivity of the two assays for detecting MCDV was comparable (D.T. Gordon, *unpublished*). Thus, the simpler, less expensive, and higher capacity micro-immune diffusion assay may be used as an alternate method for MCDV assay.

Since the maize virus survey had revealed that MDMV, as well as MCDV, was prevalent in the USA (5), an assay for MDMV was incorporated into the micro-immune diffusion assay for MCDV (D.T. Gordon and L. Negi, *unpublished*). In this combined MCDV-MDMV assay, preparations of MCDV and MDMV were concentrated and partially purified by the same procedure and treated with a pyrrolidine concentration that degraded MDMV, but apparently not MCDV, into its protein subunits. The pyrrolidine-treated antigen preparations were placed in the peripheral wells and a mixture of MCDV and MDMV antisera in the central well. The faster-diffusing protein subunits of MDMV reacted with the MDMV antibodies to form a straight precipitin line about midway between

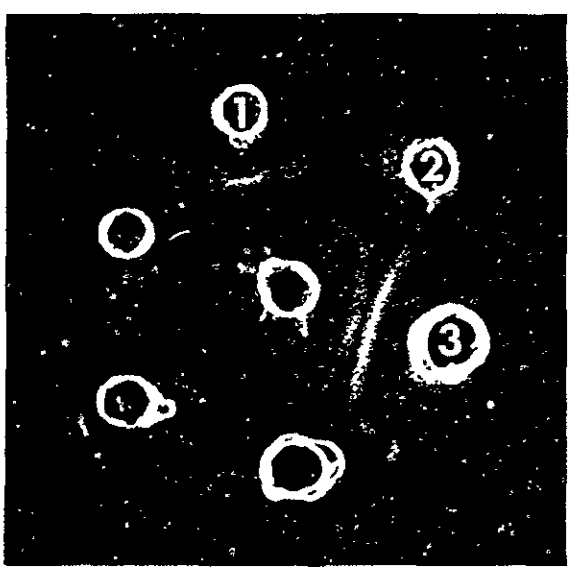

Fig. 1. Micro-immune agar-gel double diffusion test showing reactions between MCDV or pyrrolidine degraded MDMV and MCDV- and MDMV-antisera. Outer wells contained 1) MCDV, 2) pyrrolidine degraded MDMV, and 3) MCDV plus pyrrolidine degraded MDMV. The central well contained a mixture of MCDV and MDMV antisera. The remaining outer wells were empty. Photographic enlargement was 4.9 X.

the antigen and antisera wells, whereas the slower diffusing MCDV formed an arc near the antigen well (Fig. 1). Precipitin bands were sufficiently separated to avoid confusion.

The current micro-immune diffusion assay for maize viruses has two main drawbacks. First, the assay requires concentration and partial purification of the viruses. Secondly, about 24 hours elapse before reactions develop sufficiently for reliable readings.

LITERATURE CITED

1. AAPOLA, A.I.E., and W.F. ROCHOW. 1971. Relationships among three isolates of barley yellow dwarf virus. Virology 46:127-141.
2. BALL, E.M. 1974. Serological tests for the identification of plant viruses. The Am. Phytopathol. Soc., St. Paul, Minn. 31 p.
3. BALL. E.M., and M.K. BRAKKE. 1969. Analysis of antigen-antibody reactions of two plant viruses by density-gradient centrifugation and electron microscopy. Virology 39:746-758.
4. GORDON, D.T. 1977. Maize virus diseases in the United States. Pages 45-51 *in* Proc. Maize Virus Dis. Colloq. Workshop, 16-19 Aug., 1976. Ohio Agric. Res. and Dev. Center, Wooster. 145 p.
5. GORDON, D.T., and L.R. NAULT. 1977. Involvement of maize chlorotic dwarf virus and other agents in stunting diseases of Zea mays in the United States. Phytopathology 67:27-36.
6. LOUIE, R. 1977. Maize virus disease identification by symptomatology. Pages 89-91 *in* Proc. Maize Virus Dis. Colloq. Workshop, 16-19 Aug., 1976. Ohio Agric. Res. and Dev. Center, Wooster. 145 p.
7. LOUIE, R., J.K. KNOKE, and D.T. GORDON. 1974. Epiphytotics of maize dwarf mosaic and maize chlorotic dwarf diseases in Ohio. Phytopathology 64:1455-1459.
8. MC CRUM, R.C., J.C. STUDENROTH, and D. OLSZEWSKA. 1971. Microslide Ouchterlony technique for serological detection of potato virus X. Phytopathology 61:290-292.
9. NAULT, L.R., W.E. STYER, J.K. KNOKE, and H.N. PITRE. 1973. Semipersistent transmission of leafhopper-borne maize chlorotic dwarf virus. J. Econ. Entomol. 66:1271-1273.

DISCUSSION OF D.T. GORDON PAPER

V.D. Damsteegt: How long does it take to learn the serological technique that you have described so that one can feel confident about the results he obtains? Also, how do you judge the accuracy of your results?

D.T. Gordon: We have had people in our lab who no matter how long or hard they have tried could never perform the test properly. But for those people who have a knack for doing these assays, we have had no difficulty in teaching them the technique within a matter of several hours. As far as the accuracy of the serological test for MCDV is concerned, we assay a preparation by rate-zonal centrifugation as a check on the serological test. With MDMV, we do infectivity assays, so that we identify the virus by host response and compare these results with those of the serological tests. We are presently evaluating these data to see how the serological assay results compare with those of the other two. My impression is, from the results that we have obtained so far, that we do not have problems with spurious reactions. However, the sensitivity of the serological assay is below that of infectivity and centrifugation assays. We need to improve the sensitivity of the former.

T. Sutabutra: Can you use the gel-diffusion technique to detect or identify the strains of MDMV?

D.T. Gordon: No. This is one thing that we are interested in doing. We can differentiate the A and B strains in the microprecipitin test with cross absorbed antisera, but we have not attempted to do this in the immunodiffusion system.

T. Sutabutra: I would expect that with degraded virus you might be able to detect differences with the immune diffusion assay.

D.T. Gordon: Yes, I think that you are right. I expect that we will be able to show differences in an intragel absorption test and separate specific antibodies to the A or B strains.

T. Sutabutra: Have you used sodium dodecyl sulfate to degrade the virus in the immunodiffusion assay?

D.T. Gordon: We have, and we have had problems. With the salt concentrations we use, we get precipitates in the agar. These precipitates make it almost impossible to see virus-antibody precipitin bands. To avoid this problem, we have tried a number of other substances for degrading the virus. Among these, pyrrolidine is the best material. However, even with this material there are some problems. We have found that results are concentration dependent. Also, results depend on the age of the pyrrolidine. Pyrrolidine stock solutions should not be discolored. Also, we normally prepare our pyrrolidine dilution the same day or the day before we run tests. Once we learned the importance of these factors and handled them properly, assays have gone smoothly.

T. Sutabutra: Will this work for bullet-shaped or bacilliform viruses?

D.T. Gordon: We have not assayed for a bacilliform virus by serological methods, so I do not know.

R.E. Davis: Your comments on the desirability for a micro method that would be relatively rapid reminded me of a certain serological test that was developed in The Netherlands for the detection of virus infections in certain bulbs.

D.T. Gordon: Is this similar to the radial diffusion test, where you have the antiserum in the agar?

R.E. Davis: Yes. The single immunodiffusion drop test was derived from the radial immunodiffusion test. The single immunodiffusion test is a micro method that requires much less antiserum.

R. Gamez: Have you tried the use of crude extracts, without disrupting the virus, with a filamentous virus like MDMV? If you use a lower agar concentration you get some diffusion.

D.T. Gordon: We have had problems with low agar concentrations. We have used agarose, epiagar, and Ion agar No. 2, and have found that we cannot reduce the agar concentration sufficiently to allow diffusion of a long filamentous particle, such as MDMV has, and still have a firm agar matrix.

R. Gamez: This has been used with a PVY-type virus. The Florida group developed or adapted this technique. I have used PVY without any treatment of the crude extracts, and I got very good reactions. Another technique is the rheophoresis assay. This is a technique used for human viruses, but also works very well for plant viruses. You get a reaction in just a matter of a few hours.

D.T. Gordon: We have attempted other types of tests like bentonite flocculation, latex, etc. These are very rapid tests. But we have always found them to be somewhat unreliable, particularly with preparations from samples collected in the field However, we have not used the rheophoresis method. I am not familiar with it. What we have attempted is to develop an assay that is relatively simple to use and that people with little or no experience with serological testing can use in their laboratories. We have not attempted to develop a technique exclusively for plant virologists So our purposes are different from what one ordinarily expects. This dictates, to a certain extent, the type of assay that we are interested in.

T. Sutabutra: You have mentioned that in testing you have to increase virus concentration. Can you, for example, treat the extract with pyrrolidine and eliminate the concentration step?

D.T. Gordon: We have not performed assays in that way. This, we plan to test. Since we must concentrate MCDV, MDMV is concentrated as well We plan to perform the assay without concentration.

T. Sutabutra: How can you do it, straight from the crude extract?

D.T. Gordon: We have found that the concentrations of these maize viruses vary quite widely and that for some infected field-collected samples the concentration is adequate for detection without concentration but for others it is not. We have taken the conservative approach and concentrate to maximize the sensitivity and, thus, the reliability of the assay. Using extract material in the serological assay will be feasible when we improve sensitivity of the assay. In this regard, Dr R.E. Gingery is developing an immunofluorescent test for MCDV that appears as sensitive as the methods I have described and also does not require concentration of the virus to achieve this sensitivity.

Electron Microscopy as a Means for Discovery of New Maize Viruses and Virus-like Pathogens

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Research presented in this paper is the result of cooperation with many investigators, including members of the S-70 Technical Committee, the hybrid corn seed industry, and the Maize Virus Disease Research Group at Wooster.

We thank R. Scott Poethig for assistance with the light microscope techniques and R. W. Toler, Texas A&M University, for cooperation in joint surveys and sample collections.

Financial support from the U. S. Department of Agriculture, Cooperative State Research Service, Grant No. 316-15-55 and Cooperative Agreement No. 13-15-75-2-14, is gratefully acknowledged.

Approved for publication as Journal Article No. 205-76 of the Ohio Agricultural Research and Development Center, Wooster.

METHODS OF DETECTION AND CHARACTERIZATION

Transmission electron microscopy (EM) has been used in the maize virus disease research program at Wooster. Procedures currently employed are in common use in plant virus research and have been described and reviewed by others (19, 22). We shall briefly comment on application of these procedures to studies of maize viruses and agents or presumptive agents causing virus-like diseases in maize.

Negative staining. — Negative staining of plant sap taken directly from infected leaves allows detection of virus particles and provides information on their dimensions and morphology. Results of this technique are most convincing when many particles of uniform size and shape are visualized in the same electron micrograph. Often, maize viruses occur in such low frequency in this method of preparation that one must search extensively to include more than one virus particle in an electron micrograph. We have used this technique to detect flexuous rod, isometric, and rhabdovirus particles, as well as combinations of these different particles from the same maize plant. Standardization of specimen preparation and magnification calibration are necessary for critical comparison of virus dimensions.

Negative staining of purified or partially purified virus preparations provides confirmation of rate zonal centrifugation assays and a means of monitoring purification procedures. Virus particle frequency in these preparations can be made adequate (10^9 particles/ml), but particles may be broken, aggregated, or degraded. Non-volatile buffer salts interfere with the negative stain but removal of these buffer salts may cause additional particle degradation. Aldehyde fixation, prior to negative staining, may prevent degradation but may also cause particle alteration. More than one kind of virus particle is sometimes found by this method in centrifuged preparations which were presumed to be pure.

High-resolution electron microscopy of negatively stained virus preparations, usually purified, may reveal the organization of virus particle morphological subunits. Use of thin support films for specimen preparation and careful alignment and compensation of the electron microscope are necessary for high resolution. In addition, image analysis and model building may be necessary to determine virus particle substructure from electron micrographs (14).

Tissue sections. — Thin sections of fixed and embedded infected tissue from experimentally inoculated plants have been used to characterize known pathogens and pathogen-induced changes of ultrastructure in maize leaf cells. A classic ultrastructural study of viruses and their form, distribution,and pathologic effects in plant hosts is provided by Esau (13). Virus-induced inclusions and cytopathic effects may be characteristic of a virus or class of viruses and these structures may be more readily observable than virus particles. However, healthy maize cells contain several types of crystalline inclusions. Therefore, it is necessary to know the ultrastructure of healthy maize cells and to recognize artifacts produced in specimen preparation in order to distinguish pathogen-induced ultrastructural changes. Mycoplasma-like bodies (MLB) are also revealed in thin sections of infected tissue.

Detection of spiroplasmas. — Thick sections of infected tissue, as described by Davis and Worley (9), are necessary for an EM identification of helical filaments of spiroplasmas. Thin sections may reveal segments of curved, mycoplasma-like filaments suggestive of the spiroplasma helical morphology, but some MLB occur as curved fila-

ments without helical morphology. We have observed helical filaments of spiroplasmas in negatively stained plant sap from infected leaves, but this method has not been reliable. Dark-field, light microscope examination of sap from infected leaves, as described by Davis (8), has proved to be a convenient means of visualizing spiroplasmas. Non-helical MLB are not detected by this method.

Serum specific electron microscopy. — Derrick (10, 11) has developed this technique, which allows detection, identification, and determination of serological relations of maize viruses and spiroplasmas. Our initial trials confirm the usefulness of this method. Antiserum to each pathogen is required for detection, but purification and concentration of virus particles are not necessary.

IMPROVING EFFICIENCY OF DETECTION

The above methods have contributed to the discovery and characterization of new and unsuspected maize viruses and pathogens causing virus-like diseases. They have also been useful in the detection, characterization, and confirmation of known maize pathogens. Results obtained with these methods clearly demonstrate the utility of EM in visualizing virus particles and MLB. However, in the study of some diseases of unknown etiology, none of these methods nor others (centrifugation, serology, and transmission) are successful in the initial efforts to detect the pathogen. In these situations we have successfully used electron microscopy of thin-sectioned tissue from field-collected leaves.

Initially, this approach had severe limitations: 1) size and number of samples that can be examined is extremely limited; 2) the pathogen may not be easily recognized; and 3) the relation of a pathogen seen by electron microscopy to the symptoms is questionable.

By light microscope comparisons of adjacent healthy and diseased tissues of an infected maize leaf in a series of increasing magnifications, we can relate leaf symptoms to tissue and cellular alterations. These alterations detected by light microscopy can then be related to virus particles, inclusions, and cytopathic effects seen by EM. These comparisons of healthy and diseased tissue, correlated over a range of magnifications, greatly facilitate the use of EM in detecting virus particles and MLB and implicating them in the etiology of maize diseases. Within areas of gross symptoms of a leaf, the region of tissue to be examined by EM can be selected precisely by light microscopy. Then, virus particles, MLB, and cytopathic effects can be recognized by EM within diseased cells that are precisely located within the leaf symptoms. The association of virus particles, inclusions, and cytopathic effects with diseased tissue can be generalized at low magnification with the light microscope and subsequently related back to the gross leaf symptoms.

Directing the EM search for virus particles to diseased cells by light microscopy increases the probability of detecting a pathogen of expected morphology or recognizing one of unexpected morphology. What is seen by EM is somewhat dependent on what we think can be seen. Proposing pathogenicity for a novel structure may be limited by our inability to distinguish a novel pathogen from a novel structure induced by a pathogen. However, consistent location of virus particles or MLB in diseased but not healthy cells implies a causal relationship. Adjacent healthy cells may also be used for comparison to detect ultrastructural changes induced by a pathogen or to monitor the occurrence of artifacts produced in specimen preparation.

The ease with which different maize viruses or MLB can be recognized by light microscopy depends upon the size, contrast, location, and distinctive features of these pathogens or their associated inclusion and cytopathic effects. For example, rhabdovirus inclusions are easily detected by light microscopy, whereas a low incidence of MLB is difficult to distinguish from mitochrondria. Each pathogen may require correlation studies, modifications in technique, and a period of familiarization before tissue regions likely to contain that pathogen are readily recognized by light microscopy. Although we may find little evidence of some maize virus-like pathogens by light microscopy, the above approach has greatly improved the efficiency of EM in the detection of several kinds of maize virus particles and MLB.

NEW MAIZE PATHOGENS

Use of EM in the Maize Virus Disease Research Group at Wooster has led or assisted in the discovery of several new maize viruses or pathogens producing virus-like symptoms. The success of this endeavor has been dependent upon samples supplied by colleagues or, better, collected in cooperative surveys with colleagues familiar with maize diseases of a geographical region. The technical quality of microscopy and specimen preparations and familiarity with maize ultrastructure have also been important. Ultrastructural evidence for the presence of a virus or microorganism in a diseased plant depends upon how closely structures "seen" by EM resemble known virus particles or MLB. The presumption that the virus particles or MLB revealed by EM are the pathogenic agents of a disease depends on observing a unique association of these structures with the diseased plants or, better, diseased tissue. Observation of the same structures in infective vectors is also important for obligately

vectored pathogens. The strength of EM evidence for new maize pathogens has varied substantially and is inherently incomplete. This evidence has been most useful in generating and directing additional efforts to characterize a pathogen, its transmission, and its role in a disease.

Maize chlorotic dwarf virus (MCDV). — The first evidence of this isometric virus was obtained by EM (2) in sample plants collected by R. Louie and J. K. Knoke in southern Ohio. The disease, which shows symptoms of plant stunting with leaf reddening and yellowing, had been called corn stunt, implying a mycoplasma etiology. MLB were not found. Instead, two unique inclusions were located in the vascular tissue. One was a dense granular inclusion with isometric virus particles and the other was a striated sheet inclusion with some features similar to the cylindrical inclusions associated with the PVY virus group (1). The first indication that MCDV infects the widely distributed perennial weed host, Johnsongrass, and is transmitted by the widely distributed leafhopper, *Graminella nigrifrons* (Forbes), occurred when these same two inclusions were found in a corn plant supplied by T. P. Pirone (2, 25). This plant was one of several stunted plants that had been exposed to *G. nigrifrons* previously fed on field-collected Johnsongrass.

Yellow sorghum stunt mycoplasma. — The disease symptoms, extreme stunting and leaf yellowing, were first described in sweet sorghum by Zummo and coworkers (29). Samples supplied by Zummo from widely distributed sweet sorghum plots in the southeastern U.S. showed by EM the consistent presence of MLB. Inclusions associated with MCDV also occurred in some of these samples. Attempts by N. Zummo and L. R. Nault to transmit the disease agent of yellow sorghum stunt have been unsuccessful to date. There is no evidence that this MLB is a spiroplasma (4, 5).

Maize rhabdovirus(es) (MRV). — Rhabdovirus particles were first detected in maize in the continental U. S. by EM of samples from Texas, supplied by R. W. Toler (3, 6), and subsequently in samples collected during joint surveys with him. We have also found MRV in samples from Hawaii and Iowa (provided by D. R. Wilkinson) and Mississippi. Others have reported MRV in Alabama (28) and Lousiana (12). Some maize samples with rhabdovirus particles were also infected with MCDV and maize dwarf mosaic virus. Symptoms associated with MRV ranged from severe stunting with chlorotic-striped leaves to symptomless. Transmission attempts with leafhoppers and planthoppers from field samples collected in the continental U. S. were unsuccessful (L. R. Nault, *personal communication*). However, an unexpected MRV, found in a MCDV host range study, was transmitted by *G. nigrifrons* (23). Possibilities for the identity of MRV in the continental U. S. include maize mosaic virus (18) and wheat striate mosaic virus (26). Oat striate mosaic virus, a recently reported *G. nigrifrons*-transmitted rhabdovirus, may also be a possibility, but current tests do not indicate that maize is a host (20).

Chlorotic lethal disease agent of maize. — This severe virus-like disease of sweet corn was first reported by Tsai (27) in southern Florida. Transmission of the disease agent by the planthopper *Perigrinus maidis* (Ashm.) suggested to us that maize mosaic, a *P. maidis*-transmitted rhabdovirus (18), was involved. Samples were supplied for EM by J. H. Tsai and the pathogen was transmitted in the greenhouse at Wooster by L. R. Nault. Instead of rhabdovirus particles, anomalous cytoplasmic inclusions of very low density were found in tissue sections of infected leaves by light microscopy and EM (Bradfute and Robertson, *unpublished*). Attempts to find virus-like particles by EM in tissue sections and negatively stained preparations have been unsuccessful.

Wheat spot chlorosis pathogen (WSCP). — This virus-like disease was first reported in Ohio wheat by Nault and coworkers (24) and thought to be similar to wheat spot mosaic disease. The agent is vectored by the wheat curl mite, *Aceria tulipae* (Kiefer), and experimentally infects barley, oats, and wheat. Symptoms in maize start as chlorotic leaf spots, some of which later coalesce and form dashes and streaks. Double membrane bound bodies (DMB), 0.1 to 0.2 μm in diam, are consistently revealed by EM of tissue sections of diseased leaves (7). However, we do not know if the DMB is the pathogen or a cytopathic structure induced by the pathogen. Attempts to isolate and concentrate DMB from diseased maize tissue have been unsuccessful (Bradfute, *unpublished*).

Short flexuous rod virus. — Flexuous rod virus particles, less than 425 nm long, were found by EM in negatively stained preparations from unidentified weed grass samples collected in a Louisiana maize field (Bradfute, Gordon, Robertson, Toler, and Derrick, *unpublished*). The virus occurs in high tissue concentration and is mechanically transmissible to maize and other Gramineae species producing chlorotic streaks on leaves. The virus particle length suggests the existence of a new group of plant viruses.

Maize bushy stunt mycoplasma. — Mycoplasma-like bodies, as detected in tissue sections by EM, were found in stunted corn plants collected in Texas (Bradfute, Nault, Robertson, and Toler, *unpublished*). The disease agent is persistently vectored by the leafhopper *Dalbulus maidis* (DeLong & Wolcott). The symptoms, compared to Rio Grande corn stunt, develop sooner and corn plants are more severely stunted and have greater shoot proliferation. Leaves are yellowed or reddened, depending on the maize genotype, time of inoculation, and stage of plant development, but do not have the high-contrast chlorotic stripes associated with Rio Grande corn stunt. Helical fila-

ments of spiroplasma were not detected in dark-field, light microscopy of juice expressed from diseased leaves and in smears of infective vectors. However, MLB were consistently found in tissue sections of diseased leaves by EM. Previously, the morphology of MLB in maize has been either helical, as in the case of Rio Grande corn stunt spiroplasma (9), or undetermined. This is the first evidence for a non-helical MLB in maize.

Maize rayado fino virus (MRFV). — This virus, first described by Gamez (15, 16), produces leaf symptoms of fine chlorotic stripes. These symptoms were identified for Bradfute by J. Galindo on a joint maize virus survey in Mexico. Isometric virus particles were found by EM in negatively stained preparations and tissue sections of maize with similar virus-like symptoms collected in Texas with R.W. Toler (Bradfute, Robertson, Gordon, Nault, and Toler, *unpublished*). The virus is persistently transmitted by *D. maidis* and reacts with MRFV-antiserum supplied by R. Gamez. This is the first report of MRFV in the U.S.

Importance of new pathogens. — With the exception of maize chlorotic dwarf virus, for which the distribution and relative importance are reported (17), the economic or potential economic importance of new maize pathogens indicated by EM is unknown. They could represent new occurrences of pathogens that will become important in the U. S. Alternatively, they may have been present in maize in the U. S. and gone undetected due to lack of adequate detection methods, extensive surveys, and distinguishing symptoms (21). Their detection by such a selective method as EM suggests to us that they are more common than might otherwise be suspected.

