PN ABG-120

6.375

Final Report

Submitted to the Office of the Science Advisor
U.S. Agency for International Development

Covering Period
August 29, 1986 - August 28, 1989
(Two year no cost extension: August 29, 1989 - August 28, 1991)

Title of Project
Ecology of a Virus and Mollicutes of Maize and
of their *Dalbulus* Vectors in Costa Rica and Mexico

Principal Investigator
Donald T. Gordon, Department of Plant Pathology,
Ohio Agricultural Research and Development Center (OARDC),
The Ohio State University (OSU)

Grantee Institution
The Ohio State University

Co-investigators
Rodrigo Gamez
University of Costa Rica
Cellular and Molecular Biology Research Center

R. E. Gingery
USDA, ARS and the Department of Plant Pathology,
OARDC, OSU

Laurence V. Madden
Department of Plant Pathology, OARDC, OSU

Lowell R. Nault
Department of Entomology, OARDC, OSU

Raefael Rodriguez-M.
Colegio de Postgraduedos
Centro de Fitopatologia, Chipingo, Mexico

S. E. Teraguchi
Cleveland Museum of Natural History and
Case Western Reserve University, Cleveland, Ohio

Project number: 6.375

REC'D IN R&D/R
MAY 31 1995

Grant Number: DPE-5542-G-SS-6038-00

AID Grant Project Officer
Benjamin H. Waite.

Project Duration
August 29, 1986 - August 28, 1991

CC:BOSTID

TABLE OF CONTENTS

EXECUTIVE SUMMARY 1

RESEARCH OBJECTIVES 2

METHODS AND RESULTS 5
- Monitoring Dalbulus Vectors and Disease Incidences 5
  - Costa Rica 5
  - Mexico 7
- Surveys for Vectors and Pathogens within Various Vegetational Zones 18
  - Costa Rica 18
  - Mexico 18
- Hibernation of *Dalbulus* leafhoppers 21
- Tests of alternate sources of the three pathogens 23
- Environmental measurements 25
- Development and Comparison of Serological Assays 26
- Production of Monoclonal Antibodies to MBSM 27
- Production of antiserum to CSS and MRFV 30
  - Preparation of CSS antiserum 30
  - Preparation of MRFV antiserum 31
- Serological relationship between MBSM and CSS 32
- Symptom diagnosis and pathogen identification 32
  - Nicaraguan Study 32
  - Mexican Study 40
- Investigators responsible for studies 43

DISCUSSION OF EXPERIMENTAL RESULTS IN RELATION TO STUDY OBJECTIVES 43

IMPACT, RELEVANCE AND TECHNOLOGY TRANSFER 48
- Usefulness of experimental findings in Mexico and the developing countries of Central America. 48

PROJECT ACTIVITIES/OUTPUTS 49

PROJECT PRODUCTS AND OUTPUTS 50

FUTURE WORK 51

LITERATURE CITED 52

EXECUTIVE SUMMARY

Incidences of maize rayado fino, caused by maize rayado fino virus (MRFV), and corn stunting diseases, caused by the corn stunt spiroplasma (CSS) and/or maize bushy stunt mycoplasma (MBSM), three important maize pathogens in Mexico and Central America, were recorded in test plots at two locations in Costa Rica and three locations in Mexico for the years 1987-1989. The locations were in different ecological zones within the two countries. Incidences of both diseases were low at all locations during the three years. Also, surveys for these diseases in Costa Rica in 10 of the country's 11 vegetational zones also found low incidence or no diseases. Results suggest that these diseases occur sporadically, without major outbreaks each year. Further, none of the diseases predominated in specific ecological zones as expected. Also, maize rayado fino incidences were as great or greater than those of the stunting diseases, highlighting its importance. Numbers of *Dalbulus* leafhoppers, vectors of the three pathogens, within test maize fields were not correlated with disease incidences. Evidence concerning the source (local or distant) of infective *Dalbulus* leafhoppers was inconclusive, although it suggested that infective *Dalbulus* survive overseason in nearby wooded or weed areas and not in distant maize where continuous cultivation was possible due to irrigation or rainfall. Reliable, sensitive and specific assays were developed for the three pathogens and used to show that disease identification by symptoms was not reliable and that pathogen identification by assays was necessary for accurate disease diagnosis. These assays are available for use by scientists studying these diseases.