LITERATURE CITED

1. BRADFUTE, O.E., R.E. GINGERY, D.T. GORDON, and L.R. NAULT 1972. Tissue ultrastructure, sedimentation and leafhopper transmission of a virus associated with maize dwarfing disease. J. Cell. Biol. 55:25a. (Abstr.).
2. BRADFUTE, O.E., R. LOUIE, and J.K. KNOKE. 1972. Isometric virus-like particles in maize with stunt symptoms. Phytopathology 62·748. (Abstr.).
3. BRADFUTE, O E , and D.C. ROBERTSON. 1974. Rhabdovirus-like particles in stunted maize from Texas and Hawaii. Proc Electron Microsc. Soc. Am. 32:266-267.
4. BRADFUTE, O.E , and DIANE C. ROBERTSON. 1974 Mycoplasma-like bodies in stunted sorghum. Proc. Electron Microsc. Soc. Am. 32:170-171.
5. BRADFUTE, O.E., and DIANE C ROBERTSON. 1974. Nonspiral mycoplasma-like bodies in stunted sorghum from southern Ohio. Proc Am. Phytopathol. Soc. 1:96-97. (Abstr.).
6. BRADFUTE, O.E., DIANE C. ROBERTSON, and R.W. TOLER. 1974. Possible occurrence of maize mosaic virus in the continental United States. Proc. Am. Phytopathol. Soc. 1:36. (Abstr.).
7. BRADFUTE, O.E., R.E WHITMOYER, and L.R. NAULT. 1970. Ultrastructure of plant leaf tissue infected with mite-borne, viral-like pathogens. Proc. Electron Microsc. Soc. Am. 28:178-179.
8. DAVIS, R E. 1974. Spiroplasma in corn stunt-infected individuals of the vector leafhopper Dalbulus maidis. Plant Dis. Rep 58 1109-1112.
9. DAVIS, R.E., and J.F. WORLEY. 1973. Spiroplasma: Motile, helical microorganism associated with corn stunt disease. Phytopathology 63 403-408.
10. DERRICK, K S. 1973. Quantitative assay for plant viruses using serologically specific electron microscopy. Virology 56·652-653
11. DERRICK. K.S., and R.H. BOLANSKY. 1976. Assay for viruses and mycoplasmas using serologically specific electron microscopy. Phytopathology 66:815-820.
12. DERRICK. K.S , and M J. GIAMALVA. 1975. Rhabdovirus-like particles associated with a disease of corn in Louisiana. Proc. Am. Phytopathol. Soc. 2:133. (Abstr.).
13. ESAU, K. 1968. Viruses in plant hosts: Form, distribution and pathologic effects. Univ. of Wisconsin Press, Madison. 225 p.
14. FINCH, J.T., and K C. HOMES. 1967. Structural studies of viruses. Pages 351-474 in Methods in Virology, K. Maramorsch and H. Koprowski, eds., Vol. III. Academic Press, N.Y. 677 p.
15. GAMEZ, R. 1969. A new leafhopper-borne virus of corn in Central America. Plant Dis. Rep. 53:929-932.
16. GAMEZ, R. 1977. Leafhopper-transmitted maize rayado fino virus in Central America. Pages 15-19 in Proc. Maize Virus Dis Colloq. and Workshop, 16-19 Aug , 1976. Ohio Agric. Res. and Dev. Center, Wooster, 145 p
17. GORDON, D.T., and L.R. NAULT. 1977. Involvement of maize chlorotic dwarf virus and other agents in stunting diseases of Zea mays in the United States. Phytopathology 67:27-36.
18. HEROLD, FRIEDA. 1972. Maize mosaic virus. No. 94 in Descriptions of Plant Viruses. C.M.I./A.A.B. Kew, Surrey, England, 4 p
19. HORNE. R.W. 1967. Electron microscopy of isolated virus particles and their components. Pages 521-574 in Methods of Virology, K Maramorsch and H. Koprowski, eds Vol. III. Academic Press, N.Y. 677 p.
20. JEDLINSKI, H. 1976. Oat striate mosaic, a new virus disease in Illinois spread by the leafhopper, Graminella nigrifrons (Forbes). Proc. Am. Phytopathol. Soc. 3 (In press).
21. LOUIE, R. 1977. Maize virus disease identification by symptomatology. Pages 89-91 in Proc. Maize Virus Dis Colloq. and Workshop, 16-19 Aug., 1976. Ohio Agric. Res. and Dev. Center, Wooster. 145 p.
22. MILNE, R.G. 1972. Electron microscopy of viruses. Pages 76-128 in Principles and Techniques in Plant Virology, C.I. Kado and H.O. Agrawal, eds., Van Nostrand Reinhold Co., N.Y. 688 p.
23. NAULT, L.R., D.T. GORDON, DIANE C. ROBERTSON, and O E BRADFUTE. 1976. Host range of maize chlorotic dwarf virus. Plant Dis. Rep. 60:374-377.
24. NAULT, L R., W.E. STYER, D.T. GORDON, O.E. BRADFUTE, H.N. LAFEVER, and L.E. WILLIAMS. 1970. An eriophyid-borne pathogen from Ohio and its relation to wheat spot mosaic virus. Plant Dis Rep. 54.156-160.
25. PIRONE, T.P., O.E. BRADFUTE, P H. FREITAG, M.C.Y. LUNG, and C.G PONELEIT. 1972. Virus-like particles associated with a leafhopper transmitted disease of corn in Kentucky. Plant Dis. Rep. 56:652-656.
26. SINHA, R.C , and R.M. BEHKI. 1972 American wheat striate mosaic virus. No. 99 in Descriptions of Plant Viruses, C.M.I./A.A B Kew, Surrey, England. 4 p.
27. TSAI, J.H. 1975. Occurrence of a corn disease in Florida transmitted by Peregrinus maidis. Plant Dis. Rep 59:830-833.
28. ZEYEN, R.J., and R.H. MORRISON. 1975. Rhabdovirus-like particles associated with stunting of maize in Alabama. Plant Dis. Rep 59:169-171.

29. ZUMMO, N., O.E. BRADFUTE, D.C. ROBERTSON, and K.C. FREEMAN. 1975. Yellow sorghum stunt: A disease symptom of sweet sorghum associated with a mycoplasma-like body in the United States. Plant Dis. Rep. 59:714-716.

DISCUSSION OF O.E. BRADFUTE PAPER

G. Martinez-Lopez: How thick are those sections for light microscopy?

O.E. Bradfute: The sections are 0.5 to 2 μm thick compared to the 5 to 10 μm thick sections commonly used in light microscopy of paraffin-embedded tissue. To make sections 0.5 to 2 μm thick, we must use plastic embedding and an ultramicrotome or a microtome specially designed to cut sections of this thickness.

T. Sutabutra: Do you think that some day we may be able to identify flexuous rod viruses on the basis of morphology alone?

O.E. Bradfute: Identification of flexuous rod viruses on the basis of morphology alone would be very useful, but I think it is overly optimistic to expect this from our present technology. Many flexuous rod virus particles are similar in width and length and appear similar in negatively-stained preparations. The new maize viruses we have found by EM have gross morphological differences. The pitch of the helix of protein molecules composing flexuous rod virus particles has been determined by optical diffractometry of electron micrographs of negative-stained preparations. Differences in the pitch of the helix of flexuous rod viruses might provide a basis for classification. Additional developments in technology should also prove useful. But, on the basis of present technology, I believe many flexuous rod viruses will appear morphologically similar in EM

T. Sutabutra: We have studied maize dwarf mosaic, soybean mosaic, and other similar flexuous rod viruses. The virus particles have the same diameter and length, but their substructure is more or less different. I would hope that a means of virus identification could be developed on the basis of substructural differences of virus particles observed by EM

O.E. Bradfute: We see some differences in substructure of flexuous rod virus particles that appear to result from uncontrolled variables in negatively-stained preparations. We have also observed differences in radius of curvature and stain penetration that appear to be related to the type of flexuous rod virus. These differences and others obtained from improved technology may, as you suggest, provide a means of classifying flexuous rod viruses into groups, analogous to their classification on the basis of length. However, the structure of flexuous rod virus particles is determined largely by the nature of the protein coat. Because a relatively small proportion of the virus genome determines the nature of this protein and its ability to form a rod, many flexuous rod viruses could be similar in size, morphology, and structure.

Detection and Identification of Maize Viruses by Rate-Zonal Density Gradient Centrifugation

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Cooperative investigations of the Agricultural Research Service, U.S. Department of Agriculture, and the Ohio Agricultural Research and Development Center. Approved for publication as Article No. 167-76 of the Ohio Agricultural Research and Development Center, Wooster.

Grateful acknowledgement is made to R. Gamez for the generous gift of maize rayado fino virus antiserum.

ABSTRACT

Rate-zonal density gradient centrifugation was used for routine assays for maize chlorotic dwarf and maize dwarf mosaic viruses. Effects of centrifugation conditions and virus source on the assay are described. A virus, related to maize rayado fino virus, was detected for the first time in maize in the USA.

Rate-zonal centrifugation (2) is a powerful tool for the purification of plant viruses, since virus particles in plant extracts can be efficiently separated from most contaminating plant materials. However, usefulness of the technique is not limited to purification. Because a particular virus regularly sediments to the same depth in gradients centrifuged under the same conditions, the banding position after rate-zonal centrifugation can serve to identify the virus. Also, the technique provides a sensitive method of virus detection, since relatively small amounts of virus can be observed by highly sensitive gradient scanning techniques.

The paper reports the identification of maize chlorotic dwarf virus (MCDV) and maize dwarf mosaic virus (MDMV) by rate-zonal centrifugation. The detection of new viruses from maize (*Zea mays* L.) by this method is discussed.

MATERIALS AND METHODS

Viruses were partially purified from field-collected maize and centrifuged gradients were analyzed as described previously (9).

RESULTS

Figure 1 illustrates that MCDV and MDMV band at different depths in centrifuged gradients and, thus, are readily distinguishable by rate-zonal centrifugation. Furthermore, no other maize virus has a sedimentation rate similar to that for MCDV and the relative banding position in centrifuged gradients is sufficient to identify this virus. In contrast, the sedimentation rate of MDMV is

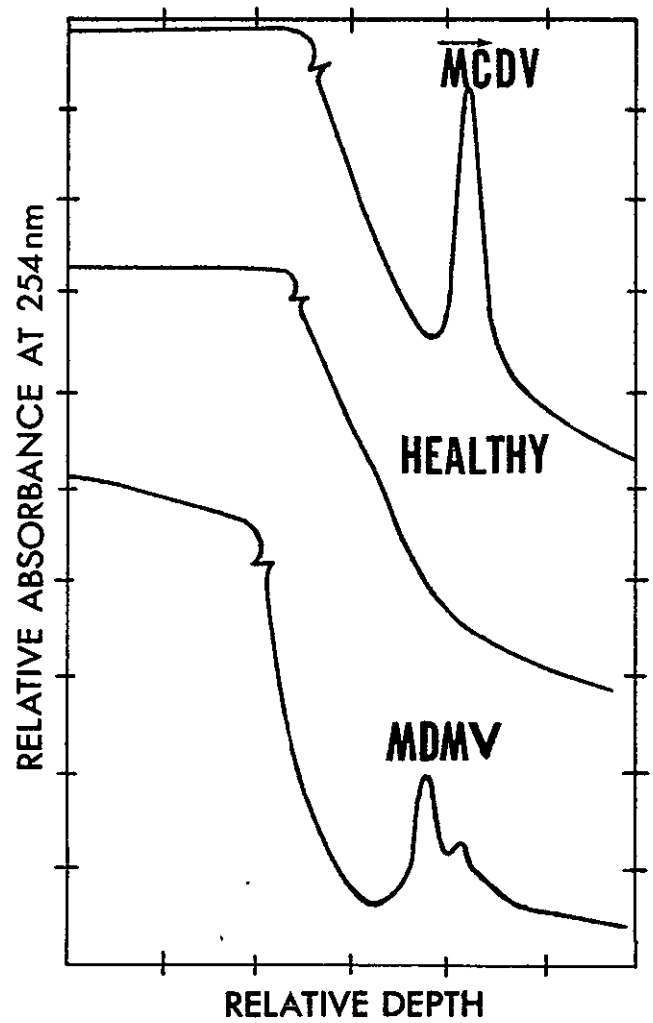

Fig. 1. Ultraviolet absorbance profiles after rate-zonal centrifugation of partially purified preparations from MCDV-infected, MDMV-infected, and healthy tissue.

similar to that of wheat streak mosaic virus, which occurs occasionally in maize (7), and identification by banding position alone is insufficient for these viruses. Rate-zonal centrifugation also provides quantitative estimates of virus because the area under a virus peak is directly proportional to the amount of virus. The profiles of preparations from uninfected plants showed no absorbance peaks in the virus region of the gradients.

The shape of a virus peak can provide preliminary evidence of particle morphology. A shoulder preceeding the main virus peak is often indicative of a filamentous-shaped virus (3, 6, 10) (Fig. 1, MDMV). A symmetrical peak suggests a spherical particle (Fig. 1, MCDV).

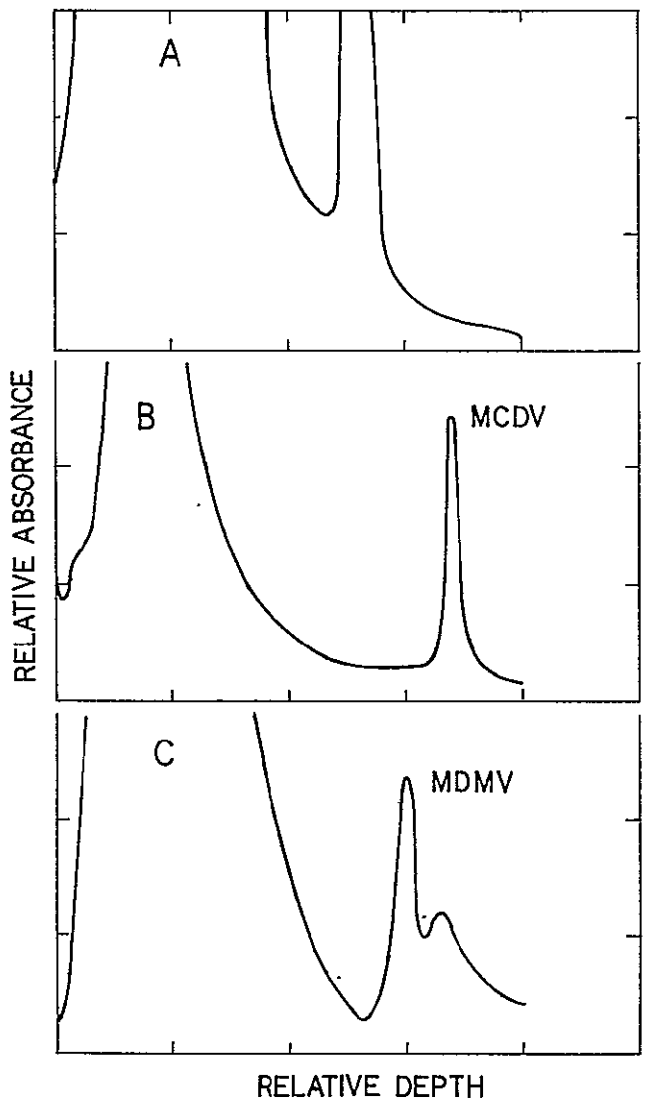

Fig. 2. Ultraviolet absorbance profiles of centrifuged gradients scanned at 254 nm. Partially purified preparations from infected leaves containing (A) unknown virus, (B) MCDV, and (C) MDMV (D.T. Gordon, *personal communication*).

To be sure that rate-zonal analysis would differentiate MCDV and MDMV, large numbers of field-infected samples were centrifuged under various conditions (D.T. Gordon, *personal communication*) to determine the effect on the ratio of the distance sedimented by each virus. Variation in the rotor, source of MCDV, and length of time of centrifugation were not significant; however, the phosphate buffer concentration in the gradient and source of MDMV were. Even so, the identification of MCDV and MDMV by relative banding position was always possible.

So far, the discussion has assumed the presence of known viruses only. An absorbance peak at a different location would strongly suggest a new virus. Even if banding positions were similar, new viruses would be detected, but other techniques would be required for identification.

An example of virus detection by rate-zonal centrifugation occurred with maize collected in Texas that exhibited symptoms different from those associated with either MCDV or MDMV (O.E. Bradfute and R. Toler, *personal communication*). Rate-zonal analysis of this material revealed a large, symmetrical peak that sedimented slower than either standard (Fig. 2). Thus, the sample was suspected of containing a new virus, spherical in shape and occurring in high concentration. Electron microscopy supported this observation by showing spherical virus-like particles in quick-dip preparations from these plants (O.E. Bradfute, *personal communication*). Disease symptoms suggested that this virus might be maize rayado fino virus (MRFV) (4). An immune centrifugation assay (5) with MRFV antiserum demonstrated a relationship to MRFV (D.T. Gordon, *personal communication*). Thus, a virus related to MRFV has been identified for the first time from diseased maize in the USA. Further tests are required to determine the closeness of relationship.

DISCUSSION

It may be appropriate to consider other methods of virus detection and identification to see how rate-zonal centrifugation may be advantageously used.

Symptomatology is the first means of detection since only plants exhibiting symptoms are usually assayed. The greatest advantage of symptomatology is that a tentative diagnosis is made immediately with little effort. However, virus identification by symptoms presents problems (8), and definitive evidence in necessary to establish a particular symptom as diagnostic. In cases where definite diagnostic symptoms have been demonstrated, symptomatology is very valuable (5, 8).

Electron microscopy is useful for the detection of virus-like particles in plants and for demon-

strating ultrastructural features of infections (1). In some cases where other techniques have failed to associate a virus particle with a disease, electon microscopy has provided this association. However, electon microscopy is of limited value for routine detection and identification because of time and effort involved and inability to distinguish viruses of similar particle morphology.

Vector specificity and mode of transmission are indispensable for virus characterization, but are not particularly suitable for routine detection and identification of viruses because of the long period before results are known and the effort and space involved.

Infectivity and host range are frequently useful for identification and detection of mechanically transmissible viruses, but they also require a long time for results.

In contrast, serology is particularly suited for routine analysis. Serological procedures are relatively rapid, remarkably specific, highly sensitive, and large numbers of samples can be easily processed. Generally, if a suitable serological technique for virus identification and detection is available, it is the method of choice for routine assays. However, with a new virus, or one to which antiserum has not been prepared, serology cannot be used.

Rate-zonal centrifugation has been useful for identification of maize viruses, particularly the obligately vectored MCDV (7). The assay is relatively rapid, and a moderately large number of samples can be processed. It is useful for routine assays for known viruses, especially if serological techniques have not been developed. If quantitative data concerning the amount of virus present are sought, rate-zonal centrifugation is suitable. Rate-zonal centrifugation is also useful for detection of new viruses. However, the virus must be stable under the conditions of extraction and centrifugation and have a sedimentation rate that permits detection in the gradient.

There are obvious limitations to each technique, and the detection and identification of maize viruses is most conclusive if several methods are used collaboratively. The most appropriate assay cannot be predicted, and all relevant procedures should be tried. Where one technique is used routinely, its reliability must be demonstrated initially by other methods.

LITERATURE CITED

1. BRADFUTE, O.E. 1977. Electron microscopy as a means for discovery of new maize viruses and virus-like pathogens. Pages 103-107 in Proc. Maize Virus Dis. Colloq. and Workshop, 16-19 Aug , 1976. Ohio Agric. Res. and Dev. Center, Wooster. 145 p.
2. BRAKKE, M.K. 1967. Density-gradient centrifugation. Pages 93-118 in K. Maramorosch and H. Koprowski (eds.) Methods in Virology, Vol. II. Academic Press, N.Y 682 p.
3. DAMIRDAGH, I.S., and R.J. SHEPHERD. 1970. Purification of the tobacco etch and other viruses of the potato Y group. Phytopathology 60:132-142.
4. GAMEZ, R. 1977. The leafhopper-transmitted rayado fino virus in Central America. Pages 15-19 in Proc. Maize Virus Dis. Colloq. and Workshop. 16-19 Aug., 1976 Ohio Agric. Res. and Dev. Center, Wooster. 145 p.
5. GORDON, D.T. 1977. Routine serological assays for diagnosis of maize virus diseases. Pages 99-102 in Proc. Maize Virus Dis. Colloq. and Workshop, 16-19 Aug., 1976. Ohio Agric. Res. and Dev Center, Wooster. 145 p.
6. GORDON, D.T., and R.E. GINGERY. 1973. Purification of maize dwarf mosaic virus by continuous-flow centrifugation. Phytopathology 63:1386-1392.
7. GORDON, D.T , and L.R. NAULT. 1977. Involvement of maize chlorotic dwarf virus and other agents in stunting diseases of Zea mays in the United States. Phytopathology 67:27-36.
8. LOUIE, R. 1977. Maize virus disease identification by symptomatology. Pages 89-91 in Proc. Maize Virus Dis. Colloq. and Workshop, 16-19 Aug., 1976. Ohio Agric. Res. and Dev. Center, Wooster. 145 p.
9. LOUIE, R , J.K. KNOKE, and D.T. GORDON. 1974. Epiphytotics of maize dwarf mosaic and maize chlorotic dwarf diseases in Ohio. Phytopathology 64:1455-1459.
10. SHEPHERD, R.J., and G.S. POUND. 1960. Purification of turnip mosaic virus. Phytopathology 50:797-803.

DISCUSSION OF R.E. GINGERY PAPER

V.D. Damsteegt: Although I agree that symptomatology may be confusing, it is often not feasible for an individual investigator to assay a larger number of samples by several techniques. Under these conditions, what tests beyond symptomatology can be done to obtain a reliable diagnosis?

R.E. Gingery: The answer depends on the disease or diseases that may be present and on the experience of the person and the technology available to him. The appropriate test beyond symptomatology need not be particularly complex as long as its reliability has been adequately demonstrated. Serological tests are often the easiest to perform and are usually suited to large numbers of samples. If antisera are not available, diagnosis of mechanically transmissible viruses can often be done by relatively simple host range infectivity tests. The problem is usually more difficult with obligately vectored viruses. In these cases, rate-zonal centrifugation, electron microscopy, or vector specificity are sometimes helpful.

Vectors of Maize Viruses

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These studies were supported in part by Grant No. 316-15-55 and Cooperative Agreement No. 12-15-75-2-14 from the Cooperative State Research Service, U.S. Department of Agriculture.

Approved for publication as Journal Article No. 194-76 of the Ohio Agricultural Research and Development Center, Wooster.

ABSTRACT

The search for vectors of maize viruses, mycoplasmas and spiroplasmas, the determination of the mode of pathogen transmission by vectors, the use of vectors as tools for studying maize pathogens, the rearing of vectors, and "trouble shooting" problems dealing with maize vectors in the laboratory are discussed. New information concerning the effects of temperature on the biology of *Graminella nigrifrons* and the transmission of maize chlorotic dwarf virus is also presented.

The major maize virus, mycoplasma, and spiroplasma diseases all have one thing in common, they are transmitted by arthropods. This feature sets them apart from most other maize pathogens. With the exception of the aphid-borne maize dwarf mosaic virus, these pathogens are obligately transmitted by their vectors. By this, I mean these pathogens cannot be mechanically transmitted. This places severe limitations on their study. Cultures of vectors must be established and then maintained and utilized to infect plants from which the pathogens may be extracted and purified. The purified pathogen can then be used for production of antiserum, physical and biochemical analyses, and other studies crucial to identification. Vectors are also used as "tools" to conduct host range studies and to screen maize inbreds and varieties for disease tolerance and resistance.