Our results indicate that the CIMMYT program of breeding resistance to corn stunting disease needs to include breeding resistance to MRFV and to employ experimental inoculations of breeding material with specific pathogens, followed by monitoring inoculated plants using assays to determine the identity of the infecting pathogen. Our results also indicate that resistant maize would not be needed in all years for disease control and that further study is needed to allow forecasting of years with potential for serious disease outbreaks. Further, breeding resistant maize adapted to all major ecological zones in Mexico and Central America seems warranted. Finally, study is needed to identify the overseasoning sites of infective *Dalbulus* for the purposes of identifying pathogen sources and allowing forecasting of serious disease outbreaks.

RESEARCH OBJECTIVES

The purpose of this study was to gain a better understanding of how three important maize pathogens, maize rayado fino virus (MRFV), corn stunt spiroplasma (CSS) and maize bushy stunt mycoplasma (MBSM), and their *Dalbulus* leafhopper vectors, have become adapted to maize grown in a wide variety of ecological zones in Costa Rica and Mexico. The answer to this question is important for development in these countries because of the importance of maize as food for their populations and the importance of these pathogens in restricting maximum yields of maize. Through a better understanding of the ecological adaptation of these pathogens and their vectors, we expected to be better able to recommend measures to reduce their impact on maize production.

The hypothesis to be tested was that the differential ecological requirements of CSS, MBSM and MRFV result in different patterns of altitudinal distribution for the three pathogens. For example, in Mexico we predicted that MBSM would be favored by higher and CSS by lower elevations. Thus, at Montecillos, our first location, with an elevation of 2,240 m, a well-defined dry season (November to March), a rainy season (April to October), an annual precipitation of ~630 mm, and a mean annual temperature of 15°C, (average maximum of 27°C and minimum of 0.7°C.), we expected an earlier appearance and greater incidence of MBSM relative to CSS. In contrast, at Poza Rica, our second location, at 60 m elevation and characterized as a humid tropical lowland with an annual precipitation of ~1300 mm, mostly occurring during the rainy season (May to October) and an average annual mean, maximum, and minimum temperatures of 24°, 35°, and 14° C, respectively, we expected a greater relative incidence of CSS compared to MBSM. Our third location, Tlaltizapan, at 940 m has a dry tropical/subtropical climate with a dry (November-April) and rainy (May-October) season, an annual precipitation of ~800 mm, and average annual temperatures of 23°C. (average annual maxima and minima of 34° and 20°C, respectively), and was expected to favor one or the other pathogen depending on the time of year. At Tlaltizapan, there are two maize plantings, one in December and a second in June. Irrigation is common during the dry season allowing maize to be grown. Based on the ecological requirements of MRFV, the latter was expected to be prevalent at each location during periods of maximum precipitation.

These predictions were based on the following previous observations. MBSM is pathogenic to *D. maidis*, but not to *D. elimatus* and *D. gelbus*. CSS is pathogenic to *D. elimatus* and *D. gelbus*, but not to *D. maidis*. *D. maidis* perennates at low elevations, whereas *D. elimatus* and *D. gelbus* perennate at high elevations. At lower temperatures, the combined latent periods of CSS in maize and *D. maidis* is 60-62 days, which when combined with the observation that maize becomes unfavorable to *Dalbulus* after about 60 days from the time of planting, would not allow sufficient time for *D. maidis* to acquire CSS from infected maize plants and to transmit it, following a latent period in the vector, to healthy maize in the same or adjacent fields. Further, the latent period of CSS may be so long at the lower temperatures that symptoms may be expressed too late for recognition. Thus, we would expect only one infection cycle of CSS per crop cycle so that at the higher elevation (Montecillos) secondary spread of CSS would be unexpected. Counterbalancing this prediction is the recognition of the possibility that migration of large numbers of inoculative, migrant *D. maidis* from lower elevations, where its survival was expected, could result in significant incidences of CSS at higher elevations. However, the most probable expectation was that corn stunt incidence would be higher at lower than higher elevations. Since the combined plant-leafhopper latent period for MBSM is about 43 days at the lower temperatures, secondary spread of MBSM at higher elevations seemed more likely than for CSS. Mitigating against higher incidences of MBSM at lower elevations was the

previous observation that at higher temperatures maize bushy stunt symptoms may be so severe that maize plants die prematurely. In addition, in maize plants and *D. maidis* doubly infected with CSS and MBSM, CSS apparently displaces MBSM, thereby limiting MBSM titer. Thus, both of these observations suggested that at lower altitudes with higher temperatures, MBSM incidence relative to that of CSS would be less, thereby reducing MBSM source and hence the potential for secondary spread of MBSM relative to that for CSS. We also expected that MBSM would be introduced into maize at the higher elevations from local sources and thus would appear sooner in the growing season.

In contrast, MRFV and leafhopper populations are favored by higher levels of precipitation during the rainy season rather than by higher or lower temperatures as true for CSS and MBSM. Thus, higher levels of precipitation would be expected to be associated with higher incidence of maize rayado fino. However, higher precipitation may directly promote leafhopper development and only indirectly maize rayado fino incidence due to larger and more active *Dalbulus* populations which develop during the period of ample moisture. Temperature may be slightly negatively correlated with MRFV incidence. It should be noted that the effects of temperature on maize rayado fino were for incidence in the field, whereas for MBSM and CSS the temperature effects were in terms of incubation period of the pathogen in maize plants and *Dalbulus* vectors and disease severity in the plant and vector. Based on the reduced impact of temperature on MRFV compared to that of temperature on MBSM and CSS, we expected to find MRFV over a wider range of altitudes than either CSS or MBSM. It is noted that the effect of moisture on corn stunt and maize bushy stunt was unknown. Factors that may favor MRFV incidence over that of CSS and MBSM are the shorter latent period and less severe effects on maize plants and *Dalbulus* vectors. Finally, in maize plants simultaneously infected with MRFV and CSS, CSS symptoms eventually predominate.

Environmental factors also affect the ecology of the *Dalbulus* vectors of these pathogens. The known geographic distributions of *D. maidis*, *D. elimatus* and *D. gelbus* correlate with known effects of temperature on their respective longevity and fecundity. For example, at 20° and 26°C, survival of *D. maidis* and *D. elimatus* was not different, whereas at 23°C *D. elimatus* lived longer than *D. maidis* but at 29°C *D. maidis* survived longer than *D. elimatus*. Fecundity of *D. maidis* and *D. elimatus* was not significantly different at 20°, 23° and 26°C, whereas at 29°C *D. maidis* was more fecund. At these temperatures, *D. gelbus* lived as long or longer than *D. maidis* or *D. elimatus* but was less fecund. Thus, high temperatures would be expected to favor *D. maidis* and this species would be expected to predominate at lower elevations and to be similarly abundant with *D. elimatus* at higher elevations. In contrast, *D. gelbus* would be expected to be less abundant than either *D. maidis* or *D. elimatus*.

Thus, in summary, CSS and MBSM would appear to be more highly specialized ecologically than MRFV and thus adapted to a narrower range of ecological habitats than MRFV. Occurrences of CSS and MBSM outside of the ecological habitats to which they are best adapted would be expected to be due to migration of CSS-inoculative *D. maidis* from lower to higher elevations and to survival of MBSM in *D. elimatus* at higher elevations. Thus, these speculations on ecological specialization of these pathogens and their vectors allow for predictions of disease occurrence in the different ecological habitats differentiated by temperature and rainfall.