THE SEARCH FOR THE VECTOR

A maize pathologist may be confronted with the situation of dealing with an unrecognized disease in the field. He has ruled out bacterial and fungal pathogens, and further, suspects that a virus or mycoplasma is involved. The problem may be further complicated if the pathogen is not mechanically transmissible. If so, the vector must then be sought before progress can be made.

The first question to be asked in the vector search is "where to start?" Certain bits of information can narrow the search. If the pathogen is mechanically transmissible, then the need to find the vector is less compelling from the viewpoint of identifying the pathogen. It is, however, imperative to identify the vector to study disease epidemiology. If the pathogen is mechanically transmissible, it is unlikely that it will have a leafhopper (Cicadellidae), planthopper (Fulgoridae), or whitefly (Aleyrodidae) vector. Mites (Eriophyidae), aphids (Aphididae), flea beetles (Chrysomelidae), or nematodes (Nematoda) would be better candidates as vectors for a mechanically transmissible pathogen (1).

Information provided by electron microscopy can also narrow the vector search. Appearance and location of viruses, mycoplasmas, and spiroplasmas can provide important clues. Mycoplasmas and spiroplasmas are known to be transmitted only by leafhoppers. Flexous-rod viruses are transmitted principally by aphids and eriophyids. Rhabdoviruses are aphid, leafhopper, or planthopper transmitted. Small isometric viruses are vectored principally by aphids, leafhoppers or flea beetles. Although Gibbs (1) work was published 8 years ago, his compilation of plant viruses based upon vector groups and virus morphology still offers valuable information in the search for a vector. A word of caution must be mentioned here. The visualization of a virus particle or mycoplasma-like body by electron microscopy does not necessarily indicate its involvement in the disease syndrome. Another pathogen, not visualized by electron microscopy, could be involved.

In searching for a maize vector, the first impulse is to collect from maize, particularly plants infected with the suspected pathogen. This may lead to success but many potential vectors may also be missed. For example, many of the aphid vectors of the maize dwarf mosaic virus (MDMV) and the leafhopper vectors of maize chlorotic dwarf virus (MCDV) prefer to feed on other hosts. As a matter of course, potential vec-

tors should be sought from grassy weeds in and around maize fields or from other nearby grain crops.

The type of vector can sometimes be predicted from the pattern of field spread of the pathogen. High incidence of disease along the edge of the field may indicate a nearby source of air-borne vectors. Scattered infections indicate a distant source of an aerial vector, whereas localized, a slowly spreading disease suggests a soil inhabiting vector. The latter could include fungi and nematodes as well as arthropods.

DETERMINING MODE OF VECTOR TRANSMISSION

Determination of the mode of pathogen transmission is as important as identifying the vector. Sylvester (10) broadly grouped vector-pathogen relationships into three categories: persistent, semipersistent, and nonpersistent. These relationships can be illustrated by the transmission of three maize pathogens. The persistence of the aphid-borne MDMV can be measured in minutes (6), the persistence of the leafhopper-borne MCDV in hours (7), and the persistence of the leafhopper-borne maize bushy stunt mycoplasma in days or weeks (L.R. Nault, *unpublished*). Another important functional characteristic is the presence or absence of an incubation period of the pathogen in the vector. The nonpersistent and semipersistent pathogens can be transmitted by their vectors immediately after they are acquired, whereas persistent pathogens must first undergo an incubation period of several days or weeks in their vectors. When testing potential maize vectors, several techniques must be tried. Following acquisition access periods, which should vary from several minutes to several days, some insects should be allowed to feed immediately on test plants while others should be held for 2 to 3 weeks before being tested as vectors.

Large numbers of potential vectors should be used with various vector transmission techniques. Pathogen titre in field-collected plants may be low, resulting in a low percentage of vectors becoming inoculative. It has been my experience that transmission from experimentally infected plants is generally easier than from field-collected plants.

VECTORS AS TOOLS

Use of vectors in the laboratory is generally not warranted in the case of mechanically transmissible pathogens. It is of interest to note, however, that after large numbers of successive mechanical transfers, the vector transmissibility of these pathogens is often lost. This has occurred in my own laboratory with an aphid-borne isolate of MDMV and an eriophyid-borne isolate of wheat streak mosaic virus. Occasional vector transfers of mechanically transmissible pathogens will ensure maintenance not only of vector transmission but perhaps of other characteristics as well. To study those pathogens that are not mechanically transmitted, laboratory use of vectors is mandatory. In my laboratory over the past several years, thousands of maize plants have been leafhopper inoculated with MCDV. From these plants, virus has been purified and used to determine its chemical and physical properites (2) and to produce antiserum (5). The antiserum has been used to develop rapid and sensitive techniques for assay of MCDV in field-collected samples, and allowed us to determine distribution and incidence of the virus in the U.S. (3). Leafhoppers have also been used to determine the host range of MCDV (8) and to test maize inbreds and hybrids for virus susceptibility (J.K. Knoke and R. Louie, *unpublished*).

The infectivity of purified or partially purified preparations of non-mechanically transmissible pathogens creates special problems. These preparations must be introduced back into the vector before their infectivity can be ascertained. Two widely used methods have been developed to serve this purpose (4). The first is the use of fine, glass needles to inject virus preparations into the vector's haemocoel. The second method employs membrane-enclosed solutions from which the vectors can feed and acquire the pathogen. Injection and membrane feeding work well for persistent pathogens, whereas only membrane feeding can be used for those that are transmitted in a nonpersistent or semipersistent fashion.

VECTOR REARING

Perhaps the most time-consuming aspect in the study of maize pathogens is vector rearing. The first hurdle is to establish a field-collected population in the laboratory or greenhouse. This is not always as easy as it first seems. A species may not adapt well to restraints of a rearing cage and its environment.

Since all vectors of maize pathogens require living plants to survive, the selection of a rearing host is of paramount importance. In the case of a monophagous species, there is little choice. A prime example of such a species is *Dalbulus maidis* (DeLong & Wolcott). Maize and the closely related *Tripsacum dactyloides* L. are the only known hosts (9).

If a species is oligophagous or polyphagous, other decisions in host selection must be made. These are: finding a host that is easy to grow from seed or other plant parts, a host in which the vector will readily deposit its eggs, and a host best suited for vector feeding and development.

The feeding host should survive well when fed upon by large numbers of developing individuals. Ovipositional and feeding hosts may not necessarily be the same. For example, I have found that the vector of MCDV, *Graminella nigrifrons* (Forbes), readily lays its eggs in wheat, barley, and oats, but seedlings of these species often succumb to the feeding of this leafhopper. On the other hand, *G. nigrifrons* does not readily oviposit in maize, but feeds and develops well on it. Maize will support larger populations of leafhoppers in rearing cages before succumbing to feeding injury.

Another important consideration is rearing the vector on hosts that are not susceptible to the maize pathogen under investigation. I use oats or barley as the ovipositional or feeding host for young *G. nigrifrons*. Neither is susceptible to MCDV (8). Use of these hosts greatly diminishes the possibility of perpetuating contaminating isolates of MCDV in the rearing colonies.

The ability to control day length and temperature cannot be overlooked when rearing maize vectors. For this reason it is preferable to maintain vectors in an environmentally controlled laboratory rather than in a greenhouse. Day length will particularly affect morph development in aphids. It is preferable to maintain apterous viviparae for transmission tests. This morph can be readily maintained if a 16-hour light/day regime is used.

Although temperature is important in the rearing of all vector species, it has its most noticeable effect on insects that have a relatively long life cycle, such as the Cicadelloidea. I shall again draw my examples from *G. nigrifrons*. An increase in temperature from 15 to 30 C resulted in a marked increase in egg laying by the leafhopper (Fig. 1). Also affected is the length of time of development from egg to adult (Fig. 2). An increase in temperature decreases developmental time. At an even lower temperature, 15 C, no egg hatch occurred, indicating that the developmental threshold for the species is between 15 and 20 C. I consider 25 C as the optimum rearing temperature for *G. nigrifrons*. Whereas development takes about 1 week longer, resultant adults are larger and hardier than those reared at 30 C.

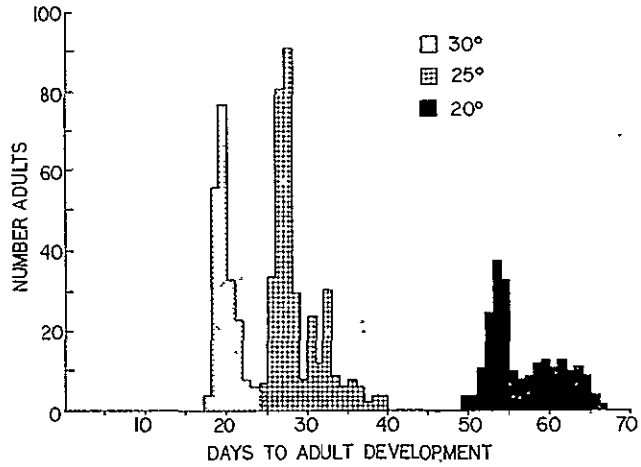

Fig. 2. Effects of temperature on time of development of *Graminella nigrifrons* from egg to adult.

Temperature also affects the transmission of MCDV by *G. nigrifrons* (Fig. 3). Although initial inoculativity of leafhoppers is not significantly affected by temperature, persistence of MCDV in the vector is affected. An increase in temperature resulted in a decrease in persistence of virus in the vector. So, while higher temperatures favor

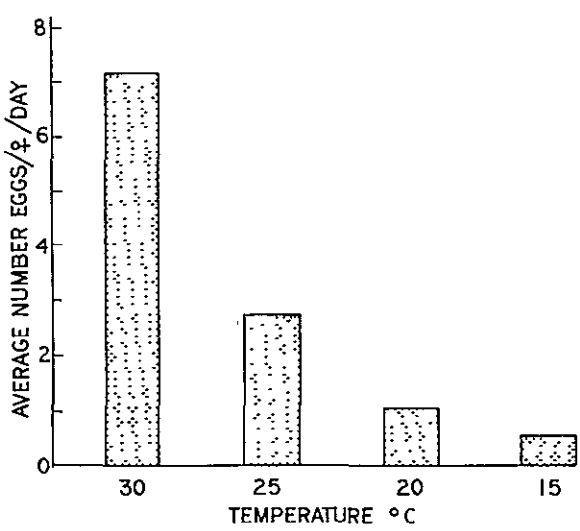

Fig. 1. Effects of temperature on egg laying by the leafhopper *Graminella nigrifrons*. Each value represents the average from 150 females.

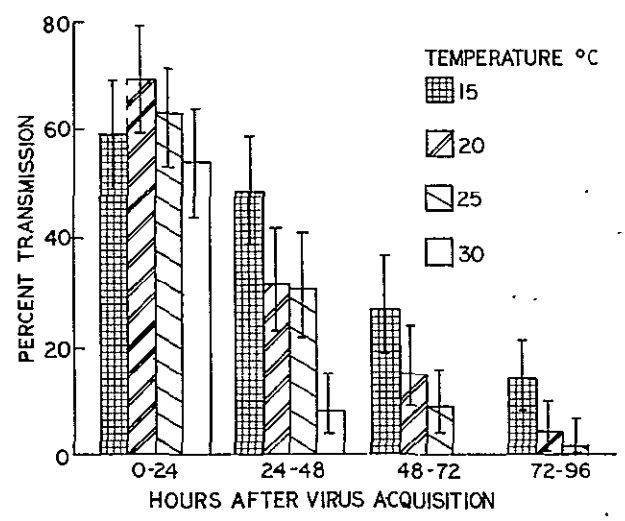

Fig. 3. Effects of temperature on transmission of maize chlorotic dwarf virus by *Graminella nigrifrons*. Leafhoppers were given a 72-hour acquisition access period on infected leaves prior to transfer at five per plant. Each value represents an average of 100 tests; bars represent standard errors.

rearing of *G. nigrifrons*, lower temperatures favor virus transmission. This information has allowed us to maximize our laboratory production of MCDV-infected maize.

"TROUBLE SHOOTING"

For lack of a more descriptive term, I would like to conclude with what I call "trouble shooting." The following are what I consider to be our most troublesome and persistent problems and how we cope with them.

The first deals with providing pest-free host plants for vectors and pathogens. These plants are maintained in the greenhouse where they are subjected to the normal complement of greenhouse pests. We are troubled principally by aphids, whiteflies, and spider mites. Each requires special attention. Since these greenhouse-grown plants will be needed to rear insects or to be used as source plants for vectors to acquire pathogens, pesticides used to control pests must be both effective and short-lived. Plictran is our most effective miticide. We have observed no harmful effects even when this compound is sprayed directly into rearing cages containing aphids or leafhoppers. Only spider mites are killed. Fumigation with Vapona is usually effective in controlling contaminant aphid species in the greenhouse. Within 48 hours of treatment we can use Vapona-fumigated plants to rear aphids or leafhoppers. Unfortunately, Vapona has little effect on whiteflies. Resmethrin, a synthetic pyrethroid, is formulated especially for whiteflies. It is also effective against leafhoppers but not aphids. Plants sprayed with Resmethrin for rearing or as test plants can be used within 24 hours after treatment.

Even though great care may be taken to use non persistent pesticides in vector-virus studies, use of more persistent agricultural chemicals by others can create a problem for vector specialists. In one instance I used plastic pots that had been previously sprayed with a persistent pesticide. Soap and water washing did not remove residues and release of absorbed pesticide produced a slow decline of vector populations. For our most sensitive vector species, I now use disposable styrofoam cups to grow host plants. Also, care must be exercised to not re-use greenhouse soils that have been treated with pesticides. Lastly, be certain that seeds have been treated only with fungicides and not insecticides.

A final problem is to maintain species pure, plant pathogen-free vectors. Periodically, each species being reared should be checked to be certain it is the one you expect it to be. More than once, we have found the closely related *D. maidis* and *D. elimatus* (Ball) intermixed in the colonies. Only a microscopic examination revealed the problem. No matter how tight you may feel your rearing cages are, leaks develop. A simple leak detector is a UV-light and suction trap. Leafhoppers and planthoppers that have escaped into the rearing room, greenhouse, or laboratory are effectively attracted to and killed by the trap. Also, as a matter of course, samples of reared vectors should be assayed to determine if they are accidentally harboring plant pathogens. Contaminated colonies should be eliminated and new colonies started from pathogen-free vectors.

LITERATURE CITED

1. GIBBS, A 1969 Plant virus classification 263-328 in K.M. Smith and M.A. Lauffer, eds. Adv. Virus Res. Vol. 14, Academic Press. 350 p.
2. GINGERY, R E. 1976. Properties of maize chlorotic dwarf virus and its ribonucleic acid. Virology 73.311-318.
3. GORDON, D.T. 1977. Routine serological assays for diagnosis of maize virus diseases. Pages 99-102 in Proc. Maize Virus Dis Colloq. and Workshop, 16-19 Aug., 1976. Ohio Agric. Res. and Dev. Center, Wooster, 145 p.
4. KADO, C.I., and H.O. AGRIWAL. 1972. Principles and techniques in plant virology. Van Nostrand Reinhold Co., New York, N.Y 688 p.
5. GORDON, D T., and L.R. NAULT. 1977. Involvement of maize chlorotic dwarf virus and other agents in stunting diseases of Zea mays in the United States. Phytopathology 67:27-36
6. NAULT, L.R., H.J. HARLAN, and W.R. FINDLEY. 1971 Comparative susceptibility of corn to aphid and mechanical inoculation of maize dwarf mosaic virus. J. Econ Entomol. 64:21-23.
7. NAULT, L.R., W.E. STYER, J.K KNOKE, and H.N PITRE. 1973. Semi-persistent transmission of leafhopper-borne maize chlorotic dwarf virus. J. Econ. Entomol. 66:1271-1273.
8. NAULT, L.R., D.T. GORDON, D.C. ROBERTSON, and O.E. BRADFUTE. 1976. Host range of maize chlorotic dwarf virus. Plant Dis. Rep. 60:374-377
9. PITRE, H.N., R.L. COMBS, JR., and W.W. DOUGLAS. 1966. Gamagrass, Tripsacum dactyloides: a new host of Dalbulus maidis, vector of corn stunt virus. Plant Dis. Rep. 50:570-571.
10. SYLVESTER, E.S. 1969. Virus transmission by aphids—viewpoint. Pages 159-173 in K. Maramorosch, ed. Viruses, Vectors, and Vegetation. Academic Press, New York. 666 p.

DISCUSSION OF L.R. NAULT PAPER

R. Gamez: I was pleased to hear that you encountered the same problems that I have during the last few years. I would like to comment that for many of these maize viruses, the virus-vector relationship provides a very subtle criteria for characterization. In my experience with the rayado fino virus, I did not have access to an electron microscope or other techniques. I relied solely on the characteristic of virus transmission. We have shown how confusing and how unreliable symptoms are, so I would like to stress that I give much importance to the virus-vector relationship.

L.R. Nault: One aspect that I did not discuss in the search for a vector is the means by which you discovered maize rayado fino. You were not looking for the disease in the field; rather you obtained the pathogen directly from viruliferous vectors. We are now attempting to use this method to discover new vectors and pathogens, but so far it has not worked. Wound tumor is another classical example of discovering the virus from field-collected vectors.

R. Gamez: We have also used this approach in the search for vectors of legume viruses.

J.M. Fajamisin: You mentioned a virus that is obligately transmitted by vectors; maize streak virus is an example. With this disease, we have had problems, and I have not found the vector What are your thoughts on how to solve this problem.

L.R. Nault: I read your article in *Plant Disease Reporter* and it certainly has a tremendous amount of information. The serological results and symptomatology indicate you have maize streak, and my suggestion would be to search for a *Cicadulina* vector. There may be several problems. I would certainly suggest that you obtain the biotype of vectors from workers in East Africa. It is possible that biotypes collected from East Africa will not transmit or will be poor vectors. Have you attempted to rear *Cicadulina*?

J.M. Fajamisin: Yes, we have in the greenhouse. But we have had problems.

L.R. Nault: As I mentioned earlier, sometimes field-collected vectors may not at first adjust well to the artificial rearing environment. It may take several generations before the adjustment is made.

R.J. Lastra: Do you find any differences in transmission when you place insects in a small plastic cage on top of the leaves or in a cage which covers the whole plant?

L.R. Nault: When we are transmitting from field-collected samples, we use a petri dish to contain vectors. The dish bottom is covered with a mixture of one part activated charcoal to nine parts plaster of paris. When wetted, a fairly high humidity is maintained in the dish, about 90-95%. Vectors are free to feed on either leaf surface. In transmissions from greenhouse inoculated materials, we use plexiglass tube cages. We prefer plexiglass over other kinds of plastic simply because pesticides can be washed off fairly easily. The disadvantage is that plexiglass is expensive. For pathogen acquisition, we place these tube cages over small, infected seedlings and in this way the vector can feed anywhere it wishes. We use the same method for inoculating test plants. We also use clip-on cages.

R.J. Lastra: It seems to me that clip-on cages which restrict insect movement may lower the transmission rates.

L.R. Nault: This may be so. However, I have no data on the effect on transmission rates.

E.E. Rosenkranz: I want to mention a couple of other problems, perhaps for the benefit of those who have not worked with vectors If cages are plastic, make sure that it is not toxic to the insects. For instance, if you use cellulose acetate, the fumes may be toxic to the insects. Thus, it is better to use either butyrate or cellulose nitrate. The other point I want to make concerns a situation where a vector is transmitting two different disease agents and contamination can very easily occur. One example is *G nigrifrons* transmitting maize chlorotic dwarf virus and what we call Mississippi corn stunt agent or what you refer to as maize bushy stunt mycoplasma. It is very easy to contaminate one with the other, since *G. nigrifrons* can transmit both, although the efficiency of the transmission varies greatly.

L.R. Nault: I have found exactly the same thing. Cellulose acetate is toxic not only to leafhoppers but also to aphids, although aphids are less sensitive. We do not like cellulose nitrate because it is flammable. Cellulose butyrate works very well for aphids, but we have had problems with leafhoppers Either this plastic is toxic or insecticides, *e.g*, Vapona, absorb to it when we fumigate cages and test plants.

E.E. Rosenkranz: In the case where a vector can transmit two disease agents, it is a good idea to start leafhopper and planthopper cultures from eggs which can be excised from the plant.

V.D. Damsteegt: We rear leafhoppers in a greenhouse. The greenhouse temperature stays the same, but day length varies. Will that give us the seasonal variation that you find outside?

L.R. Nault: Yes, day length and light intensity will affect the plant which will in turn affect nutrients the vector receives from the plant. There may also be a direct effect of day length on vector diapause, etc.

G. Martinez-Lopez: We have been talking about a situation where the same vector transmits different disease agents in the laboratory. How do you prevent contamination?

L.R. Nault: This is the greatest problem I face. If I am working with pathogens that are vector specific, I really do not have much of a problem. However, if a single species is the vector for several pathogens, you will need to rear these vectors in separate plant growth chambers, rearing rooms or greenhouses. At least your stock cultures of pathogen-free vectors should be maintained in isolation. Contamination of the stock colony can occur. The only way to eliminate contaminants is to do as Gene Rosenkranz suggested, that is, to excise eggs, since these maize pathogens are not transovarially passed.

Virus Disease Epiphytology: Developing Field Tests for Disease Resistance in Maize

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Approved for publication as Article No. 193-76 of the Ohio Agricultural Research and Development Center, Wooster. Based on cooperative investigations of the Agricultural Research Service, U.S. Department of Agriculture, and the Ohio Agricultural Research and Development Center, Wooster.

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The authors express appreciation to L.M. Russell for assistance in aphid identification.

ABSTRACT

Principles related to characterization of maize virus disease epiphytotics are discussed. Techniques suitable for evaluating or measuring individual factors contributing to disease outbreaks are described. Data on disease intensity, vector populations, and vector infectivity in Ohio are presented. Field techniques suitable for evaluating maize lines for resistance to maize chlorotic dwarf virus and strains of maize dwarf mosaic virus are described.

Under the subject of virus disease ephiphytology, we wish to discuss some principles related to the character, ecology, and causes of outbreaks of maize virus diseases. Since our primary interest is in maize dwarf mosaic (MDM) and maize chlorotic dwarf (MCD), the discussion will relate more directly to these two major virus diseases of maize in the U.S.A. Why do these diseases occur? Why do epiphytotics develop in specific areas of southern Ohio, Georgia, and Mississippi, but not in northern Ohio, Iowa, or Wisconsin? Although we have data to supply some answers to these questions, we still do not have those diseases completely controlled.

A first step toward the development of satisfactory control techniques is an adequate characterization and understanding of factors that lead to disease outbreaks. With insect-vectored maize viruses, our knowledge must relate to the vectors, viruses, the host plants, and their interactions with each other and the environment. Information needed to understand and properly manipulate the insect vector under both field and laboratory conditions includes: insect identity, infectivity in nature, transmission efficacy in various environments, population and seasonal occurrence, alternate and overwintering hosts, and the influence of environmental conditions on vector biology and behavior. We also need similar information for the virus. Knowledge of its identity, the presence or absence of strains, seasonal occurrence, and overwintering hosts may lead to the development of suitable disease control techniques. Additional information on virus purification, serology, and particle morphology may aid in detection and identification of the pathogen.

The most essential information related to the maize host plant is the development of suitable methods for detecting and measuring resistance or tolerance to vectors and viruses. Once resistance is detected, its basis and method of inheritance or perpetuation must be established.