As to predictions of pathogen occurrence in Costa Rica, the first location, Alajuela, was a tropical premontane moist forest with two clearly defined seasons, dry from December to April and rainy from May through November. Average annual precipitation was ~2100 mm distributed within two

distinct periods during the rainy season. Elevation was ~1,100 m and the mean annual temperature was between 20-30°C with high temperatures of 24.5-31°C and low temperatures of 15-19°C. The average relative humidity was between 72 and 95%. There were two main planting dates, May and September. Small maize fields were replanted throughout the rainy season and, to a lesser extent, during the dry season under irrigation. Given the moist conditions of this location during the growing season, MRFV would be expected to be favored and is endemic at this locations. Also the moderate temperature would be expected to favor MBSM over CSS. Further, these environmental conditions should promote abundant *D. maidis* populations.

Tabago, our second location, had a tropical dry forest with two clearly defined seasons, dry from November to June and rainy from late June to November. Elevation was 100 m and the mean annual temperature was 27°C with average minimum and maximum temperatures of 17° and 38°C, respectively. The annual precipitation was ~1400 mm. There was one maize planting in June, but some maize was grown year round, made possible by extensive irrigation. Given the high temperatures, CSS would be expected to be favored over MBSM and MRFV. In fact both CSS and MBSM are both present and MRFV is only occasional.

In summary, predictions of pathogen occurrence at all five locations, based on the ecological adaptation of the three pathogens, indicated that MRFV would be expected to be prevalent in maize grown within the higher moisture zones with relatively high, medium or low temperatures. Locations in this study with these climatic features are Alajuela and Tabago in Costa Rica and Poza Rica in Mexico. However, prior to this study, MRFV was present at Alajuela and Montecillos, occasionally at Tabago, and absent or seldom observed at Tlaltizapan and Poza Rica. CSS would be expected to be prevalent at locations with high temperatures. These are Tabago and Poza Rica. Prior to this study, CSS was reported prevalent at Tabago and Poza Rica, at the end of the rainy season at Alajuela, and at Tlaltizapan. MBSM would be expected to be prevalent at cooler locations (higher elevations) and possibly at locations at mid elevation during cooler periods. These are Alajuela, Montecillos and Tlaltizapan. Prior to this study, MBSM was more common at Alajuela than CSS, showed high incidence at Montecillos in past but not during recent years and occurred occasionally at Tabago. Thus, for these predictions seven were correct, five incorrect and three questionable based on past reports.

Insofar as is known, except for the rare and endemic rhizomatous and perennial teosinte species from Jalisco, Mexico, there are no perennial hosts of CSS, MBSM or MRFV in Mexico and Central America. Consequently, these pathogens likely overseason only in their *Dalbulus* vectors. Thus, the question of how these pathogens overseason becomes a question of how their *Dalbulus* vectors overseason. During the dry period between growing seasons, approximately from November to May for Mexico and Central America, live maize plants are not available to sustain *Dalbulus*, principally *D. maidis*, with the exception of a few areas where irrigation allows year round maize production. In answer to the question of what happens to *D. maidis* during the rain-free (actually low rainfall) period in the absence of locally irrigated maize, we proposed two hypotheses: 1) *D. madis* migrates to and from other regions where irrigated maize is grown during the dry season, or 2) adults disperse to nearby overseasoning locations and remain in a facultative resting state until conditions return (rainy weather) that coincide with the planting of the new year's maize crop. Experiments were conducted to obtain evidence for acceptance or rejection of these hypotheses.

Realization of our objective to better understand the ecology of these pathogens and their vectors was

expected to enhance the effeciency and relevance of the CIMMYT program of breeding for resistance to these pathogens. Controlling these pathogens would be expected to increase markedly maize yields throughout the region.

To our knowledge, this study was the first to attempt to test the impact of known interactions of plant viruses and mollicutes (CSS and MBSM), insect vectors and host plants, as influenced by the environment, on disease occurrence. The study also represented a significant departure from previous studies in that it attempted to explain how these interactions influenced the ecological specializations of pathogens, vectors and plant hosts. These attempts were clearly principal innovative aspects of the project.

METHODS AND RESULTS

Monitoring Dalbulus Vectors and Disease Incidences

Costa Rica

Incidences of diseases caused by CSS, MBSM, MRFV and numbers of vector *Dalbulus* leafhoppers were determined at two locations in Costa Rica. The locations were the Alajuela Experiment Station of the University of Costa Rica and the Tabago Experiment Station at Canas, Guanacaste. The principal features of these locations were presented in the RESEARCH OBJECTIVES and are summarized in Table 1.

At each location, two maize plots were planted with a genotype susceptible to the three pathogens. The plots were square and 30 m on a side. At each location, planting was done in October-November and in May-June (Table 2). Disease assessments were made approximately weekly by observing 400 randomly chosen plants for symptoms of maize rayado fino and corn stunting diseases caused by CSS and/or MBSM. Leafhoppers were monitored by placing two yellow sticky cards (12x17 cm) vertically in the canopy (at about canopy height) which were moved up as plants grew. Cards were changed weekly and the numbers of male and female *Dalbulus* leafhoppers were counted on both sides of the cards.

Low incidences of stunting disease (maize bushy stunt and corn stunt) were observed in all test plots at both locations (Table 2, Figs. 1-4). The highest incidence was 15% which was observed at Alajuela in the October, 1987 planting. Stunting disease incidences were considerably higher at Alajuela than at Tabago and were lower for the May-June than the October planting. Maize rayado fino disease incidence paralleled stunting disease incidence at Alajuela, but the former disease was absent from Tabago. MBSM and CSS infected plants could not be distinguished because diagnostic symptoms for each were unknown.

D. maidis was the only *Dalbulus* species trapped on yellow sticky cards at the two locations (Table 2, Figs. 1-4). *D. maidis* populations were similar in size at the two locations. *D. maidis* populations were generally low, especially for the May - June planting (Table 2). Female *D. maidis* were more abundant than males in five of eight plantings with as many as 77% being females. Disease incidence and leafhopper numbers were not consistently nor directly related. For example, the highest density of leafhoppers (562) was recorded for the 1988 October planting at Tabago but disease incidence there

Table 1. Ecological, seasonal, topographical, climatic, maize cropping, diseases, and survival of maize and *Dalbulus* characteristics of locations in Costa Rica (Alajuela and Tabago) and Mexico (Montecillos, Tlaltizapan and Poza Rica)

Characteristic	Alajuela	Tabago	Montecillos	Tlaltizapan	Poza Rica
1. Ecological zone	Tropical premontane moist forest	Tropical dry forest	Temperate	Dry tropical/ subtropical	Humid tropical lowland
2. Seasons	Dry: Dec-Apr. Rainy: May-Nov.	Dry: Nov-Jun. Rainy: late June-Nov.	Dry: Nov-Mar. Rainy: Apr-Oct.	Dry: Nov-Apr. Rainy: May-Oct.	Dry: Nov-Apr. Rainy: May-Oct.
3. Ave. Annual Precipitation	~2100 mm: Two distinct periods during rainy season.	~1400 mm	~630 mm	~800 mm	~1300 mm
4. Elevation	~1100 m	100 m	2240 m	940 m	60 m
5. Mean Annual Temperature	20-23°C	27°C	15°C	23°C	24°C
6. Ave. High Temp. Ave. Low Temp.	24.5-31°C 15-19°C	38°C 17°C	27°C 0.7°C (subfreezing temp in Dec. and Jan.)	34°C 20°C	35°C 14°C
7. Ave. RH	72-95%	-	-	-	-
8. Maize plantings	May and Sept. Small fields year round-with little irrigation.	June. Some fields year round with irrigation.	April.	Dec and June. Some fields year around with irrigation.	June and Jan.
9. Virus or disease present	MRFV (endemic). Corn stunt at end of rainy season. MBS more common than CS.	MRFV-occasional. CS-present. MBS - present.	MRFV-present MBS-high in past yesrs. Little recently.	CS-present MBS-not present.	CS-common. MBS-seldom found MRFV-seldom found
10. Survival of *Dalbulus* during the dry season.	*D. maidis* found in nearby refuges	*D. maidis* found in nearby refuges.	*D. maidis*: May-Nov. *D. elimatus*: year round.	*D. maidis* and *D. guevarai* both present.	*D. elimatus*, *D. maidis* and *D. longulus*.