A simplified relationship between the vector, virus, host plant and environment is presented in Fig. 1. The terms inoculation pressure and disease potential are relative and may be equated with the probabilities that a host plant will be inoculated and that disease will occur. A disease epiphytotic is likely when large numbers of susceptible maize plants are present and inoculation pressure is high. Inoculation pressure will be high when large numbers of viruliferous vectors are present and when they are actively moving from host plant and feeding or probing. All that is required to prevent an epiphytotic is to eliminate the susceptible host (grow a resistant variety), or reduce the inoculation pressure to zero by removing the vector or the virus source. If resistant hosts are not available, attempts to control the disease should be concentrated on reducing the numbers of viruliferous vectors in maize fields. For both MDM and MCD control, the elimination of Johnsongrass (*Sorghum halepense* [L.] Pers.) in and around maize fields may be effective. By limiting

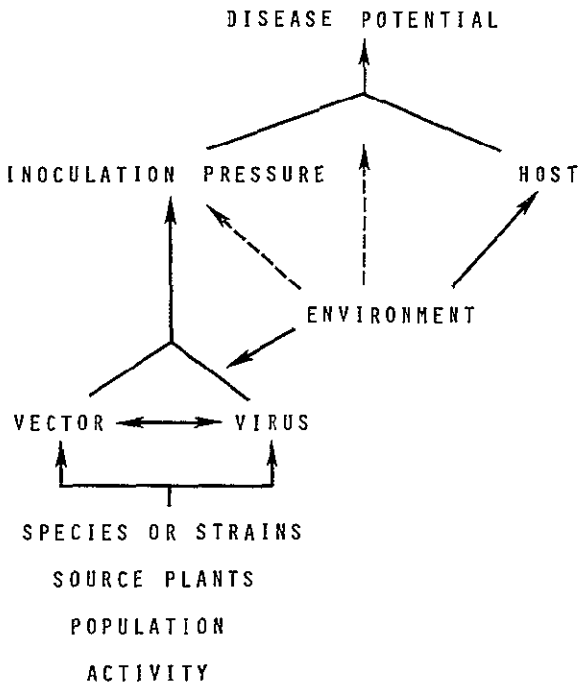

Fig. 1 Relationship between vector, virus, host plant, and environment in the development of maize virus disease epiphytotics.

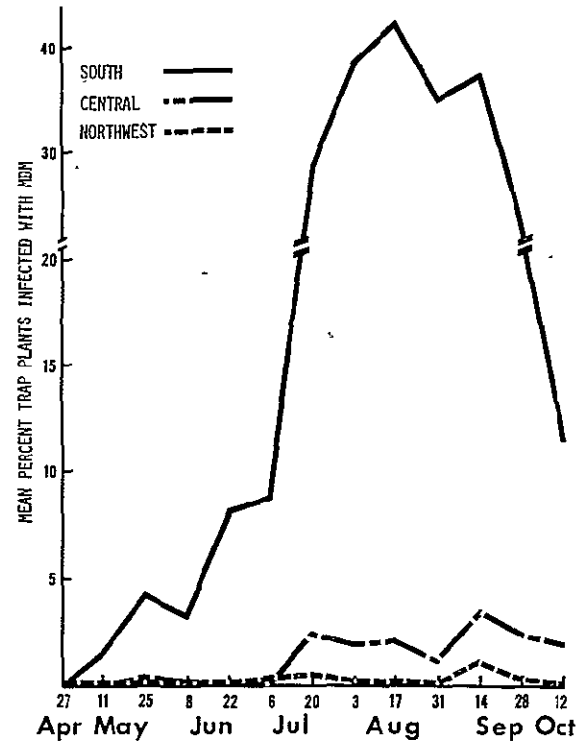

Fig. 2. Inoculation pressure for MDM in three areas of Ohio as measured by trap plants.

this overwintering host for MDMV and MCDV, the numbers of viruliferous aphids and leafhoppers would be minimized. Treating maize with certain systemic insecticides also can reduce disease incidence of the semipersistent and persistent types of viruses (4).

To evaluate the effectiveness of any control directed towards the vector or virus source, the inoculation pressure must be accurately measured. Furthermore, in valid attempts to detect resistant varieties, the inoculation pressure must also be standardized for individual viruses or virus strains. This will permit results from different tests to be uniformly compared and evaluated.

The method we use to measure inoculation pressure consists of exposing trap plants at field sites (3). These trap plants are groups of 14-day-old, virus-susceptible maize lines exposed in a bare soil area of a field in 4-inch pots for a 7-day period. Each week throughout the growing season new plants are placed in the field and those previously exposed returned to the greenhouse where the number of diseased plants is recorded for a 3-5 week period.

This trap plant technique provides the type of information shown in Figure 2. As indicated, the inoculation pressure for MDM was high in southern Ohio from mid-July until late September, with a very low percentage of infection in trap plants exposed in central or northern Ohio. MDMV-susceptible maize planted in southern Ohio would most likely become infected. Maize planted

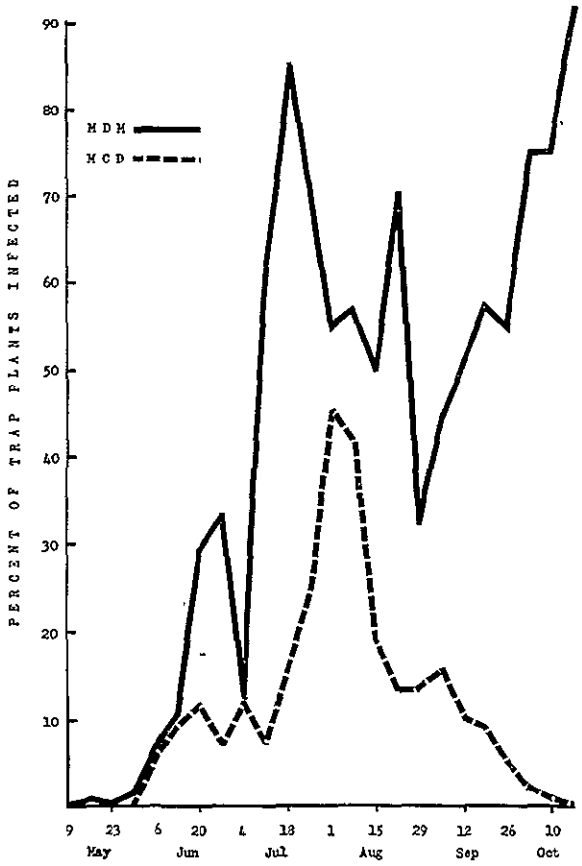

Fig. 3. Inoculation pressure for MDM and MCD in a field area of southern Ohio.

in central or northern Ohio would probably remain healthy. If diagnostic symptoms for two or more diseases can be distinguished in the same trap plant, or if trap plant lines used are differentially susceptible to the viruses present, then inoculation pressures for more than one disease can be measured (5). Inoculation pressure values in Figure 3 indicate that MDM was more prevalent than MCD at a field site in southern Ohio. However, the relative incidences of the two diseases, as measured by trap plant lines, do not necessarily coincide with their incidences in field plots. This is because the amount of infection in trap plant lines depends not only on the amount of inoculum present, but also on the relative susceptibility of these trap plant lines to the various viruses. By using the same carefully selected trap plant lines at various test sites, a valid measure of inoculation pressure can be obtained that will permit comparisons between different locations, or between different years at one location, for each of the recognized virus diseases.

Individual factors that contribute to the high inoculation pressure may also be measured. For example, aphid populations can be estimated by using yellow pans filled with water (9). Aphids are attracted to the yellow color, trapped in the water, and easily removed and counted. Typical aphid population curves, based on data from yellow pans (Fig. 4), suggest that there may be more aphids present during mid-summer in northwestern Ohio than in central or southern Ohio. However, the more critical, early-season peak in aphid population in the south represents the type of aphid vector activity that could account for the development of MDM epiphytotics in that area. Conversely, the lack of early-season aphid activity in more northerly areas would not favor MDM epiphytotics.

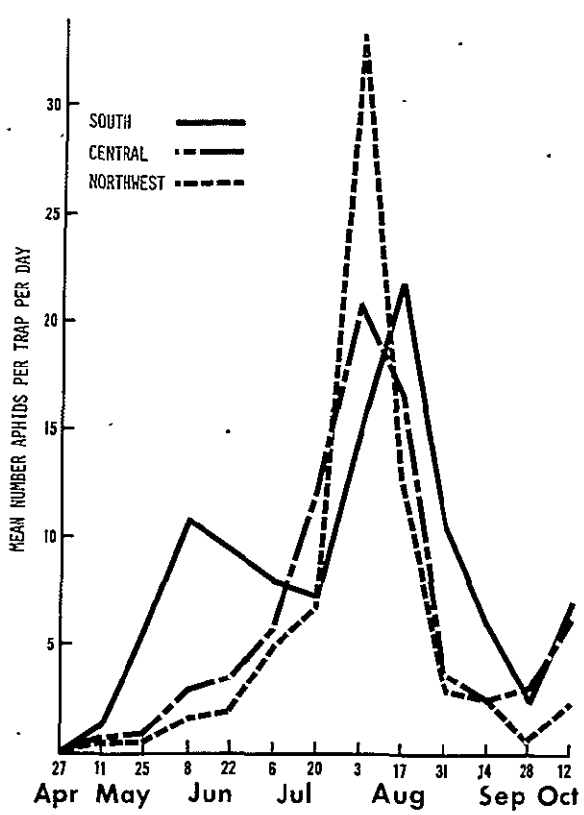

Fig. 4. Seasonal aphid population curves as measured by yellow pan traps exposed in three areas of Ohio.

A comparison of temperature, aphid, and trap plant data from one site in southern Ohio (Fig. 5) suggests that the following events may lead to the development of natural MDM epiphytotics. As temperature increased in the spring, aphids emerged from overwintering hosts and migrated to maize fields. During this time, aphids fed on MDMV-infected Johnsongrass, acquired virus, and inoculated young maize plants in the field and adjacent trap plants. This spring migration reached its peak in early June. By July, aphids had more or less settled on their summer hosts. However, the inoculation pressure, as measured by infection in trap plants, continued to increase steadily. This was because many of the infected young maize plants and annual weeds common to maize fields (13) now served as a source of inoculum. Thus, more of the few aphids present became viruliferous and contributed substantially to secondary spread. By mid-August, when the summer aphid population reached its peak, the infection in trap plants reached over 90%. It then remained relatively high until a killing frost eliminated the MDMV-infected source plants and aphids could no longer acquire the virus. From mid-August until about mid-September, a direct relationship between temperature, aphid numbers and inoculation pressure was apparent. Warmer periods resulted in greater aphid activity and increased virus transmission.

Another example of an attempt to measure individual factors contributing to the inoculation pressure value is the direct assay of aphids. Throughout one summer we assayed alate aphids that were flying in a field heavily infected with MDMV near Portsmouth, Ohio. To identify those viruliferous individuals captured, single aphids that had alighted in a yellow pan trap were transferred with a small brush to a small clip-on plastic cage containing the youngest exposed leaf of a 14-day-old WF9xOh51A maize seedling. This hybrid had previously been selected for its susceptibility to MDMV. Each aphid was confined to its test seedling within 10 minutes after a flight that normally occurred in the evening, no earlier than 2 hours before sunset. After a 24-hour inoculation access period, the aphid was removed from the cage, stored in alcohol, and later identified. Test plants were held for 3 weeks in a greenhouse to observe the development of MDM symptoms.

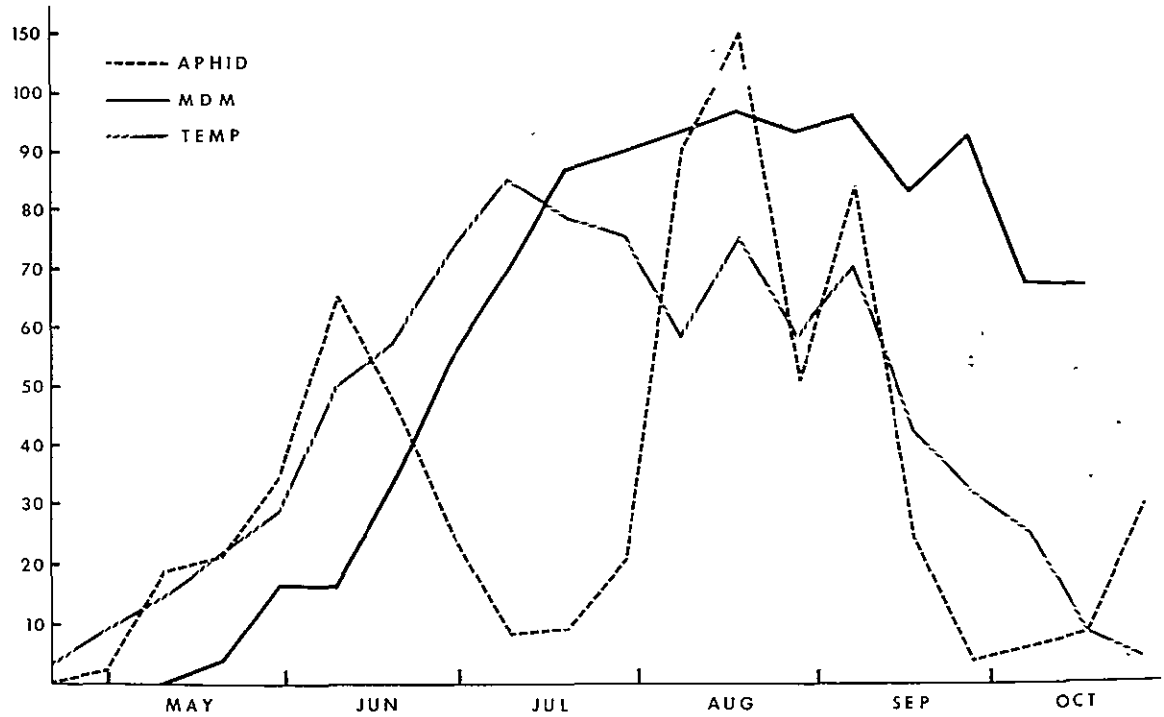

Fig. 5. A seasonal comparison of aphid populations, MDM inoculation pressure, and average temperature for one test site in southern Ohio.

At least 13 species of aphids were found to carry MDMV in the field (Table 1). About one third of the individuals captured were *Myzus persicae* (Sulzer), and accounted for just over one half of the MDMV transmission. The "number infected" column in Table 1 probably reflects the relative importance of various aphid species in MDMV transmission for this location and sample period. The ubiquitous (7) and polyphagous (14) *M. persicae* accounted for 7-8 times more MDMV transmission than *Rhopalosiphum maidis* (Fitch) or *Aphis maidiradicis* Forbes, two species that develop on maize (14). Of these 13 field-positive vectors of MDMV, six species had been recorded as vectors of MDMV in the laboratory (1,6,8,10), and several species were previously found in a similar MDM field area in southern Ohio (2). One species, *Hyadaphis erysimi* (Kaltenbach), was a vector here but failed to transmit all six strains of MDMV in the laboratory (6). Aphids in this study that failed to transmit MDMV are included in Table 2. Three of these species, *Schizaphis graminum* (Rondani), *Macrosiphum euphorbiae* (Thomas), and *Acyrthosiphon pisum* (Harris), are known vectors of MDMV in the laboratory (1,6,10). Since *S. graminum* is an efficient vector of MDMV-A in the laboratory (10), and relatively large numbers were captured in the field early in the season, this vector may be important in initiating MDM epiphytotics in southern Ohio. Although early season MDMV transmission does occur in this area (3), none of the 489 aphids captured during May and June transmitted MDMV. Those captured and assayed during July, August, and September transmitted MDMV at a rate of 9.5, 7.0, and 6.0%, respectively.

TABLE 1. Viruliferous alate aphids captured and assayed in an MDM field area near Portsmouth, Ohio

Aphid	No. plants	
	assayed	infected
Myzus persicae (Sulzer)	459	33
Rhopalosiphum maidis (Fitch)	114	5
Hyadaphis erysimi (Kaltenbach)	49	4
Aphis maidiradicis Forbes	28	4
Aphis gossypii Glover	49	2
Dactynotus ambrosiae (Thomas)	48	2
Hyalopterus atriplicis (L.)	21	2
Aphis craccivora Koch	20	2
Rhopalosiphum fitchii (Sanderson)	31	1
Drepanaphis sp.	22	1
Longistigma caryae (Harris)	2	1
Rhopalosiphum nymphaeae (L.)	2	1
Acyrthosiphon dirhodum (Walker)	1	1
Unidentifiable	226	6
Others (nonviruliferous)	311	
TOTAL	1383	65

At least two factors may have contributed to the lack of transmission by *S. graminum* (and possibly other aphids) in this study. This species rapidly loses its ability to transmit MDMV after its

TABLE 2. Nonviruliferous alate aphids captured in MDM field area near Portsmouth, Ohio

Aphid	No. assayed	Month most aphids captured
Schizaphis graminum (Rondani)	101	5
Aphis rumicis L.	68	5
Macrosiphum euphorbiae (Thomas)	60	5
Capitophorus spp.[a]	33	7
Chaetosiphon sp.	7	8
Macrosiphum kiowanepum (Hottes)	7	7
Masonaphis sp.	6	6
Acyrthosiphon pisum (Harris)	5	6
14 species (1-4 aphids)	24	
TOTAL	311	

[a] Approximately equal numbers of *C. elaegni* (Del Guercio) and *C. hippophaes* (Walker).

acquisition feeding (11). Also, they were assayed after a flight of unknown duration. If this flight lasted for several minutes, many of the aphids may have become nonviruliferous during flight.

We also have made estimates of the population and infectivity of *Graminella nigrifrons* (Forbes) and *Macrosteles fascifrons* (Stål) leafhoppers, as they may relate to field occurrence of MCDV. To measure leafhopper populations we used a modified Johnson-Taylor VI-12 insect suction trap (15). The trap was altered by installing a 15-watt fluorescent blacklight vertically over its opening, surrounding this light and opening with one-eighth-inch mesh hardware cloth to exclude large insects, removing the disc-release mechanism and guide rod, and attaching the thread portion of a mason jar lid directly below the collection tube of the trap. A mason jar containing 70% alcohol was attached to this lid and usually removed at 24-hour intervals. Leafhoppers were then sorted and counted to determine their daily population.

To assay leafhoppers captured in a field area where MCD epiphytotics are prevalent, leafhoppers were collected one or two nights each week with the modified Johnson-Taylor trap. However, these insects were introduced into a holding cage containing corn seedlings rather than into the alcohol jar. Each morning after collecting, the holding cage was transported to the laboratory and the leafhoppers were removed, anesthesized with CO_2, and sorted to species. They were then confined to maize test plants, as described for aphid assays. However, 5 or 25 leafhoppers were introduced into each cage and exposed to test seedlings within 12 hours of their capture. Following an inoculation access period of 48 hours, test seedlings were held in the greenhouse 4 weeks for symptom development. These time periods are suitable for detecting the semipersistent transmission of MCDV (12).

During one summer more than 4,100 *G. nigrifrons* adults were caged on about 550 test plants. Assuming that only one leafhopper was viruliferous for each infected assay plant, only 13 individuals or about 0.3% of the *G. nigrifrons* transmitted MCDV. None of more than 3,000 *M. fascifrons* transmitted MCDV.

Field studies involving individual viruses or virus strains must be conducted outside the area of natural virus occurrence if a virus complex exists in the naturally infected area. In northern Ohio, host plant reactions to one virus may be observed in isolated plot areas without natural contamination from other viruses. Observations on the spread of one virus in a maize field may be made by planting a susceptible variety in a grid pattern, inoculating a group of plants in the center of the plot, and recording, by position, the presence of infected plants in the plot at various time intervals after introduction of the virus source. At Wooster, studies of this type suggested that plants immediately adjacent to an MDMV source became infected first. Also, most of the infected plants were east or north (downwind) from the initial source of virus and, in plots containing the test line WF9xOh51A, MDMV-B was transmitted about twice as often as MDMV-A.

Field trials have also been conducted near Wooster to detect and measure resistance of maize lines to individual virus strains and aphids. These field trials were of three types: (1) the mechanical inoculation of test lines with MDMV strains, (2) the mechanical inoculation of source rows to allow naturally occurring aphids to transfer virus from source row to test lines, and (3) the uniform distribution of a source of MCDV in a field plot to permit natural *G. nigrifrons* vectors to inoculate test lines.

To mechanically inoculate test lines or source rows with MDMV strains, a tractor-mounted air compressor, and an artist air brush were used to spray plants with a suspension of buffer, carborundum, and plant sap from maize seedlings infected with one MDMV strain. Plants were normally inoculated twice, at 14 days after planting and again 1 week later. Periodic observations of inoculated plants to record the presence of symptoms can provide information on resistance of maize lines. These plots were planted in May or early June so that plant growth would be near normal and plot readings for infection could be completed before the seasonal aphid populations increased and possibly resulted in additional virus transmission that may confound the data.

Plots in the second type of field trial were to aid in detecting resistance to aphids or to aphid transmission of MDMV strains. In these plots, each two rows of test lines were alternated with one row of WF9xOh51A hybrid. When this MDMV-susceptible hybrid was inoculated with the air brush, it served as a uniform virus source for

naturally occurring aphids. By comparing percentage infection in a line after aphid inoculation with percentage infection in that same line after direct mechanical inoculation, lines possessing some aphid resistance may be detected.

Planting date for these plots was based on the mean seasonal aphid population curve for this area. Planting at Wooster was delayed until the first week in July so that source plants and the test lines would be in a susceptible physiological state when they were exposed to peak aphid populations in late July or early August.

The third type of isolated field trial at Wooster was to measure resistance to MCDV in maize lines. Since this virus is not mechanically transmitted, we must rely on the natural population of *G.nigrifrons* to effect transmission from rows or areas of MCDV-infected source plants uniformly distributed within the test plot. To serve as this virus source, both maize and Johnsongrass seedlings were inoculated with MCDV by *G. nigrifrons* in cages in the greenhouse and later transplanted in the field test area. Planting time for these lines was governed by the *G. nigrifrons* population peak for this area. Barley planted in the field area immediately surrounding the test plot may assure that vector populations are sufficient to produce local epiphytotics.

In field studies with viruses that cannot be mechanically transmitted, it is not possible to distinguish between host plant resistance to the vector and to the virus. In these cases, additional field or laboratory trials must be conducted on the maize lines to establish their resistance to vector feeding and determine if they attract or repel the vector.

These three types of field trials have two major advantages over host plant resistance trials conducted only in field areas where a virus complex exists. First, by including similar maize lines in each of these isolated field trials, and exposing them to a uniform inoculation pressure, we can compare maize lines for resistance to individual viruses. Second, we can detect maize lines that are resistant to one virus but hightly susceptible to another. These resistant lines and their potentially valuable germplasm may be lost or undetected if field testing is conducted only in a multiple virus area and one virus severely damages the plant.