Table 2. Total number of *Dalbulus maidis* leafhoppers and final incidences of maize stunting (corn stunt and/or maize bushy stunt) and maize rayado fino (MRF) diseases at two locations in Costa Rica.

Expt.	Location	Planting Date (Day of year)	Total *D. maidis*[a]	Females (%)	Disease Incidence (%)	MRF Incidence (%)
1	Alajuela	10/01/87 (274)	342	60	14.5	7.2
	Tabago	10/13/87 (286)	266	77	3.0	0.0
2	Alajuela	6/09/88 (160)	62	55	7.0	3.5
	Tabago	5/25/88 (145)	75	41	0.2	0.0
3	Alajuela	9/30/88 (273)	268	43	10.8	10.0
	Tabago	10/04/88 (277)	562	74	1.2	<0.1
4	Alajuela	6/09/89 (160)	219	36	2.3	<0.1
	Tabago	5/28/89 (148)	43	60	0.0	0.0

[a] Total per week for four traps (bothsides).

was only 1.2%, whereas a *D. maidis* density of 62 and a disease incidence of 9.5% were recorded for the 1988 June planting at this location. Further, plots of *D. maidis* (male and female) numbers versus day of year when collections were made revealed no relationship to disease incidence (plots not shown).

Mexico

Incidences of diseases and numbers of *Dalbulus* leafhoppers were determined at three locations in Mexico. These were the Montecillos campus of Chapingo University; Tlaltizapan, Morelos; and Poza Rica, Veracruz. The latter two locations are stations run by CIMMYT. The principal features of these locations were presented in the Research Objectives and are summarized in Table 1.

At the three locations, two maize plots were planted with a genotype susceptible to the three pathogens. The plots were square and about 30 m on a side. At Montecillos, one planting per year

was made; at the other locations there were two plantings. Disease assessments were made every 1 to 2 wk by observing 400 randomly chosen plants for symptoms of the three diseases. Leafhoppers were monitored by placing two yellow sticky cards vertically in the canopy, at canopy height. Cards were changed weekly and the number of leafhoppers were counted on both sides of the cards. Environmental data were collected at three locations from standard weather stations.

Disease (maize rayado fino) incidences were low at all three locations (Fig. 5). The greatest final incidence was less than 20% for all years, and for one year and location the final incidence was less than 8%. Tlaltizapan had the greatest incidences in three of the four seasonal plantings and Poza Rica in the remaining fourth seasonal planting. Montecillos had lower incidences than at the other two locations for all plantings. For 1987, Montecillos was the only location were disease incidence data were recorded. At Tlaltizapan and Poza Rica, disease incidences were greater during the dry season (30 to 150 day of year) than the rainy season (190-310 day of year) plantings. At all locations and for all years diseased maize showed symptoms of maize rayado fino but incidences of diseases caused by corn stunt or maize bushy stunt were not reported.

Protein A enzyme-linked immunosorbent assay (PAS-ELISA) was used to assay the following samples collected from Montecillos: grass and weed samples collected during the dry season, April 1988: samples of maize with MRFV symptoms collected, July 1988; grass and weed samples collected during the dry season, April 1989; grass and weed samples collected during the rainy season - Plot I, 1989; and grass and weed samples collected during the rainy season - Plot II, 1989 (See Tables 1-4 in "Tests of alternate sources of the three pathogens").

For 35 maize samples collected from plants with maize rayado fino symptoms at Montecillos, July 1988, and tested by PAS-ELISA, 49% reacted positively with CSS antiserum, 86% with MBSM antiserum, and 29% with MRFV antiserum. It is noted that both CSS and MBSM were detected by PAS-ELISA, whereas symptoms incited by the two pathogens were not recorded.