LITERATURE CITED

1. BANCROFT, J.B., A.J. ULLSTRUP, MIMI MESSIEHA, C.E. BRACKER, and T.E. SNAZELLE. 1966. Some biological and physical properties of a Midwestern isolate of maize dwarf mosaic virus. Phytopathology 56:474-478.
2. BLAIR, B.D. 1970. Aphids collected from a Scioto County, Ohio corn field and areas bordering the field. J. Econ. Entomol. 63:1099-1101.
3. KNOKE, J.K., RAYMOND LOUIE, R.J. ANDERSON, and D.T. GORDON. 1974. Distribution of maize dwarf mosaic and aphid vectors in Ohio. Phytopathology 64:639-645.
4. KNOKE, J.K. 1977. Control. The use of pesticides for vector management. Pages 129-131 in Proc. Maize Virus Dis. Colloq. and Workshop, 16-19 Aug. 1976. Ohio Agricultural Research and Dev. Center, Wooster. 145 p
5. LOUIE, RAYMOND, J.K. KNOKE, and D.T. GORDON. 1974. Epiphytotics of maize dwarf mosaic and maize chlorotic dwarf diseases in Ohio. Phytopathology 64:1455-1459
6. LOUIE, RAYMOND, and J.K. KNOKE. 1975. Strains of maize dwarf mosaic virus. Plant Dis. Rep. 59:518-522.
7. MEDLER, J.T., AND A.K. GHOSH. 1969. Keys to species of alate aphids collected by suction, wind, and yellow pan water traps in the North Central States, Oklahoma and Texas. Univ. of Wisconsin Res. Bull. No. 277. 99 p.
8. MESSIEHA, MIMI. 1967. Aphid transmission of maize dwarf mosaic virus. Phytopathology 57:956-959.
9. MOERICKE, V. 1950. Uber das Farbsehen der Pfirsichblattlaus (Myzodes persicae Sulz.). Z. Tierpsychol. 7:265-274.
10. NAULT, L.R., and R.H.E. BRADLEY. 1969. Acquisition of maize dwarf mosaic virus by the greenbug, Schizaphis graminum. Ann. Entomol. Soc. Amer. 62:403-406.
11. NAULT, L.R., H.J. HARLAN, and W.R. FINDLEY. 1971. Comparative susceptibility of corn to aphid and mechanical inoculation of maize dwarf mosaic virus. J. Econ. Entomol. 64:21-23.
12. NAULT, L.R., W.E. STYER, J.K. KNOKE, and H.N. PITRE. 1973. Semipersistent transmission of leafhopper-borne maize chlorotic dwarf virus. J. Econ. Entomol. 66:1271-1273.
13. NAULT, L.R., D.T. GORDON, DIANE C. ROBERTSON, and O.E. BRADFUTE. 1976. Host range of maize chlorotic dwarf virus. Plant Dis. Rep. 60:374-377.
14. PALMER, M.A. 1952. Aphids of the Rocky Mountain Region. The Thomas Say Foundation. Vol. V. The A. B. Hirschfeld Press. Denver, Colorado. 452 p.
15. TAYLOR, L.R. 1951. An improved suction trap for insects. Ann. Appl. Biol. 38:582-591.

DISCUSSION OF J.K. KNOKE PAPER

V.D. Damsteegt: We set our yellow pans on the ground, where they stay all year. Yours were on a stand. Do you raise their height as the canopy goes up?

J.K. Knoke: It is most important to get the yellow pan off the ground, or in some way cover the surrounding areas so that soil does not splash into it. Presence of soil in the trap decreases trapping efficiency. We finally settled on a 2-foot height for yellow pans. Rather than worry about raising it up as the canopy increases, we found it best to clear an area of foliage about 5 feet around the yellow pan. If the pan is hidden under tall corn, aphids would not find it. All you would catch in the pan in that case are possibly a lot of wingless forms that are developing on adjacent corn plants.

V.D. Damsteegt: Do you keep the pans out throughout the season? How often do you collect from them?

J.K. Knoke: That depends on the available help and where the plots are located, but the yellow pans work best if you remove the insects daily. We collect every day near Wooster. In southern Ohio, when our summer help is not there, we attempt to extend the collection over 7-day periods. This longer period can be used if you clean out the pans and add fresh water after every collection. Even at 95 F temperatures and low humidity, the water will generally last a week.

G. Martinez-Lopez: How do you handle the manipulation of aphids to assure that you can detect those that are viruliferous

J.K. Knoke: Certainly, we have no control over what the aphid has done from the time it has left the plant and flown into the area where we are collecting. If the aphid comes from 5 miles away on a gentle breeze, it may have taken 30 minutes to get there. If it has taken 30 minutes to get there, the aphid probably is not carrying virus when we trap it. To detect viruliferous aphids, we must assay in the field where the insects are flying. First of all, good aphid flights normally occur about sunset, so you need to be there in the evening. You will not trap many insects if you have too much wind. With strong winds, they will be blown past your collection site. Therefore, you cannot assay every day. Our assay technique consists of placing several yellow pans around a bare soil area where we have test plants, cages, and everything else needed to make the assay. When an aphid lands in the yellow pan, we pick it up with a small brush, place it in a cage, clamp the cage over the maize leaf, and put a rubber band around the cage and the supporting stake. It takes less than 1 minute to capture the aphid and confine it on the test plant. Again, we have no control over whether the aphid feeds or not. However, it is exposed to a test plant and has had a period of fasting immediately before capture when in flight. If many insects are coming in, we select aphids from only one yellow pan. If few aphids are flying, we use several yellow pans or a larger yellow painted surface. The important point is to confine the aphid on a test plant immediately after it flies into the collection area.

R. Gamez: Is the yellow color on your pans equally attractive to all different species of aphids or leafhoppers?

J.K. Knoke: We capture essentially no leafhoppers in the yellow pans. I am sure that any given color is not equally attractive to all aphid species. For that reason it is not an entirely accurate sampling tool. But we catch many aphid species in the pan, and I believe it is the best tool available. The yellow color is important. We use canary yellow enamel paint, produced by Pratt and Lambert. I have tried more orange and more green shades, but canary yellow is the best.

R. Gamez: Do you have information on the aphid feeding pattern during the day? You mentioned that they were active in late evening. Is this correlated with wind velocity or light intensity?

J.K. Knoke: We have only a little information on this question and probably more for leafhoppers than for aphids. I am sure that it would be correlated with wind velocity, since during the day we generally have more wind than during the evening. At Wooster, we collect aphids for assay in the afternoon. On some days, we get the desired number of 50 aphids per day in a period of 15 minutes. On other days, we may collect from 1.00 pm to 5:00 pm and only catch 2 to 3 aphids.

L.M. Josephson: Do you use carborundum in your mechanical inoculations?

J.K. Knoke: Yes. We use a buffer suspension of carborundum and plant sap extracted from corn plants that were inoculated with the individual strains of viruses.

L.M. Josephson: Is it necessary to use 120 psi to get the virus particle in the plant?

R.W. Toler: We find that we are quite successful when we use carborundum at 60 psi Without carborundum, we do as well at 100 to 120 psi. With very young corn plants, three-leaf stage, inoculated in the field, we tend to kill too many cells.

J.K. Knoke: At very low pressure, you will get nozzle blockage more often than at higher pressures.

L.M. Josephson: That was our difficulty with the carborundum, even though we used a very fine grade We now get as good results by not using carborundum.

R.W. Toler: Another thing I have found with carborundum is that it wears out nozzels and tips very rapidly. We now use higher pressures and no carborundum, but if we wish to inoculate older plants we use carborundum.

V.D. Damsteegt: I have one comment on pressure. Talking about 100 psi is not significant unless you measure pressure at the orifice For example, let us compare a hose about 1 inch in diam with 100 psi versus a hose like an artist's airbrush that has about one-eighth inch diam. With a one-eighth inch hose, 100 psi will give a good percentage of infection, but the same pressure with a 1-inch hose will blow the plant off the bench.

R.W. Toler: Orifice to leaf distance also is critical.

Breeding Corn for Resistance to Virus in Ohio

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Cooperative investigations of the Agricultural Research Service, U.S. Department of Agriculture, and the Ohio Agricultural Research and Development Center. Approved for publication as Journal Article No. 190-76 of the Ohio Agricultural Research and Development Center, Wooster.

ABSTRACT

Breeding corn in southern Ohio for resistance to the virus disease complex, known to comprise several strains of maize dwarf mosaic virus (MDMV) and maize chlorotic dwarf virus (MCDV), was begun in 1965. Breeding methods included selfing in crosses of highly tolerant x susceptible inbreds, backcrossing to develop highly tolerant versions of some widely used inbreds, and developing breeding populations with a high frequency of genes for tolerance to the virus diseases. Performance trials have shown several hybrids with good tolerance to the virus disease complex found in southern Ohio. Genetic studies, involving mechanical inoculation and exposure to aphid inoculation of MDMV strains A, B, E and F, showed that resistance in inbred Pa405 was due to a single dominant gene, although in one cross with mechanical inoculation two dominant genes appeared to be required for resistance. In crosses involving inbred Oh07, resistance noted on the early rating date later broke down. The shift to susceptibility was attributed to increased virus concentration in the plants.

Breeding corn for virus resistance in Ohio was initiated in 1965, following a year of screening germplasm for virus reaction. In 1964, large numbers of inbred lines and hybrids and some exotic materials were rated for virus reaction near Portsmouth, Ohio. Fortunately, a good tolerance to the virus diseases present was found in a few adapted inbred lines and hybrids.

METHODS

Methods of breeding for virus resistance include backcrossing, followed by selfing to incorporate resistance into a few widely used inbred lines; selfing in crosses of resistant lines with lines possessing complementary agronomic traits; and developing breeding populations with high-frequencies of genes for virus resistance.

In breeding plots near Portsmouth, desirable plants were self-pollinated. When family structure was present, the pollinated plants determined to be most desirable for virus resistance were selected from progeny rows. Of course, attention was also given to other desirable agronomic characteristics. At harvest, ears were saved from selfed plants that rated best for virus reaction. Many recent selections were also tested for reaction to the maize dwarf mosaic virus (MDMV) strains and to maize chlorotic dwarf virus (MCDV) in the greenhouse.

PROGRESS IN DEVELOPING RESISTANCE

Success in breeding to improve the virus tollerance in inbred lines has been limited. However, three inbred lines with virus tolerance, developed in the Ohio program, have been released to private plant breeders and seed producers. Oh514 was released in 1968 and Oh509A and Oh513 in 1974. Oh509A did not show the tolerance to virus in the recent 1976 test plots that was observed in previous years. Apparently, during the advanced selection of Oh509A virus incidence was lower or different viruses of virus strains predominated.

Inbred lines differed in tolerance to virus. A few lines showed high tolerance, but most showed low or medium tolerance. Several inbreds have been found to differ in reaction to known strains of MDMV. For example, in field tests near Wooster, inbreds Oh7B, T232 and Ky61-2335 were resistant to strain A but susceptible to strain B. In early 1976 readings, inbred GA209 appeared resistant to strains A, B and E but susceptible to strain F; and inbred Oh513 appeared resistant to strains A and F and susceptible to strains B and E. However, later in the season, virus symptoms of strains E and F were indistinct in Oh513 and GA209, respectively. Tests to strain D were inconclusive due to poor plant growth. Pa405 was the only inbred tested that was most resistant to all known strains of MDMV; however, it showed local lesion reaction to MDMV-A in greenhouse tests.

We are still searching for high tolerance to MCDV, and breeding and selection appear to be bringing us closer. Breeding populations involved in our virus improvement program have narrow and broad genetic bases. Some populations involve only U.S. germplasm and others involve U.S. germplasm combined with germplasm from Central America.

The principal method for improvement of breeding populations has been the half-sib and full-sib selection methods. Full-sib or S_1 progenies of half-sib plants selected for virus resistance were also tested for yield, and resistance to stalk- and root-lodging, *Helminthosporium turcicum* Pass., and European corn borer *Ostrinia nubilalis* (Hübner), leaf feeding. Progenies found most promising were continued for further inbreeding and selection and recombined to reconstitute the population.

Oh(MDM)S1 and Oh(MDM)S2 are the two synthetic populations which have been in the virus program the longest. Preliminary progress from selection for virus tolerance in these two synthetics was tested in 1976. Four replicate tests were grown of cycles C_0, C_1, C_2 and C_3. Cycles C_1, C_2 and C_3 of Oh(MDM)S1 were from sib-matings of selected selfed plants. Cycle C_1 of Oh(MDM)S2 was from four generations of sib-matings of selected plants and cycles C_2 and C_3 were from sib-matings of selected selfed plants. Each cycle was represented by approximately 100 plants.

Analyses of variance of percentage MDM, MCD and healthy plants showed significance at the 5% level for the latter two traits in both synthetics. Selection in Oh(MDM)S1 (Fig. 1) was most consistent for MCD tolerance, 74% for C_0 and 43% for C_3. Healthy plant percentages decreased from 18% to 14% from C_0 to C_1, increased to 30% in C_2 but did not change from C_2 to C_3. Little progress was realized for MDM tolerance, C_0=36% and C_3=34%.

Progress from selection in Oh(MDM)S2 (Fig. 2) was good for MCD (60% in C_0 and C_1 to 26% in C_3) and healthy plants (28 to 59%) but most consistent for the latter. Progress for MDM tolerance was consistent but slow (22% to 10% from C_0 to C_3).

A yield trial including commercial and open-pedigree combinations has been grown in the virus plots near Portsmouth since 1968. Several hybrids have been found to have good tolerance to the virus diseases present. However, these hybrids, generally, do not stand or yield as well as adapted hybrids lacking virus tolerance. Earlier maturing hybrids with more virus tolerance than those presently available are needed for replanting in areas where flooding occurs.

Our challenge is to develop parent lines of hybrids with high tolerance to all known viruses

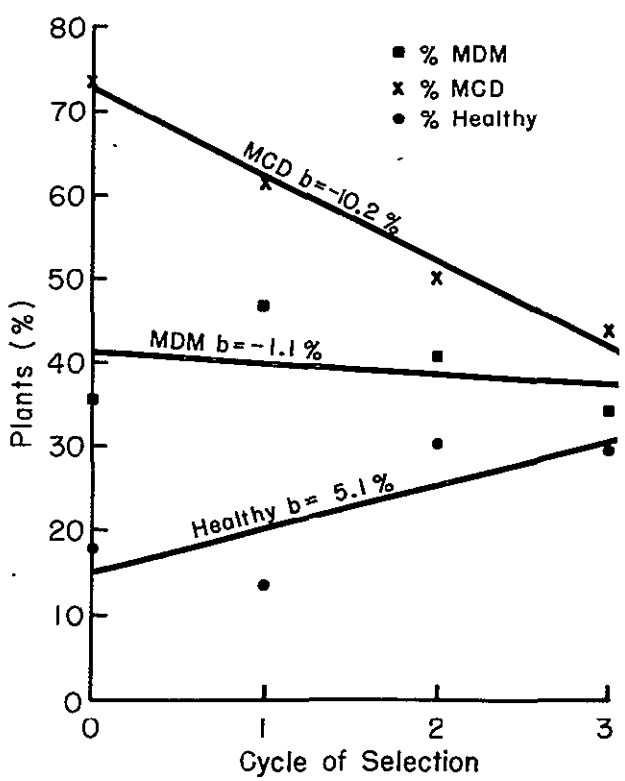

Fig. 1. Improvement in Oh(MDM)S1 for percent MDM, MCD, and healthy plants in three cycles of selection.

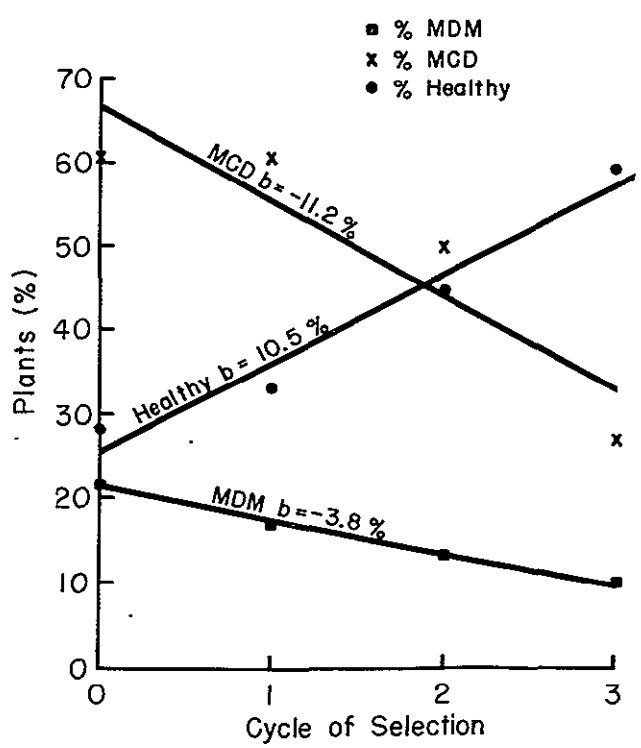

Fig. 2. Improvement in Oh(MDM)S2 for percent MDM, MCD, and healthy plants in three cycles of selection.

and virus strains that occur in the area of production, and to combine the tolerance with resistance to the common production hazards, such as stalk rots, *H. turcicum*, and European corn borer leaf feeding.

Regardless of hybrid planted, under high incidence of virus some yield loss undoubtedly will be experienced. A certain number of plants will be virus infected sufficiently to reduce yield, and some so severely as to yield little or no grain. Early virus infection and virus concentration in the plants appear to affect degree of host reaction. Or the hybrid may be susceptible to a virus or strain to which its reaction was previously unknown.

GENETICS OF RESISTANCE

Resistance inheritance to the MDM disease complex has been studied in segregating progenies by Loesch and Zuber (4); Josephson, Hilty and Arnold (3); and Dollinger, Findley and Williams (1). Resistance was found to be partially dominant to dominant and controlled by relatively few major genes (probably two), but several minor genes appeared to be required for a high degree of resistance or tolerance.

Using reciprocal translocations, Findley, Dollinger, Louie and Knoke (2) found major genes for resistance in inbred Oh07 associated with both arms of chromosome 6 and the short arm of chromosome 8. Other associations were found with the long arm of chromosomes 1 and 2, short arms of 3, 7 and 8, and both arms of 10. Similar associations were found in inbred Mo22, but with the possible exception of the short arm of chromosome 10, it appeared to lack major genes for resistance. Scott and Nelson (5) found resistance in inbred GA209 to be associated with both arms of chromosome 6.

We concluded that, although resistance inheritance to the MDM disease complex was difficult to explain on a Mendelian basis, inheritance to the components of the complex should be interpretable on a relatively simple genetic basis. Therefore, experiments were conducted on resistance inheritance to strains A, B, D, E and F of MDMV.

In 1974, generations P_1, P_2, F_1, F_2, B_1, B_2, B_1S and B_2S of the crosses Oh28xOh07 and Oh28xPa405 were tested to MDMV strains A and B, respectively. Generations B_1 and B_2 were backcrosses to the susceptible parent Oh28(P_1) and resistant parent Oh07(P_2) or Pa405(P_2), respectively, and B_1S and B_2S were selfs of the respective backcrosses. Test plants were either mechanically inoculated or exposed to aphid vectors. Seeds of the test generations and inoculum-source rows of WF9xOh51A were planted on July 8 so that plant growth coincided with peak aphid populations. One inoculum-source row was alternately planted to two test-plot rows throughout the experimental area. Separate fields for each virus strain prevented contamination. The experiments were conducted at Wooster, where MDMV does not occur naturally.

On July 29 and August 5, mechanical inoculations of the test plants with each virus strain were made at 7-8.5 kg/cm^2 with a tractor mounted artist's air brush. At the time of inoculation, plants were in the 3- to 5-leaf stage. All plants in test rows in the areas involving mechanical inoculation were inoculated; in areas involving natural aphid transmission of virus, only plants in source rows were mechanically inoculated.

Individual plants inoculated with MDMV-A or -B were observed for chlorotic streaks, mottle, flecks, rings and mosaic virus symptoms on August 12 (August 13 for MDMV-B), August 26 and September 9. Virus symptoms were not evident on test plants in the aphid transmission areas on the early dates.

TABLE 1. Percent of virus-infected plants on three dates (1974) after mechanical inoculation with MDMV-A

Generations	Date 8-12	8-26	9-9	Total plants
	Percent infected plants			
P_1 - Oh28	86.3	100.0	100.0	73
P_2 - Oh07	0	72.7	80.5	77
F_1 - P_1xP_2	2.7	79 3	98 2	111
B_1 - $(P_1xP_2)xP_1$	51 8*	95.2	97 6	83
B_2 - $(P_1xP_2)xP_2$	1.3	72.7	93.5	154
B_1S - $[(P_1xP_2)xP_1]$-S	61.9*	93.8	100.0	97
B_2S - $[(P_1xP_2)xP_2]$-S	13.9*	70.1	86.9	137
F_2 - (P_1xP_2)-S	32.3	80.4	98.3	235

*Single dominant gene for resistance, X^2 P > .05.

TABLE 2. Percent of virus-infected plants on two dates (1974) after exposure to aphid inoculation with MDMV-A

Generation	Date 8-26	9-9	Total plants
	Percent infected plants		
P_1 - Oh28	91 9	98.4	62
P_2 - Oh07	0	5.3	57
F_1 - P_1xP_2	1.8	22.5	111
B_1 - $(P_1xP_2)xP_1$	58.1*	74.4	86
B_2 - $(P_1xP_2)xP_2$	1.0	19.0	100
B_1S - $[(P_1xP_2)xP_1]$-S	57.0*	80.2	86
B_2S - $[(P_1xP_2)xP_2]$-S	12.1*	27.1	107
F_2 - (P_1xP_2)-S	29.6*	55 2	203

*Single dominant gene for resistance, X^2 P > .05.

On the August 12 ratings, ratios of diseased to healthy plants in generations B_1, B_1S and B_2S of Oh28xOh07 mechanically inoculated with MDMV-A (Table 1) indicated that resistance was controlled by a single dominant gene. By the August 26 and September 9 ratings, resistance in all generations broke down and susceptibility became dominant. Plants of the resistant parent Oh07 and F_1 generation were 80.5 and 98.2% infected, respectively.

On August 26, diseased to healthy plant ratios in segregating generations of Oh28xOh07 that were exposed to aphid inoculation (Table 2) indicated that resistance was controlled by a single dominant gene. By September 9, the percent of diseased plants in the F_1 generation increased to 22.5%. There also was an increase in diseased plants in all other generations.

In the crosses involving the highly susceptible inbred Oh28 with Oh07, the resistance gene(s) in the latter inbred apparently were overcome by increased virus concentration in the plants from multiplication and/or repeated inoculations by aphids. Recent greenhouse seedling tests of Oh07 and Oh07xOh28 also showed that the percentage of plants infected with MDMV-A increased with virus concentration in inoculum (R. Louie and W.R. Findley, *unpublished*).

In the MDMV-B mechanically-inoculated generations of plants of Oh28xPa405 (Table 3), the ratios of diseased to healthy plants on August 26 and September 9 indicated two dominant genes for resistance, except for generation B_1. Segregation in the B_1 generation indicated a single dominant gene for resistance. The percentage of diseased plants increased after the first rating date in all generations. Apparently, virus concentration had not reached a level in all susceptible plants sufficient for symptom expression by the first rating date.

TABLE 3. Percent of virus-infected plants on three dates (1974) after mechanical inoculation with MDMV-B

Generations	Date 8-13	8-26	9-9	Total plants
	Percent infected plants			
P_1 - Oh28	85.2	96.2	98.7	78
P_2 - Pa405	2.2	4.4	2.2	45
F_1 - P_1xP_2	0	4.8	3.6	83
B_1 - $(P_1xP_2)xP_1$	42.5	52.5	50.0	80
B_2 - $(P_1xP_2)xP_2$	0	1.4	.7	146
B_1S - $[(P_1xP_2)xP_1]$-S	50.9	85.5*	90.9*	55
B_2S - $[(P_1xP_2)xP_2]$-S	8.3	19.4*	26.4*	72
F_2 - (P_1xP_2)-S	19.7	45.1*	45.1*	122

*Two dominant genes for resistance, X^2 P > .05.

TABLE 4. Percent of virus-infected plants on two dates (1974) after exposure to aphid inoculation with MDMV-B

Generation	Date 8-26	9-9	Total plants
	Percent infected plants		
P_1 - Oh28	100.0	100.0	44
P_2 - Pa405	0	0	54
F_1 - P_1xP_2	0	0	96
B_1 - $(P_1xP_2)xP_1$	47.9*	47.9*	94
B_2 - $(P_1xP_2)xP_2$	0	0	153
B_1S - $[(P_1xP_2)xP_1]$-S	69.1*	76.4	55
B_2S - $[(P_1xP_2)xP_2]$-S	13.7*	19.2*	73
F_2 - (P_1xP_2)-S	29.8*	29.8*	161

*Single dominant gene for resistance, X^2 P > .05.

The aphid-exposed plants in generations of Oh28xPa405 segregated in ratios of diseased to healthy plants, indicating that resistance to MDMV-B was controlled by a single dominant gene (Table 4).

In 1975, generations P_1, P_2, F_1, F_2, B_1, B_2, and F_3 progenies of the cross M14xPa405 were inoculated mechanically and exposed to aphid inoculation of strains A, B, D, E and F of MDMV. The plots for mechanical inoculation were planted on May 9 and 12 and those for aphid inoculation on July 2. Inoculations of pure virus strains were made as described above. The mechanically inoculated plots were inoculated on May 27 or 28 and June 4 or 6, and the aphid plots were inoculated on July 15 or 16 and 22. Plants mechanically inoculated and exposed to aphid inoculation were observed for virus symtoms on June 30 and August 27, respectively. Data from the generations of plants exposed to aphid inoculation were less reliable due to a dry period that resulted in uneven plant emergence and are not reported. Plant growth was abnormal in the mechanically inoculated strain D plot due to low soil fertility.

TABLE 5. Results of mechanical inoculations with MDMV strains A, B, D, E, and F on June 30, 1975

Generations	Percent infected plants A	B	D	E	F	Total plants (range)
P_1 - M14	70	93	90	95	96	78-86
P_2 - Pa405	0	0	0	0	0	70-88
F_1 - P_1xP_2&P_2xP_1	0	1	0	0	0	161-178
B_1 - $(P_1xP_2)xP_1$	44*	39	27	57*	52*	214-237
B_2 - $(P_1xP_2)xP_2$	0	0	0	0	0	222-234
F_2 - (P_1xP_2)-S	16	25*	18	25*	28*	323-353

*Single dominant gene for resistance, X^2 P > .05.

Segregation in the generations of mechanically inoculated plants indicated that, in general, resistance was controlled by a single dominant gene (Table 5). Deficiencies in numbers of susceptible plants for the single-gene hypothesis occurred in F_2 for MDMV-A, B_1 for MDMV-B and F_2 and B_1 for MDMV-D.

Two replicate plots of 12 seeds each were planted of the F_3 progenies. Considering the data across strains of the 120 mechanically inoculated progenies, 23 were resistant, 28 susceptible and 69 segregating. The chi-square value for a 1:2:1 ratio was 3.09 ($P = 0.22$).

Reactions of resistant and segregating progenies were rechecked in 1976 for 51 MDMV-A, 47 MDMV-B, 11 MDMV-D, 16 MDMV-E, and 16 MDMV-F progenies. In almost every case the hypothesis established for the progeny in 1975 was confirmed. Exceptions were explainable by results of aphid inoculations in 1975. Additional studies of the F_3 progenies are planned to attempt to further substantiate the results, and to correlate progeny by virus strain reaction.

LITERATURE CITED

1. DOLLINGER, E.J., W.R. FINDLEY, and L.E. WILLIAMS. 1970. Resistance inheritance to maize dwarf mosaic virus in maize (Zea mays L.). Crop Sci. 10:412-415.
2. FINDLEY, W.R., E.J. DOLLINGER, RAYMOND LOUIE, and J.K. KNOKE. 1973. Locating genes for maize dwarf mosaic resistance by means of chromosomal translocations in corn (Zea mays L.). Crop Sci. 13:608-611.
3. JOSEPHSON, L.J., J.W. HILTY, and JERRY ARNOLD. 1967. Inheritance of tolerance of corn inbreds to the maize dwarf mosaic virus. Agron. Abstr p. 13.
4. LOESCH, P.J., JR., and M.S. ZUBER. 1967. An inheritance study of resistance to maize dwarf mosaic virus in corn (Zea mays L.). Agron J. 59:423-426.
5. SCOTT, GENE E., and LLOYD R. NELSON. 1971. Locating genes for resistance to maize dwarf mosaic in maize seedlings by using chromosomal translocations Crop Sci. 11:801-803

DISCUSSION OF W.R. FINDLEY PAPER

E.E. Rosenkranz: It is interesting that Pa405 is the only inbred line that is resistant to all six strains of maize dwarf mosaic virus and yet is very susceptible to maize chlorotic dwarf virus.

J.M. Fajamisin: Is there any relationship between Pa405 and inbred Oh07?

W.R. Findley: I do not think so. Oh07 is from the cross CI 540 x Ill.L., and Pa405 comes out of NY3 x Pa54

J.M. Fajamisin: I am interested in population improvement. I have tried the full-sib method for developing resistance to rust and blight, but I did not find it very easy. So I discontinued it and am now using S_1 progeny selection. This gives me the opportunity to check the progeny for the percentage infection in the S_1. Do you think one would get a better result by combining percentage infection with a severity scale when rating for maize streak?

W.R. Findley: Yes, I think an S_1 progeny evaluation is good. We are leaning this way. I think you need to select the best S_1 progenies and recombine these with other progenies showing additional desirable traits

J.M. Fajamisin: Is yield considered too?

W.R. Findley: Yes, yield is also considered. For our 20 progenies that are recombined to reconstitute a population we cannot expect to get every one of the S_1 selections to have good virus, blight, and corn borer tolerance. So, what we do is to take the outstanding progenies for each trait. These will go back into the population. What we are trying to do, but have not progressed far enough to know how well it will work, is to maintain a high frequency of all the favorable genes in the population.

G.E. Scott: Do you think that your method would be more effective than cyclic selection?

W.R. Findley: I do not know if we can do that. This program is relatively new. We plan to use S_1 or full-sib progenies to reconstitute the population. However, if this method does not work, we will change.

G.E. Scott: One other question on the strain reaction. Are you keeping track of your F_3's, so that you can tell if the same genes are operating for reaction to strain A and strain B? These genes could be different.

W.R. Findley: Yes. We have identified the F_3 progenies. We plan to determine if the same gene(s) control reactions to the different strains.

J.M. Fajamisin: I think from my little experience that cyclic selection does not work very well, because of the difference in evolution of genes for yield. In your populations you are breeding for resistance.

G.E. Scott: But, if you try to breed for five characters at a time, your rate of progress on any one has to be slower.

B. Tsotsis: What is the size of your reconstituted source population, and how many plants do you maintain in the reconstituted population?

W.R. Findley: We try to start with 400 progenies.

B. Tsotsis: I notice that you are using the Oh(MDM)S1 and Oh(MDM)S2 populations. Does this indicate that more lines are common?

W.R. Findley: Yes.

B. Tsotsis: Are you trying to separate these into heterotic pools?

W.R. Findley: No. These are special populations developed specifically for sources of virus resistance.

B. Tsotsis: Your success in maintaining yield will depend on what success you have maintaining heterotic populations. It has been proved time and time again that the amount of heterosis is proportional to the amount of genetic diversity. If you have two separate pools and these pools are related, the expected heterosis would be lessened. I think it is possible to combine yield and traits. We have been doing some of this work, and we are now in the 19th cycle. But you have to use very sizable populations and select large numbers of plants for use in combining traits in long-term selections. In our program, we have about 8,000 plants per composite per cycle. Then, we superimpose various stresses on the populations. We find mass selection to be quite effective and when conducted in the presence of viruses many plants are eliminated. Since for many virus diseased plants there is no seed set or the plants die before pollination, this gives us the opportunity to work with larger numbers of potentially desirable plants.

W.R. Findley: I think that is right, except you soon reach the point where the loss of plants, due to susceptibility, no longer permits effective progress using mass selection. This occurs. because the level of susceptibility of the population approaches equilibrium.

B. Tsotsis: Yes. You have to make adjustments after awhile. I think that sib testing would be significant, since these exotic strains are highly heterozygous. You get a tremendous amount of inbreeding depression after selfing two or three cycles. Then you have to begin sibbing or reconstituting the population. One of the procedures that we have followed for several cycles is to assess these exotics or unadaptable materials. We generate and evaluate the S_1 and reconstitute the synthetic using S_1's. Then we let the synthetic be the basis for improvement of the original accession. We have done this several times, but after the S_3 generation we have great difficulty maintaining some of these synthetics in the program.

J.M. Fajamisin: The S_2 modification is also being used is it not?

B. Tsotsis: Well, this is not unorthodox, but it has to be fitted to the results or your interests. You can use several breeding schemes to salvage some of the selfed material. We are reconstituting the original accessions as candidates for the developmental nursery.

B.L. Renfro: In your virus resistance heritability studies, do you recognize only two classes, systemically and nonsystemically infected?

W.R. Findley: Yes.

B.L. Renfro: Do you think it would be worthwhile to look at your intermediate class? We did this in Raychaudhuri's report.

W.R. Findley: Do you mean plants that were rated as tolerant?

B.L. Renfro: Yes. Those plants with mild symptoms. You are not considering all the genes.

W.R. Findley: I am not sure whether genes or virus concentration is involved.

B.L. Renfro: Did you notice any change in virus effect in different generations?

W.R. Findley: Yes. We notice a difference, particularly from the S_1 to S_2 generation.

B.L. Renfro: Did you notice any virus effects associated with vigor?

W.R. Findley: Yes. Frequently, the S_1 showed good tolerance, and the S_2 was not worth saving.

Control: The Use of Pesticides for Vector Management

J.K. Knoke

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Approved for publication as Journal Article No. 192-76 of the Ohio Agricultural Research and Development Center, Wooster.

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ABSTRACT

Principles and problems related to the control of plant viruses by using insecticides to reduce vector populations are discussed. Attempts to control maize viruses with insecticides suggest that some systemic materials may effectively reduce field incidence of persistent or semipersistent viruses.

Logic behind the use of insecticides for virus control is very simple. It goes like this: Insects carry viruses; insecticides kill insects; therefore, insecticides will prevent virus spread. Control of plant viruses by insecticides has been attempted for about 50 years. Insecticides have been applied directly to the crop to be protected, to grassy areas around crops, to trap crops, and to permanent and temporary feeding and breeding sites of vectors at some distance from the crop to be protected. Since there is probably an effective pesticide available for all known vectors, control of insect-borne viruses should be readily accomplished. However, it was soon learned in early virus control investigations that insecticides frequently were of little use (1).

Problems of controlling insect vectors and, therefore, the viruses they transmit, are different from direct control of insect pests. With pests causing direct damage to the crop, the injury sustained is roughly proportional to the number of insects present on the crop multiplied by the total time that the insects are present (1). In contrast, direct feeding damage to a crop by an insect vector in the act of transmitting a virus is relatively rare. Major crop losses are normally caused by the virus rather than the vector.

For an insect transmitted virus to spread within a crop, we must have not only the presence of the vector for a significant time but the vector must be actively feeding and moving about and there must be a virus source. It would seem that these additional requirements of vector activity and presence of virus would provide additional time or opportunity to contact the vector with pesticide before virus transmission can occur. In certain vector-virus-crop associations, however, the most effective pesticides act too slowly to prevent transmission.

Several basic conclusions relative to pesticide efficacy for virus control have evolved after years of testing against many different vector-virus associations. These may be summarized as follows. First, if both the vector and the virus originate from within a crop, then virus control by pesticides will be relatively easy. Simply apply an insecticide to eliminate the vector or an herbicide to eliminate the alternate host of the virus. Most virus problems do not fit this easy-to-control situation. Second, foliar-applied insecticide sprays give poor virus control. This is because they kill too slowly, have short residual activity, do not protect new plant growth, and may kill the normal parasites or predators of the vectors. In some cases applications of pesticides may stimulate the vector to move around more and, thereby, increase virus spread (2). A third basic conclusion of a more positive nature is that soil-applied, systemic insecticides give better vector control than foliar sprays since they have long residual activity, protect new plant growth, and normally kill only insects feeding on the plant but not parasites or predators. However, since the built-in insecticide kills insects only after they feed on the plant, it has the disadvantage of not protecting against virus inoculation which can occur before sufficient toxicant is ingested to kill the vector. A fourth basic and well established conclusion is that insecticides give relatively good control of viruses which require a considerable time period for acquisition by the vector, incubation in the insect, and inoculation of the plant. In contrast, insecticides give poor or no control of nonpersistent or stylet-borne viruses that are acquired and transmitted in a short period of time.

Attempts to control maize viruses with insecticides began about 1960. Typical results and

conclusions that have been published in the last 15 years suggest only partial success. Results with foliar sprays indicated that DDT, malathion and parathion gave only erratic control of a persistent virus (maize rough dwarf) and that these sprays upset the balance of nature (5). Other research suggested that five weekly sprays of azodrin, methyl parathion, carbaryl or baygon gave no control of corn stunt or maize dwarf mosaic (MDM) (9). Even though many of these toxicants are good insecticides for control of leafhoppers. planthoppers or aphids, I believe that we must conclude that foliar sprays generally fail to protect maize from infection by persistent and nonpersistent viruses.

On the positive side, in Italy it was found that some control of maize rough dwarf could be obtained by spraying grass borders around corn fields to eliminate overwintering planthopper vectors (4). This technique required less pesticide than would be necessary for spraying the maize fields. The obvious conclusion: sprays may be of some use in virus control if directed to the proper site at the proper time.

Better and more consistent control of maize viruses may be obtained by using certain systemic insecticides applied in a band near or in the furrow at planting time. For example, in Rhodesia, it was found that about 30 kg/ha of 10% granular aldicarb applied to the planting furrow gave almost complete protection of maize from the maize streak virus for 90 days (11). In Missouri, about a 70% reduction in a persistent type of virus disease [probably maize chlorotic dwarf (MCD)] was obtained by applying granular formulations of carbofuran to the furrow at planting time at 2.2 to 3.3 kg a.i./ha (6). Five other systemic materials, including the aldicarb that controlled maize streak, were less effective than carbofuran in virus control. In Mississippi, as little as 1.1 kg a.i./ha of carbofuran in the furrow resulted in a 70% reduction in corn stunt (also probably MCD) through 98 days after planting (3,10). Two other systemic insecticides were less effective in limiting the dissemination of this semipersistently transmitted virus (8). More recently, in Georgia, about 75% reduction in MCD was obtained 91 days after planting with 2.2 kg a.i./ha of carbofuran applied as 10% granules in the planting furrow (7). No significant reduction in MDM was obtained with this treatment. This type of information on soil applied systemics suggests in general that many systemic materials give poor control of maize virus diseases; MDM will not be controlled, even with the best toxicants, and at least one material (carbofuran) but possibly a second (aldicarb) are effective in reducing the field incidence of persistent or semipersistent types of maize viruses.

Carbofuran is not officially registered, recommended or being used in this country for control of the leafhopper vectors that transmit maize viruses. Carbofuran is registered on maize for control of armyworms, corn borers, rootworms, wireworms and flea beetles. Carbofuran at 1.1 kg a.i./ha applied as a band over the seed furrow is recommended in Ohio for control of corn rootworms. It is also recommended in Mississippi for control of the southwestern corn borer (H. N. Pitre, *personal communication*). Where it is used as recommended for these insects, growers may be obtaining significant additional benefits for vector and virus disease control in maize.

LITERATURE CITED

1. BROADBENT, L. 1957. Insecticidal control of the spread of plant viruses. Annu. Rev. Entomol. 2:339-352.
2. CARTER, W. 1973. Insects in relation to plant disease. John Wiley and Sons, New York. 2nd ed 759 p.
3. GORDON, D.T. and L.R. NAULT. 1977. Involvement of maize chlorotic dwarf virus and other agents in stunting diseases of Zea mays in the United States. Phytopathology. 67:27-36.
4. GRANCINI, P., and A. CORTE. 1969. Il nanismo ruvido del mais. Stato attuale delle conoscenze sulla malattia e prove di lotta contro i vettori. Maydica 14:79-93.
5. HARPAZ, I. 1972. Maize rough dwarf. Israel Universities Press, Jerusalem. 251 p.
6. KEASTER, A.J., and M.L. FAIRCHILD. 1968. Reduction of corn virus disease incidence and control of south-western corn borer with systemic insecticides. J. Econ. Entomol. 61:367-369.
7. KUHN, C.W., M.D. JELLUM, and J.N. ALL. 1975. Effect of carbofuran treatment on corn yield, maize chlorotic dwarf and maize dwarf mosaic virus diseases, and leafhopper populations Phytopathology 65:1017-1020.
8. NAULT, L.R., W.E. STYER, J.K. KNOKE, and H.N. PITRE. 1973. Semipersistent transmission of leafhopper-borne maize chlorotic dwarf virus. J. Econ. Entomol. 66:1271-1273.
9. PITRE, H.N. 1968. Effect of insecticidal sprays on stunt and mosaic virus diseases of corn in small field plots in Mississippi. J. Econ. Entomol. 61:585-587.
10. PITRE, H.N. 1968. Systemic insecticides for control of the black-faced leafhopper, Graminella nigrifrons, and effect on corn stunt disease. J. Econ. Entomol. 61:765-768.
11. ROSE, D.J.W. 1974. The epidemiology of maize streak disease in relation to population densities of Cicadulina spp. Ann. Appl. Biol. 76:199-207.

DISCUSSION OF J.K. KNOKE PAPER

V.D. Damsteegt: Did you say 1 lb carbofuran per acre as a band? A seed company representative in our area mentioned studies in Virginia where they used 10, 20, 30, and 40 lbs per acre.

J.K. Knoke: I was referring to 1 lb active material per acre. The representative was probably referring to a 10% granular formulation.

V.D. Damsteegt: They were recommending carbofuran, not for disease control but for better yield. They increased yields 8 to 10 bushels per acre by applying it to the soil.

J.K. Knoke: I do not think that Environmental Protection Agency (EPA) regulations will allow the recommendation of a pesticide unless it is for a specific use, such as for leafhopper control on corn

V.D. Damsteegt: If a material is registered for control of other insects, why cannot it be registered for leafhopper control?

J.K. Knoke: A person interested in this type of control has to request the manufacturer to seek registration for this specific use So far, this has not been accomplished for carbofuran control of leafhoppers. Disulfoton, a less efficient systemic material on corn, is registered on corn for leafhopper control. I presume you could, therefore, use it for control of *Graminella nigrifrons* and MCD. But disulfoton does not do as good a job as carbofuran. There are some other materials registered as foliar sprays for leafhopper control on corn. We know that the foliar sprays generally do not control virus diseases.

L.M. Chilson: On the registration for leafhoppers, as you pointed out, first of all you go to the EPA. They will request efficacy and environmental impact data. Unless the company feels that there is going to be enough pesticide sold, they are not going to spend a lot of money on research for efficacy data against leafhoppers. They will probably turn back to you and ask you to provide the data that EPA requested.

J.K. Knoke: This is being done now for carbofuran. But apparently the company is not too enthusiastic about registering the material. They must have already obtained the data for a lot of these required things. Otherwise, they could not have registered these materials for control of borers and the other insects on corn.

L.M. Chilson: Usually, you can add on another insect species to the label without getting too involved, particularly if there is a precedent, such as, the material is already being used on the crop for another purpose.

J.K. Knoke: I think it all depends on how many of us want to use carbofuran and how willing we are to go to the company and provide them with the relatively little extra information they need for the efficacy data.

L.M. Josephson: Since carbofuran is cleared for the southwestern corn borer, and all of our farmers have the possibility of getting this borer in their corn, they are applying carbofuran as a general practice. They are getting increased yields.

Practical Means of Solving Maize Virus Disease Problems in the Less Developed Countries

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INTRODUCTION

This is really an amplification on one part of my paper on "CIMMYT's Role in Maize Improvement in the Less Developed Countries" presented earlier to this Colloquium and Workshop. That is, in principle, we in CIMMYT believe successful resolution of any maize disease problem in a farmer's field will only come if the disease is dealt with as an integral part of an overall improvement program.

PRINCIPLES FOR ACTION PROGRAMS

To begin this presentation, I first plan to lay out a few principles that we in CIMMYT consider to be of importance.

1) We believe that the development of resistant or tolerant cultivars is the only practical means of solving any maize disease problem, including viruses, in the less developed countries of the world. In fact, we believe that resistance is the only practical answer to diseases of any low-value per unit crop anywhere in the world.

2) We are convinced that breeding for resistance to any disease cannot be done effectively in isolation. By this I mean one must always keep in mind the ultimate user—the farmer. Suffice it to say we all have known cultivars that have been developed with resistance or tolerance to specific diseases or with other desirable characteristics, and yet they were never accepted by the farmer. Other available cultivars which were not as resistant to a particular disease may have had a combination of characteristics he preferred.

3) Experience has proved that plant improvement through breeding is a numbers game. At CIMMYT we believe that any of our breeding programs must be based on testing and selection from a large number of segregating progeny derived from genetically diverse populations tested over a large number of sites representing the range of environments in which the cultivar must eventually fit. CIMMYT's maize program is basically an attempt to produce germplasm that will produce stable yields of grain over a period of years and a range of locations and under different temperature and moisture regimes, as well as different pests. We are seeking more stable performance, hence fewer risks for the farmer. And, the final testing must be done on the farm in cooperation with the farmer. He, as well as the researcher, must be convinced that the new cultivar is better than what he has been growing. No matter how much the researcher likes the new cultivar or how well it may perform on the experiment station, if the farmer is not convinced of its value to him under his conditions, all of the researcher's time, effort, and funding will have gone for naught.

4) We at CIMMYT are convinced that we must concentrate our efforts on the development of open-pollinated cultivars of maize for use in the developing countries. Most maize-growing areas in developing countries are affected by factors of a very practical nature that tend to diminish the advantages a hybrid may have. For example, in many areas where good hybrids are available, it is the practice of a relatively large proportion of farmers who do purchase hybrid seed to plant back the resulting seed from one to several generations. Other limitations of hybrids include the complex and precise logistics of producing good seed and getting it to the farmer.

These and other factors have suggested that the major thrust of our maize germplasm development program be toward open-pollinated varieties that do not require sophisticated hybrid seed production and distribution facilities for their increase and use.

We are also convinced that there are several breeding systems for producing the desired cultivars. The system CIMMYT uses makes it possible to develop hybrids quickly in the event the national program should wish to do so. CIMMYT's efforts to develop disease-resistant maize cultivars are based on the concept that most of the disease resistance we need is polygenic. Thus, we must utilize systems to efficiently accumulate and pyramid the effects of numerous additive genes. This generally means a long-term program involving some type of recurrent testing in conjunction with selection and subsequent recombination of the more resistant genotypes.

5) At CIMMYT we have attempted to set our priorities to take into account the major constraints in improving maize production in the developing countries. As all of you realize, there are

numerous important constraints having a technological base. Within technology, some factors are more important than others. Within diseases of corn, some are more important on a worldwide basis, whereas others are more important in a specific country or region.

At present, CIMMYT's maize program is working on two virus or virus-like diseases of corn:

1. Maize streak, of major importance in tropical Africa.

2. Corn stunt, now considered a spiroplasma-induced disease, of major importance in the tropical Americas and particularly in certain countries of Central America.

We are aware that scientists have reported other maize virus diseases to be of moderate to severe intensity in certain developing countries. Two examples are maize mosaic virus I and fine stripe virus. CIMMYT has not considered these virus diseases to be of sufficient importance in the less developed countries of the world to justify mounting special efforts leading to the development of resistant germplasm.

CIMMYT'S PROGRAMS FOR DEVELOPMENT OF GERMPLASM RESISTANT TO INSECTS AND DISEASES

In the remainder of this presentation I plan to go into some detail on how we attempt to coordinate our activities in developing insect and disease-resistant maize cultivars that combine other characteristics inportant to the ultimate user — the farmer.

We actually have two major groups of activities. The first group involves those insects and diseases prevalent in Mexico. The maize program staff work as part of an overall interdisciplinary maize improvement team. As part of their responsibilities they seed three Companion Nurseries. One of the Companion Nurseries is for studies with insects, the second involves studies of maize diseases, and the third studies response to high density plantings. Companion Nurseries are grown in Mexico to evaluate resistance of the materials to major insects and diseases indigenous to Mexico and to assist breeders in making selections.

In my earlier paper, I mentioned that international evaluation of the advanced-unit populations was done on the basis of trials involving 250 full-sib progenies from each population. In addition, a plot of each of these progenies is seeded in each of the three Companion Nurseries on a CIMMYT station in Mexico. In the case of insects, we have developed a mass rearing and artificial-infestation program. At present two insects, the fall armyworm, *Spodoptera frugiperda*, and the sugarcane borer, *Diatraea saccharalis*, are reared on artificial diets. Egg masses laid on paper are cut out and incubated in the laboratory. When close to hatching, they are taken to the field. In the field, newly hatched larvae are mixed with ground corn cobs and applied to plants at the proper stage. Damage is evaluated following infestation, and maize families appearing most resistant or tolerant to insect attack are selected and used for improvement of the population undergoing development.

In the disease-resistance nursery, one-half of each row is inoculated with stalk-rot pathogens, *Fusarium moniliforme*, *Fusarium roseum*, and *Cephalosporium acremonium*; and the entire row is inoculated with ear rot pathogens, *Diplodia maydis* and *D. macrospora*. Rows are rated for disease damage several times during each growing season.

I would now like to illustrate briefly what is being obtained from our cooperative international trials, which I mentioned earlier involving progenies and experimental varieties produced within this system.

In 1975, we sent 138 trials of 250 full-sib progenies derived from 23 advanced unit populations to 22 countries for testing. At the same time, we shipped 177 trials of experimental varieties (varieties produced at CIMMYT by crossing remnant seed of full-sib progenies chosen by national programs in 1973 or 1974) to 41 countries for testing. By mid-June 1976, we had received data from 208 of both kinds of trials mentioned above, 66% of those sent out originally. We summarize these data at CIMMYT and prepare a bound report for use by all collaborators. The data clearly illustrate that there are many progenies and several experimental varieties equal to or better than the best check varieties in yield and in most other agronomic and disease characteristics for which data were reported. These results were from 80% of the countries involved in the trials. I must add that much remains to be done in certain regions before better varieties will be available for farmer use.

Another major activity involves what we have named "collaborative research on exotic diseases and insects." Three damaging diseases and two insect pests of maize, none of them prevalent in Mexico, are found in important maize-producing areas of the developing world. To identify maize with genetic resistance or tolerance to these diseases and insects and to assist in the development of agronomically desirable populations, CIMMYT has entered into collaborative research with strong national programs in areas where these problems are most intense. These exotic diseases are maize downy mildew, a fungus disease found mainly, until fairly recently, in Asia from Indonesia to India and now reported in several countries of Africa and the Western Hemisphere; maize streak virus, a disease found in tropical

Africa; and corn stunt, a spiroplasma disease of maize in Central America. The two important groups of insects not found in Mexico are the African and Asian corn borers.

In 1975, CIMMYT sent seed of 4,000 experimental lines of maize to six national programs. One set each was sent to Thailand and the Philippines to be tested for resistance to downy mildew; one set each to Nigeria and Tanzania to be tested for resistance to maize streak; and one set each to Nicaragua and El Salvador to be tested for resistance to corn stunt. These lines were derived from crosses of three lowland tropical materials representing, broadly, world tropical maize germplasm plus other CIMMYT tropical and subtropical pools that carry downy mildew and stunt resistance. After each cycle in the six countries, sibbed or selfed seed of selected plants (apparently carrying resistance) is returned to CIMMYT for recombination and generation of new sets of progenies (March sowings). These new sets of progenies are subsequently retested at the various sites to carry out the next cycle of selection. Initially, various kinds of subpopulations will be attempted; i.e., resistance to each disease singly, resistance to any combination of two of the three diseases, and finally, resistance to all three.

By repeating this process for several cycles, CIMMYT and the national collaborating scientists expect to develop adequate resistance to the three diseases and to integrate the resistance into improved populations of maize for the tropics. In fact, as soon as any subpopulation has been identified as carrying some significant degree of resistance or tolerance, it will be integrated into appropriate advanced populations through what we call the "side car" approach. This involves, essentially a backcross procedure but using the improved population as the recurrent parent rather than returning to the original. We are not interested in resistance for its own sake; we want to use it as soon as available, to improve our breeding populations and increase their yield stability under adverse conditions.

In conclusion, we see the development of maize germplasm resistant to virus diseases to be just one spoke in the wheel of CIMMYT's international cooperative maize improvement program.

DISCUSSION OF R.D. OSLER PAPER

J.M. Fajamisin: Sometimes we do not get materials in time due to delays in international shipment. This is a problem. But, we have benefited very much from the CIMMYT program despite this complication. What we do is to look at these materials and we put the very good ones into our maize improvement program.

R.D. Osler: We hope that some materials will be useful to national programs. These are available to anyone who can and will make use of them We are delighted that you found something useful.

W.R. Findley: I would like to ask about the kind of selection pressure on populations, for example in El Salvador or Nicaragua where there is corn stunt Do you have a high incidence of corn stunt in the field? Is it difficult to find resistant or tolerant lines and are they very numerous?

R.D. Osler: I did not actually see them in the field. However, as I understand the situation, there was a very high incidence of corn stunt in Nicaragua. Selection occurred, but we did not do too well. In El Salvador the pressure was not quite as great, and we got quite a lot of material. This concerns us a bit in terms of the first cycle. We do not want to reduce the genetic variability too much in the first cycle, that is to point where we have to take anything we can get. We then have to broaden the genetic base again and repeat the process. This is a problem.

W.R. Findley: We also are very grateful for the CIMMYT materials we have used. We identified a couple of populations that look promising. We have made some progress in integrating these populations into Corn Belt material. We would like to get some more populations that have been handled in this way. Are there several populations that you plan to develop in this kind of a program, especially for corn stunt resistance?

R.D. Osler: No. Actually, we have two basic populations that we are working on. Genetically, they are very broadly based and relatively new. They bring together a number of populations initially and these are mixed before they go into the nursery. Any showing progress in the field will be available upon request. Right now, I think that it might be a waste of time to use these materials until we have determined their performance. However, these are available now.

L.R. Nault: Dr M. Guterriez also sent me seed from CIMMYT. I was alerted to his work through one of CIMMYT's publications. If you are not familiar with this work, he is attempting to recover genes from *Tripsacum* species and from *Zea perennis* for incorporation into maize. He sent seed of *Tripsacum* spp. *Zea perennis*, and teosinte. I was encouraged that maize chlorotic dwarf virus could not be transmitted to either *Zea perennis* or the Tripsacums. However, teosinte is highly susceptible to this virus. Unfortunately, *Zea perennis* is susceptible to the corn stunt spiroplasma. At least with the *Tripsacum* spp., it appears as though they are not just tolerant but immune to these pathogens. I hope that his work will continue and that Dr. Guterriez will incorporate some of the resistant genes into agronomically useful corn lines or populations.

R.D. Osler: His work will continue, but Dr. Guterriez recently left to go to Brazil. Dr. James of our staff will assume his responsibilities. She has been at CIMMYT as a postdoctoral associate for almost 2 years. Unfortunately during this past cycle, we obtained very little seed because of the changeover. Dr. Guterriez had many crosses, but these were not planted in the field this past cycle because he was leaving and we did not have any way of handling the material. These crosses are in the germplasm bank

B. Tsotsis: Are you aware of the work along these lines at the University of Illinois? Dr. Jack Harlan and others are interested in the evolution of maize and *Tripsacum* They have some very interesting types.

L.M. Josephson: As you may know, Dr. Harlan has two populations, one he calls tripsacoids and the other maizoids. We are growing these this year. They are made up of *Tripsacum* x Corn Belt crosses They are not completely resistant, and we have not grown them long enough to know whether we can isolate some usable plants. But, if we do find them, I think we could transfer the resistance to some of our lines that are more agronomically usable. If

you are interested in Tripsacum resistance, this would be a much better source than that from CIMMYT.

R.D. Osler: Actually, many of Dr. Harlan's early crosses were made in the block that we maintained in Mexico I am not sure how broad a sample he was able to obtain in terms of his crosses. But he certainly has made many of them.

B. Tsotsis: For those of you who are interested in using teosinte in the improvement of corn, Dr. George Beadle is carrying on a program at the University of Chicago in which he has contructed a series of intermediate types that can be used in a study of the evolution of maize.

L.R. Nault: I notice that you are rearing two insects at CIMMYT. By using these insects, you can ensure uniform infestation of resistant materials. In your test sites for corn stunt, how do you ensure uniform natural infections so that you are selecting for tolerance or resistance and not escapes?

R.D. Osler: Well, this is the reason why I think we should take this material back to the field this year Because of the location of our test plot in Mexico we are testing in an area where we know there is no corn stunt. In the fields in both Nicaragua and El Salvador, almost 100% of susceptible plants are killed with corn stunt. In one plot in Nicaragua 100% infection occurred even in the so-called resistant hybrids that has been involved in the El Salvador program.

Discussion of Needs for International Cooperation in Solving Maize Virus Diseases

J.M. Fajemisin: One of the most important aspects is additional training. We need training in screening for resistance, epiphytology, serology, and electron microscopy. (The electron microscope laboratory here, is very good, perhaps the best I have seen.) I believe that the tropical environment is conducive to introduction of insects on various grasses that may be virus infected. Since the tropics are continuously warm and humid it is likely that maize viruses occur wherever the crop is grown. We lack the ability to detect them. This colloquium and workshop is very important because it has permitted me to talk with OARDC researchers about getting antisera from them and sending specimens to them for electron microscopy. I am talking about using the services of the OARDC. Screening maize germplasm for resistance is very important and we need additional programs. However, if we test our materials in the U.S. or yours in our country, the problem of adaptation arises. Your germplasm may not survive in Nigeria. So, we may need CIMMYT to provide assistance in developing and evaluating germplasm. I have stressed the problems of detection, but additional training in other techniques of solving virus diseases also needs emphasis. I would also recommend intermittent meetings of this type. They would serve us well.

R.D. Osler: I think that Dr. Fajemisin's ideas are the same as CIMMYT's. All of us are aware of the many examples of extremely useful international cooperation. This has taken many forms. I believe that there are some good concepts involved, and some of these have been discussed during this colloquium and workshop. There is a tremendous resource here at Wooster and in the other people attending this colloquium and workshop. We in CIMMYT have to consider the major constraints in any area in which we are working. I wonder if we do not need better systems of virus disease identification that can be used more broadly in determining problems in countries in which we are working. U.S. researchers have their maize virus disease problems pretty well sorted out. We have not done this in countries where we work. I think, basically, we have to go back into these countries and if there are nontechnical problems we have to face these first. Periodic meetings such as this one, I think, would have been extremely useful to our group, particularly if some of the people who are directly involved in research could have attended. I would make a plea for information exchange, not just at meetings, which is tremendously important, but at an earlier time. Somehow, a mechanism for this exchange needs to be developed. Some of the experiences discussed at this colloquium and workshop need to be communicated to interested people in other countries. I make a further plea for germplasm exchange. As resistant materials are found in various areas of the world, we in CIMMYT and our colleagues in national programs would like to have access to them. We at CIMMYT hope that the expertise represented at this colloquium and workshop and the people who could not be here will continue the cooperation begun at this meeting. Perhaps this cooperation could focus on training, information exchange, and movement of people and materials. We need to develop more foolproof means of identification of virus disease problems in the less developed countries and of determining the magnitude of yield loss. When you are able to assess these, you are in a better position to assign priorities to these constraints.

R.I. Brawn: I suggest that we begin the information exchange with a newsletter. It would be of immediate service to the whole group. I would suggest that it not be too scientific but one that allows each contributor to speculate and say things not necessarily acceptable in a reviewed publication. The newsletter should indicate that its data are not for publication and it should be available only to researchers who are vitally interested in and understand the subject. To obtain contributions will require some urging and cooperation by this group.

R. Gamez: I also see a great need for a continuing exchange of information by a newsletter. Also, meetings of this sort should be held on a regular basis. One of this particular quality would be very valuable. As Dr. Osler mentioned, much improvement is needed in pathogen identification. We have identified some of the pathogens, but many remain to be identified. It is also very obvious that we have disagreements in pathogen identification. We need to develop some kind of cooperative research effort in this area. There are some excellent facilities in virology in our countries, and others should be developed. But at the same time we need collaboration with other places like the OARDC.

R.E. Ford: A good model for the newsletter might be the one initiated by the international working group on legume viruses. That newsletter contains an annual mimeograph listing of publications, research projects, and seed supplies for various important hosts. Each scientist maintains seed for two to five host species, produces his own antiserum, etc. It has been an extremely helpful system in maintaining contact around the world. Perhaps, you could have a very informal type of rotation of officers and someone in charge of getting the newsletter off once a year. Participants could send in the abstracts of their past year's work. Perhaps, this would include information ready for publication but mostly pre-publication information. Also, lists of maize virus disease publications that came out during the year could be included.

S.P. Raychaudhuri: It would be most useful if sources of resistance could be tested at a central place and data made available to all. A newsletter should be helpful in communicating this as well as other information to maize virus disease workers. Perhaps other diseases should be included. I would recommend that a newsletter be semiannual to enable information to be exchanged quickly. It could be modeled after the rice or legume virus newsletters. I also recommend establishment of a central location for virus collections. More than once, original cultures of pathogens have been lost and there is no reference material available. We have heard descriptions and have seen slides of a large number of mosaic virus diseases these past few days. But, these mosaic symptoms are not always indicative of the same virus. Therefore, it is important to characterize their causal agents and have reference collections for use in comparisons. Finally, this colloquium and workshop was one of the best I have ever attended, because of the depth of papers and discussion. Also, the workshops conducted were excellent and very useful. If we could arrange to have this type of meeting, perhaps every 2 years, and meet in different countries where corn is important, it would be very useful. Perhaps, we could meet when corn is in season and observe symptoms in the field. This would be helpful not only for people from those countries that have made much progress, put particularly for those from countries like India where we have a lot more to do.

O.R. Exconde: I fully support the idea set forth by Dr. Osler that economic threshold levels of maize virus diseases must be determined in countries where they are problems.

I believe that a key to studying economic threshold levels is the proper identification of the respective causal viruses. One way that the OARDC can provide assistance is through virus identification so that precise economic threshold levels can be established. One way of providing this assistance would be to prepare and supply antisera. I fully support the newsletter idea as a form of communication on various maize viruses. There is nothing new about cooperative nurseries. In terms of entries to nurseries in which we have been involved for the past 10 years, various countries would send the best germplasm they had, so that it could be evaluated for resistance to various virus diseases in the Philippines. Perhaps, cooperation similar to this is needed. With so few leading places, as far as research on viruses in concerned, I think that the OARDC is in a better position to help developing countries in terms of training. Participants from a training program could then truly become leaders in virus research in their areas of the world. This would be a great contribution to developing countries. Also, I fully support a workshop idea and think that it would be very useful for a group like this to meet every 2 or 3 years.

T. Sutabutra: I am a little concerned about virus introduction with seed exchanges. Ordinary treatment of seed is aimed at insects and fungi. We do not have any program for ensuring virus-free seeds in international distribution. I am concerned about this because in Thailand we do not have a serious incidence of maize dwarf mosaic, but the potential is there. A small amount of seed carrying a severe strain could cause a serious disease problem. Could there be any kind of program for exchanging virus-free seed?

D.T. Gordon: We do not have a good answer to that question. This is an area that needs investigation. We use seed that is increased in other parts of the world, for example Hawaii and Puerto Rico. Maize mosaic virus is in Hawaii. This virus may be seed transmitted, and we may risk introducing it into the U.S. This, probably, is a common problem in many countries where seed exchanges occur. I do not think, at this time, we either have means of treatment to eliminate or a simple means to detect seed-borne viruses. Research on this problem is needed.

L.E. Williams: Perhaps seed could be grown in isolated blocks and indexed for infection. This method would take considerable labor. It could be done by periodic examination and roguing of diseased plants for seed production. Then, harvested seed could be certified as disease- or virus-free.

P.A. Harvey: Regulations on the exchange of seeds of all kinds are a concern to a number of people. This was discussed at the Sorghum Improvement Conference I attended recently. Some workers would like to move seed in and out of the country. There was mention of isolation area, perhaps an island off Florida. The problem is not only with viruses but all diseases and insects as well. It is very fortunate that corn has had as little restriction in seed movement or exchange as any crop. But, this situation needs to be reviewed. As we discussed earlier, it is important for us to discuss some of the regulations that we are now using and, perhaps, recommend changes. The Animal and Plant Health Inspection Service (APHIS) of the USDA may be modifying its regulations in the near future and your ideas, particularly in plant pathology and entomology, could be very helpful. I was concerned in 1971 when a large amount of corn seed was brought into the U.S., essentially with no inspection or quarantine. As far as I know, nothing new was introduced. We need to be very cautious. I know that other countries are concerned with the same problem.

A.J. Ullstrup: Dr. Renfro has had much experience with seed exchanges in southeast Asia. Dr. Renfro, how do you prevent the spread of pathogens?

B.L. Renfro: We take the normal precautions. Many agencies produce seed for internal use or export during dry winter seasons with the aid of irrigation. During these seasons, they have high light conditions and cool nights. Under these conditions, they get better seed production, cleaner and plumper seed, and no ear rots or other diseases that occur in the rainy months when temperatures are high and insects are prevalent. This seed is produced in small lots Breeders and others are conscious of the threat of exporting seed. They like to harvest the seed from clean plants and then treat it properly. Most use drying facilities. They treat the outside of the seed; however, that does not eliminate internal pathogens nor several external ones. Nevertheless, we do not know of any case where exported pathogens or insects have become established in other countries.

L.M. Josephson: After listening to the papers and discussions this week, I cannot help but be impressed by the importance of virus and virus-like diseases of maize. I think that it would be important to catalog materials resistant to each of these agents. I suggest we should attempt to get these materials and have them available in various locations for testing. We should not wait until we get the disease, and then try to find resistance.

R.W. Toler: I would like to mention some of the contributions of the people at OARDC with whom I have worked cooperatively on corn virus diseases. Many contributions can be made in maize virology by a center like the one here in Ohio, where you have many people engaged in a multidisciplinary approach to a problem. To illustrate my point, I would like to present a little historical background on corn virus research in the U.S. Dr. L.E. Williams' discovery of maize dwarf mosaic virus had indicated the importance of virus diseases of corn in Ohio. Subsequently, the same was established elsewhere in the U.S. In 1967, breeders, entomologists, pathologists, and virologists in the South were concerned with diseases that apparently were virus or virus-like in nature. One of the first problems encountered was the safe interchange of corn material throughout the South. We found that even within corn nurseries in various southern states that there were virus diseases that appeared to be different. We did not understand what these differences represented. It became obvious that it was necessary to have contributions from many disciplines to solve this problem. Based on this awareness, we organized a southern regional research project. It soon became obvious that the diseases, in reality, were different in different states and geographical regions of the U.S. Therefore, we decided that we must have an interregional approach and that research should be multidisciplinary. We also realized that we were faced with problems of proper detection and identification of diseases and pathogens. We did not know with certainty the causal agents of symptoms that we were observing. We were seeing different reactions to what we had assumed previously to be the same causal agent. On the same corn genotypes, we were getting different reactions in Texas, Virginia, Ohio, Mississippi, and other states. How could we assess disease yield losses, if we could not properly identify the disease or pathogen? And how could we locate resistant germplasm? With the development of the interregional cooperative effort, we were able to see the performance of germplasm in different locations and to identify causal agent(s) of various symptoms. We soon found that through cooperation with the group at the OARDC, where there was expertise in electron microscopy, virology, biochemistry, entomology, breeding, and epidemiology, we could all make contributions to solving the problem of disease identification. OARDC researchers were willing to handle and identify diseased materials from throughout the U.S. in cooperation with the others. Thus, to improve our breeding programs we developed a cooperative program with the aid of the Ohio expertise. Once we began identifying the pathogens we soon found out that we had interactions of pathogens involving two or three viruses or mycoplasma-like pathogens in the same

plants. We also found new and unanticipated pathogens that we had not differentiated by symptomatology. For example, when Dr. Bradfute and I were surveying corn in Texas for one particular set of symptoms, we found a rhabdovirus-infected maize plant, that from symptoms we would not have detected. This discovery involved the use of electron microscopy. Again, through this type of cooperation, involving electron microscopy, vectors, and virology, we detected a maize rayado fino-like virus during the summer of 1976. By having a center of expertise like the OARDC, we have put together a cooperative system by which we can come up with rapid detection and identification of diseases. As Dr. L.M. Josephson mentioned, one thing that concerns us is that we do not wish to wait until a virus disease becomes epidemic before we initiate control measures. We should have corn materials evaluated for resistance and, hopefully, have sources of resistance available before an epidemic happens Based on these experiences, I think that the OARDC group has the expertise to be of value to an international program for solving maize virus disease problems.

J.M. Fajemisin: We should also establish research priorities. I know that this is a prerogative of institutions and governments, but I think that a gathering like the one here at this colloquium and workshop can influence programs in other countries. There are many research problems needing attention; the problems of virus detection and the effects of fertilizers and farming systems on disease As I mentioned during my presentation, in Nigeria 25% of maize production is in sole cropping, but the other 75% is produced in combination with cowpeas, peas, other legumes, casava, or yams. As Dr. Sutabutra mentioned, the interactions of viruses with other diseases need attention. I think that it is very important for us to establish research priorities or guides.

R.E. Davis: In our discussion of needs for an international cooperative program for solving maize virus problems, we have heard from our colleagues from other countries that they would benefit from the expertise that is available in the United States. We must also acknowledge the benefits that we would receive from collaboration with them. Some of these points have already been touched on by Dr. Josephson and Dr. Toler. We have the opportunity to receive resistant germplasm and to study new disease agents in collaborative efforts. This type of work places in perspective our own research in the U.S

J. Castillo-Loayza: We need technical advice and assistance in Peru, particularly in the areas of serology and vector identification We also need assistance in pathogen and disease identification. We are willing to exchange our knowledge of different pathogens and the diseases they cause.

G. Martinez-Lopez: We have just started work with maize viruses in Colombia. Everywhere we look we find problems. We need more hands and people to survey for these diseases and for follow-up research. I am very interested in cooperation with any of you around the world to improve our methods of identification and to get information on disease control. I also would like to receive published information on maize virus disease problems. Our research is often published in Spanish, generally as short publications. I do not know how to reach all who work on maize viruses with this information. How can we handle this? Also, there may be problems in translation. How can we here assist with this problem?

L.E. Williams: We at the OARDC are not seeing some journals in which you people from other countries are publishing. Also, I could not help noticing that you are missing some of ours. We have the OARDC Maize Virus Information Service which could serve to ensure that these publications in Spanish are made known to all maize virus researchers.

D.T. Gordon: We have a number of problems with the Maize Virus Information Service (MAVIS). We have a good record of locating relevant international journal articles for inclusion in MAVIS. When these articles appear in foreign national journals or other publications that have a very limited circulation, it is difficult for us to locate them. Also, even though we are sometimes aware of titles, we are not of the contents. We need to know contents so that we only include articles that are relevant. At the moment, we rely on several literature survey systems to provide us with titles, then we try to obtain copies. It would be valuable to us if people in other countries would send us copies of their articles and alert us to the existence of others, particularly those that do not appear in international journals. We would all benefit from this type of cooperation.

R.E. Ford: Translation may be more of a problem than distribution.

R. Louie: Does the USDA have a translation service available?

V.D. Damsteegt: I believe that the USDA National Agricultural Library has a free translation service. Although they have many journals, I do not think that they carry a lot of the national journals from other countries. Maybe they could send a list of these journals to the OARDC library.

R.J. Lastra: Most national journals have an English summary. We could send you the reprint, and you could look at the summary and decide if the paper is relevant. If so, then you could have it translated. I would like to say something about collaboration, in general. Of course, any collaboration is very important, and we appreciate very much the help that you can give us with our problems. I believe that collaboration would be more efficient on a lab-to-lab basis. I think it is important that we have contact with the persons who work in the same field in different labs and try to make arrangements for collaborative efforts among ourselves.

L.E. Williams: If we enter into an international program, many of the countries would not be able to furnish funding. Funding is the key, of course, for us to get an international program started and to do it well. We at the OARDC could continue to do many of the things that we are presently doing, on a limited basis. But we are under constraints about bringing in diseased materials from foreign countries. We cannot conduct biological studies such as host ranges and vector studies on pathogens from those samples. We can do some serology, electron microscopy, and centrifugation After these are done, we must destroy the sample.

C. Johnston: The OARDC maize virus disease group has indicated an interest in working in the international area. I hope that this colloquium and workshop can indicate to USAID, the Rockefeller Foundation, or others the need to support an international program. The problem one encounters with any public agency, at least in this country, is that budgets are formulated 2-3 years in advance. Thus, we need to be as definite as we can in our proposals and try to anticipate needs for training and research in the future. It will take much preparation and work, and you have to justify requests. The main facility that is required for virus disease work at the OARDC is one where we can rear vectors and propagate diseased plants in safety.

L.R. Nault: It appears that a primary objective of importance to everyone at this meeting is disease and pathogen identification. I wish to comment on the idea of a center for virus collections, mentioned by Dr. Raychaudhuri. This is where one of the greatest cooperative ventures might occur. Maize dwarf mosaic virus, which is mechanically transmitted, is the only virus of major importance in maize that we can lypholize and save in infected tissue for a great period of time. Most of the other viruses are vector transmitted and cannot be stored in lypholized tissue. Dr. Martinez-Lopez has established tissue cultures of *Dalbulus*. This is an important development because, per-

haps for the first time, we can easily maintain some of these *Dalbulus*-transmitted viruses. I know that Dr. Lastra has been trying to establish tissue cultures of *Peregrinus*. By cooperation, we can accomplish this goal and establish a center for a collection of maize virus and virus-like pathogens that are obligately vector transmitted. Developing a tissue culture system is a problem. This has to be overcome or otherwise a world collection of these pathogens will not be possible. I indicated in my presentation that even the mechanically transmissible pathogens, after a number of serial transfers, lose their ability to be vector transmitted. If we cannot infect plants then how useful are these cultures for screening maize germplasm? So, while we have techniques to assist us in maintaining virus cultures, we still need periodic transfers. Herein lies one of the major problems that has led us to discuss with the people from APHIS about the development of a quarantine facility. This facility would allow us to maintain foreign vectors and periodically transfer pathogens which can be maintained in tissue culture. Until tissue culture techniques are perfected, we must rely on passage of these obligately vector-transmitted pathogens from plant-to-plant by their vectors. Maintenance of a world collection of maize pathogens requires a collection of their vectors, and these vectors will in turn require a quarantine facility

R.J. Lastra: I know that what you have described means less confusion. But, what we can do at present is that a laboratory in a country where the disease is present and which has facilities could be responsible for maintaining a certain virus. They could also provide it to the others. Maize mosaic virus could be maintained by us and, perhaps, rayado fino virus by Dr. Gamez. This could be done until quarantine facilities are developed. Although you cannot import the virus for biological studies we can send a small, leaf sample from which you can compare virus materials by physical and serological procedures.

L.R. Nault: I think that until this facility can be developed, it would be very good for individuals in certain countries to maintain indigenous viruses. However, I am still hopeful that we can develop a world collection, for several reasons. One is for dealing with strains of pathogens. Serologically, we cannot separate most of the maize dwarf mosaic virus strains, and yet they produce different symptoms and infect various maize lines in different ways. In strain comparisons, we need to study the viruses in one place where they would be tested uniformly by the same transmission techniques on the same maize materials. I do not think that we could do this well if we had to depend on different people in different countries.

G. Martinez-Lopez: I would like to add more information about the insect tissue culture. It would be necessary to maintain the virus, vector, and plant at all times. As mentioned, one of the main problems is identification. Insect tissue culture would be a very useful tool for identifying some viruses. We could use insect tissue culture techniques in identification, at any place without quarantine. It would be very easy to handle once we developed techniques for viruses, spiroplasmas and mycoplasmas of maize.

L.E. Williams: We have had some cooperative work with Dr. Damsteegt. Would an international center compete with any of his programs? Also, would there be any areas in which we could still continue to cooperate?

V.D. Damsteegt: At Frederick, Maryland we have the kind of quarantine facility that has been mentioned at this meeting. But it is not designed, necessarily, for the kind of work that you have described. I do not think that because ours exists it would, necessarily, preclude or be a problem for having one here. Several things come to mind concerning a quarantine facility. One basic problem that we have at Frederick, is the development of a protocol or philosophy of working with pathogens in an area where susceptible crops are grown and yet you wish to infect living material in the facility. We have found that with a quarantine facility you do not have to worry about containing pathogens and vectors as much as you do about containing people who work with them. No one can go into a quarantine facility and suddenly walk out, accidentally. A working research nucleus exists in Ohio. This nucleus is what we need to make an international program work; we do not have enough people and space at Frederick. Our facility cost almost $2 million 20 years ago and was not designed to meet all of our present needs. Today, it might cost $10 or $15 million. There is a possibility, in terms of immediate use, that we could do some service work. As an example, INTSOY is interested in the white fly (*Bemisia*). Dr. Bob Goodman of INTSOY and I hope to get some of the white fly-transmitted viruses and work cooperatively on their transmission There would be some possibility, at least as a beginning, for us to become involved in some cooperative work on maize viruses. Dr. Bradfute and I have a little cooperative work on maize streak virus. I am not saying that you should not put a quarantine facility at Wooster because we have one at Frederick. There should not be a conflict; only cooperation and complementation. With the nucleus you have at OARDC, and if you could obtain a quarantine facility, then we would benefit as well as everyone else around the world. I am for it from that standpoint. There are a lot of things that have to be worked out, and it will take time to realize its implementation. Dr. Lightfield of APHIS will play a role in how the facility develops. I do not know the attitude of State of Ohio quarantine officials. In Maryland they do not necessarily like the introduction of foreign pathogens because of fear of escapes from the quarantine facility. Several people mentioned the fear of introduction of new pathogens through seed exchanges between countries. We bring seeds into the U.S. and if they come from an area where a serious pathogen occurs, plants from these seeds are kept inside a closed facility until we are sure that they are not infected. I am not against importation of foreign seed, provided adequate inspection is practiced.

J.W. Lightfield: I think that plant quarantine officials work as a team, involving federal and state regulatory units and research people. Each person feels his responsibility for what he is charged to do, and we each try to determine what work can be done safely. Dr. Damsteegt's research efforts on maize streak virus are restricted to the winter months when no corn is in the field and, hopefully, not the vector. If something drastic does happen, the pathogen will not survive out-of-doors. Dr. Damsteegt has agreed to these restrictions and works very well within this protocol. I do not recall of ever hearing about any pathogens escaping from the Frederick quarantine facility. I think this speaks well of the facility. If a similar facility were built at the OARDC, I am sure we would interact with quarantine officials in Ohio to see that proper safeguards were included. In setting it up, we all would have to establish procedures. We would have a part in getting diseased materials into the U.S. and in seeing that the proper safeguards were followed. I am keenly interested in the activities at Frederick. They have given the USDA serious quarantine problems, but I favor this type of work. I think that they are going to provide us with the answers we need. Of course, I have the responsibility that the work is done safely. Perhaps APHIS can help in the exchange of germplasm and in problems of receiving seed from foreign countries as well as problems the foreign countries face. Dr. Fajemisin mentioned the problem that he has in getting materials into Nigeria. APHIS is charged with the responsibility of certifying shipments going out of the U.S. and, of course, shipments coming in. I have talked with Dr. House of FAO when he was trying to get material certified for shipment into the U.S. He provided statements that he had not observed any disease of concern to the U.S. This same information is needed also in sending germplasm out of the

U.S. It is very useful to whoever is writing up the certificate to be furnished a statement of the disease conditions. Each country has its own regulations. Some countries want seed produced free or certified free of southern corn leaf blight. I think that the USDA inspects seed, but other federal agencies do not. If pest information is furnished, it expedites getting seed through quarantine inspection.

L.E. Williams: Do the representatives from seed corn companies represented here have anything to add to the advantages or the dangers to their international operations in selling seed produced in foreign areas or comments on international cooperation?

D.R. Wilkinson: Virus disease identification has been our biggest problem: If we have, for example, some germplasm in Nicaragua that is resistant to virus diseases there, will it also be resistant in Costa Rica or some of the other neighboring countries; are the virus diseases different? Anything that would help us to determine what diseases exist in different countries or areas of countries would certainly be beneficial to us in the commercial companies. I would like to take this opportunity to say that we have appreciated the cooperation we have had with researchers here at the OARDC in disease identifications.

B. Tsotsis: I concur with what has been said already concerning the need for assistance in identifying disease problems. Also, I am interested in the development of delivery systems for deploying resistance to the field. It may be proper at this time to refer to a couple of things related to deployment of resistance to meet new situations. We have faced two or three new situations in recent years, starting with MDM here in Ohio and race T of *Helminthosporium maydis*. More recently, attempts to contend with sorghum downy mildew have been made. When the corn virus problems became apparent, industry people and others began evaluating a very extensive collection of germplasm in the area where the problem occurred: In this context, I think biological materials can also be of diagnostic value. They can tell you many things that you may not recognize precisely at the time the problem first appears. Principally, they tell you what you will be able to do in a crisis situation. This was done here in Ohio, and within a matter of not more than 2 years introductions of significantly virus-resistant, commercial materials were made available to farmers. This involved propagating foundation seed and making available the quantity needed. Perhaps one of the reasons that we do not hear more about viral disease problems in Ohio and other states is because of the development of relatively resistant hybrids. In the case of southern corn leaf blight, the situation was of national scope. It involved cytoplasmic as well as a genetic factors in corn. Again within one season, the entire cytoplasmic base of the U.S. corn crop was changed. More recently with downy mildew, we and others located the problem either on the Mexico side of the border near Matamoras or near Corpus Christi, Texas. Again, the philosophy was that solutions to disease problems can be anticipated and developed rapidly. But when a disease strikes in epidemic proportions, there is precious little you can do in the year of initial damage. If we do not have the disease organism present at breeding sites, we will have to endure the problem. The way we will attempt to deal with it is to amass a germplasm collection, evaluate it under known disease conditions, and see what is resistant among existing or developmental hybrids and inbred lines. Work with the above diseases are three recent examples of what can be done One comment I would make, in general, particularly to people from foreign countries who may not be relying as extensively on resistance as we have. In seed production systems or even breeding programs, if problems are to be solved through resistant varieties or hybrids, you had better allow considerable time before you can expect to have solutions. If you take the varietal approach and you are dealing with a cross-pollinated species, the rate of progress in breeding for resistance and the cleaning up of genetic material will be a lot slower than when all these lines are designated as to exact genotypes and you had better allow for considerably more time. So I think that this situation could be a very important consideration for any country that faces disease problems. I happen to believe in the "numbers game" as Dr. Osler mentioned. By that I mean, you should have enough material to evaluate and let it tell you as much as it can about disease resistance. If some materials fail due to conditions that exist in a specific environment, it is not mandatory that you save them. This is one premise of a breeding program; you select and throw away unsuitable lines. Genotypes that appear to be marginal may be of limited interest, and they do not have to be kept in your program. A lot of assumptions are made about delivery systems and who is responsible for them at present. We have some hybrids with resistance in the U.S., and it took several years to develop them. I think that they are important materials and should be utilized.

R.E. Ford: I suggest that an international maize virus newsletter be established and that because of the expertise in maize virus diseases at Wooster, this group at OARDC take the responsiblity for it. It should be sent annually to each of the people attending this colloquium and workshop. If the need is greater, then it should be semi-annual. The newsletter could follow the format of others, and include abstracts of information and mention things that each scientist plans for the future

D.S. Stanley: I think that the classification and identification of maize virus and virus-like diseases around the world are very important, not only to the commercial people but also to governmental workers. Most commercial companies are probably doing maize work in many countries from where our speakers come, and others as well. I think that the commercial people would be willing to cooperate with governmental people, and that this cooperation should occur. I would like to see the newsletter developed

R.W. Toler: An international newsletter is usually sponsored by an international working group. For example, the international citrus virus working group has a newsletter. Does our group feel that the establishment of an international maize virus working group is desirable? Dr Davis, would you like to mention something about the international citrus virus group?

R.E. Davis: I am not a member of the citrus virologists working group, but I understand that citrus virologists have an organization called the International Organization of Citrus Virologists (IOCV). They meet periodically and publish proceedings. As far as I am aware, this organization has provided a means for the exchange of information and for enhancing collaborative efforts on an international basis.

S.P. Raychaudhuri: I have been a member of the IOCV for a long time We will hold meetings every 3 years. A meeting was held in Athens, Greece last year. This organization has committees, for instance, on nomenclature, identification, vectors, etc. People who are working in these areas are responsible for bringing out important announcements in the newsletter. These committees also help the workers in collaborative efforts that have brought to light many important findings, like the citrus stubborn and citrus greening diseases. The IOCV has been very effective. The maize virus newsletter could use the format of the legume or IOCV newsletter, because both are concerned with one crop.

L.E. Williams: What sort of a mechanism should we use to institute the newsletter? Do we elect or appoint a committee?

S.P. Raychaudhuri: Those who are interested in doing work, for example, on maize mycoplasmas or spiroplasmas should be responsible for this topic. If we have three or four people active in each subject matter area then, probably, we can finalize the establishment of an international maize virus working group in the near future. The

newsletter probably should be brought out by the OARDC group. Finally, there should be a central depository where we can compare and identify pathogens.

R.E. Ford: I suggest that there be two or three officers to bring the group together and that they identify committees that might be needed. I suggest D.T. Gordon as Chairperson, R.W. Toler, vice chairperson, and L.R. Nault, secretary, for example.

R.W. Toler: We should not forget in a proposal for an international work group to include all of the disciplines and countries involved.

R.I. Brawn: A very successful group, for example, is the maize genetic cooperation group centered partly at the University of Illinois and partly, I believe, in Indiana. They have operated for many years without any formal structure. There are no officers. It takes a Center like the OARDC where there is a continuing interest in the subject and where you are able to pass the responsibility around internally, to handle such an undertaking. I am not refuting what Dr. Ford has said, but I do not think it necessary to be so highly structured. In regard to the newsletter, preliminary information or ideas could be printed, and the reader could accept or reject them, based on his experiences. Without such a newsletter I think that we could rapidly encounter a situation with these virus diseases in which certain information that workers need to know would not be acceptable to editors of official journals and, consequently, not widely distributed. The newsletter helps to circumvent this problem.

A.J. Ullstrup: The success of the maize genetic newsletter is dependent upon the ability and dedication of one man, and this is, I think, an essential quality that you would be looking for.

R.I. Brawn: The Drosophila Newsletter, which has been in existence for a long time, also has been successful by having a rotation of good men assume responsibility for it I think we have here at the OARDC the kind of men with the abilities needed for this undertaking.

S.P. Raychaudhuri: To run a newsletter you will need money also. Maybe in the beginning, we should have a nominal subscription. That is how newsletters usually are started. This would mean that we would need a membership fee, and you may need support from other organizations. Several people at this meeting have stated that they are interested in pathogen characterization, virus maintenance, and being kept informed of new information. All of these items are important.

L.E. Williams: I do not know about sources of money. I know that the OARDC is accepting the cost of MAVIS. I do not know how much money that publishing and mailing newsletters would cost. I know it will take a lot of work. As long as it is unedited and involves only duplicating information sent in, then work will be reduced. I am not sure what expenses the OARDC could underwrite for a newsletter. The compilation, publication, and mailing of MAVIS is already a lot of work. I do not know how much more we could do.

C. Johnston: I can, with some qualifications, volunteer the OARDC to print the newsletter on a cost basis.

L.E. Williams: Then, I suggest that we print it at the OARDC.

J.M. Fajamisin: There are several countries that are not represented at this meeting, and if you omit them, it eliminates several of the poor countries.

L.E. Williams: The newsletter should be sent to all maize virus workers in the less developed countries around the world. We will need to get a mailing list of persons to whom the newsletter should be sent and who can make the most use of it. We may have to depend on many of you for this information. We at the OARDC would be willing to attempt publication of a newsletter on a temporary basis.

R.E. Davis: To return to Dr. Ford's suggestion, should we attempt to formalize some kind of network for communication. I think this might be most efficiently carried out if there were a nucleus of people to handle the network, in addition to the newsletter. That is to say, that the newsletter would be an arm of the network. Perhaps, we should consider the suggestion of Drs. Toler and Raychaudhuri to formalize the network and to consider as officers those who would represent the various areas of research in maize virology. These people could serve for a fixed term.

L.E. Williams: What do others think about Dr. Davis' suggestion that perhaps, in addition, to a newsletter, we should have other activities? Perhaps this should be handled by an elected committee that might include people outside of the U.S.

L.R. Nault: I think that it would be well to have representatives from a number of countries and the three major disciplines involved in maize virus diseases, viz. breeding, entomology, and plant pathology. We will need a committee of more than three people; perhaps six are needed.

L.E. Williams: Could we have Drs. Gordon, Toler, and Nault serve as an Executive Committee, and they in turn would select committees to develop these other things? Could we have these people be the nucleus in the beginning as the Chairperson, Vice-Chairperson, and Secretary, respectively?

I.W. Deep: Could we say that the Maize Virus Group at the OARDC will do something about the newsletter? Dr. R.I. Brawn has suggested that a more casual approach might be effective for communications. I think this approach might be just as good. Also, I think that the OARDC maize virus group will volunteer to consider this approach and to initiate, at least, some plans on this matter. Then we can see how they develop.

L.E. Williams: I believe that Dr. Davis said that there might be some things in addition to the newsletter that might need some coordination and planning. Dr. Davis, is that what you had in mind?

R.E. Davis: What I had in mind might parallel and be quite consistent with Dr. Deep's suggestion. For instance, this group could act as an interim ad hoc committee which would then formalize some kind of network to enhance the total international cooperation along all possible lines. I think that the effort might benefit from some kind of structure. My proposal was that the newsletter, which could be an entirely independent activity, might incorporate information exchanged throughout this network or might even be a means of information exchange for the hypothetical international organization. I was thinking, somewhat, along the lines of Dr Raychaudhuri's description of the citrus virologists group. Also, if we feel that it is beneficial, to meet periodically in different countries, so that those of us who are interested in these various virus diseases and other problems in maize could see on site the diseases in various countries. I think the group may need some structure that could carry on the activities from year to year.

I.W. Deep: I think that all of your recommendations are very good. I was thinking in terms of the limitation that we have on time, as far as developing something specific here. We have a lot of ideas. The OARDC group obviously will use these ideas and develop them into some recommendations. At this time, I am not sure exactly what they will be.

G.E. Scott: It seems to me that there are two or three ideas involved. One is a committee for organizing a maize virus group, another is the question of do we want a center of research at the OARDC, and a third, do we want a newsletter? You could have a committee for the newsletter and another for the desirability for a center of research, etc. The same committees would not have to handle all these things.

L.M. Josephson: I am not so sure that we are quite ready to become highly structured. I believe it is important that we go ahead with the newsletter. It seems to me that the

OARDC group would be the logical group to proceed with it. I think that contributions to this newsletter will more or less formulate future plans. If it develops that meetings should be held and that we should be structured into an international group, then I think the OARDC group could take the initiative.

L.E. Williams: Perhaps as this newsletter develops, all of you could provide opinions on whether we need to be formally structured.

T. Sutabutra: The newsletter would be very useful, but I would like to see the OARDC serve as a center for antiserum collected from all the countries. This would be useful for identification of viruses. Also, it is difficult for us in foreign countries to get specific chemicals that must be used for certain viruses. If the OARDC could serve to provide these chemicals, it would be very useful. But the thing that we are most interested in now, is service work in research to enable us to do things which we cannot do at all

V.D. Damsteegt: Concerning the newsletter, is it possible for a committee to devise a questionnaire that would inquire about the various possible directions we need to go? This questionnaire could be to all of us. We could then respond with formal comments.

L.R. Nault: Dr. Davis asked for a consensus, and I think that we need it before we do anything formally. Many maize virus workers are not here and perhaps this questionnaire could be expanded and the consensus come from additional countries.

V.D. Damsteegt: You might add a request for names of other people who might be interested in our activities.

L.R. Nault: We have been talking about two means of communication; one is the newsletter and the other is getting together on a regular basis. We discussed funding the first, which is relatively simple. But, funding the second is more difficult. How can this be done?

R.E. Ford: The legume virus workers have never sought funding. They usually try to convene at an international congress or a major national meeting about every 3 years and meet a day before or a day after the meeting. This arrangement usually brings together a significant number of the members. But as multidisciplinary as the maize virus group appears to be, I do no think that this arrangement in this case will work as well. We would not have entomologists and maize breeders at virology congresses, and we would not have virologists at entomology congresses

L.R. Nault: Often, it costs a great deal to send people to one location and usually state or federal governments do not have money for this. Funding must be sought from other sources.

R.W. Toler: One thing we have been talking around, but have not mentioned directly, is a site for a possible future meeting

R.E. Ford: We could take care of that in the questionnaire

D.T. Gordon: Could we come to a consensus to allow us to proceed further? My suggestion would be that we take Dr. Damsteegt's idea of developing a questionnaire, and based on responses to it set forth the possible directions for future development of a network of maize virus workers. Also, the OARDC group, in an unstructured manner, could put the questionnaire together, distribute it among the people attending the meeting, and get their responses and additional names of interested people. Once we have all of this information, then we could prepare a final document, and see if we can develop a viable international program.

L.E. Williams: The problem of accomplishing the things that we have discussed is funding. I think that you all know that one institution cannot assume the funding for an international program and still carry out its local missions. The OARDC group works nationally because it has received federal funds to do so. We need similar funding to initiate an international program. We have discussed the need for a quarantine facility and Dr Damsteegt has indicated that it is very expensive. Such a facility is one thing we must have if we are to serve as a center for an international program. We are on the eastern edge of the Corn Belt and must have total security In addition to such a facility, we would need funding for new personnel and equipment, travel, and supplies. This would make possible the implementation of a cooperative international program in all of its phases. This would include training, disease and pathogen identification, screening germplasm, information exchange networks (workshops, newsletter, MAVIS, and international work group) and determining economic thresholds of disease. We would give a percentage of our time and make available our present facilities and equipment.

R.I. Brawn: I believe that there is one point on which there is consensus. This has been one of the finest meetings that I have ever attended. I think I can speak for the group when I commend you for the meetings. I am glad to have been invited.

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D.C. Robertson
W.R. Findley
O.E. Bradfute
R.E. Davis
R.E. Gingery
L.E. Williams
V.D. Damsteegt
J.A. Foster

Second Row
J.K. Knoke
R.J. Lastra
R. Gamez
T. Jarapat
J. Castillo-Loayza
C.O. Grogan
D.S. Stanley
L.M. Josephson
J.M. Fajemisin
P.A. Harvey
G.E. Scott
L.M. Chilson
R. Louie
C. Johnston
R.W. Toler

Back Rows
A.J. Ullstrup
S.P. Raychaudhuri
T. Sutabutra
B.L. Renfro
B. Tsotsis
I.W. Deep
W. Kositratana
C.W. Donoho
R.I. Brawn
E.E. Rosenkranz
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