PAS-ELISA did not perform satisfactorily in tests of most samples and only selected results, most representative and best, are presented. PAS-ELISA problems occurred mostly with the MRFV and MBSM antisera which did not give consistent results. Consequently, PAS-ELISAs for many maize and leafhopper samples from Montecillos were confusing. For example, although maize showed MRFV symptoms, not every sample gave a positive reaction with the MRFV antiserum. Further, satisfactory results were not obtained for samples stored in the freezer. Also, in some cases healthy maize tissue gave positive results. Almost every sample reacted with MBSM antiserum which indicated that this antiserum also was not satisfactory. The best PAS-ELISA results were obtained for assays of *Dalbulus* species.

Numbers of four *Dalbulus* species at the three locations for three year period, including both males and females, varied considerably with location, month of year and year (Table 3). No one location had overall higher numbers than the other two locations, although Montecillos and Tlaltizapan had single major peaks of 171 and 149, respectively. Maximum numbers of *Dalbulus* species at Poza Rica occurred near or at the end of the dry season planting in both years. Maximum numbers of *Dalbulus* species at Tlaltizapan occurred at the beginning of the rainy season planting for both years. A second peak was observed at the end of the rainy season planting during the second but not the first year. Maximum numbers of *Dalbulus* species at Montecillos occurred at the end of the single yearly planting. In 1987 *Dalbulus* numbers were recorded only after the growing season. A plot of final

Table 3. Monthly totals of weekly averages of numbers of *Dalbulus* species in each of 3 yr (1987-1989)[a] at the three test sites in Mexico.

		Location								
		Montecillos			Tlaltizapan			Poza Rica		
Month	Day of year	87	88	89	87	88	89	87	88	89
1	1- 30	--[b]	--	--	--	3.2	8.8	--	1.0	3.2
2	32- 59	--	--	--	--	4.2	3.8	--	16.6	3.2
3	60- 90	--	--	--	--	5.0	8.0	--	25.0	11.2
4	91-120	--	3.0	2.2	--	15.0	11.9	--	22.6	21.0
5	121-151	--	8.9	3.6	--	17.0	24.5	--	--	--
6	152-181	--	7.0	1.2	--	149.3	48.4	--	6.8	5.2
7	187-212	8.6	15.9	1.0	--	7.7	5.2	--	14.8	9.8
8	213-243	20.1	14.6	9.6	--	2.9	1.0	--	6.0	6.4
9	244-273	32.7	--	--	11.8	2.8	--	--	3.3	20.8
10	274-304	171.3	--	--	33.5	9.7	--	--	2.8	--
11	305-334	85.4	--	--	38.0	--	--	--	--	--
12	335-365	11.7	--	--	2.5	77.1	--	11.0	9.3	--

[a] Totals of all four species, males and females. Numbers are densities per yellow sticky card totaled for the listed month.

[b] Not collecting.

Table 4. Monthly totals of weekly averages of numbers of four *Dalbulus* species (*D. maidis*, *D. elimatus*, *D. guevarai* and *D. longulus*) at the three rest sites in Mexico[a] for the three years

		Location								
		Montecillos		Tlaltizapan			Poza Rica			
Month	Day of year	*maidis*	*elim.*	*maidis*	*elim.*	*guev.*	*maidis*	*elim.*	*long.*	*guev.*
1	1- 31	--[b]	--	3.6	1.8	2.3	2.8	--	--	--
2	32- 59	--	--	2.8	--	1.3	10.8	--	1.0	--
3	60- 90	--	--	4.9	--	1.7	13.3	2.0	5.9	1.0
4	91-120	1.0	1.7	10.8	3.0	1.3	21.4	--	1.0	--
5	121-151	3.4	2.8	18.8	--	1.0	--	--	--	--
6	152-181	2.0	3.1	54.8	3.6	15.9	4.9	--	1.0	--
7	182-212	6.8	1.1	4.1	1.0	1.9	4.9	--	1.4	6.0
8	213-243	11.0	1.2	1.9	--	1.0	5.9	5.9	1.0	--
9	244-273	29.4	3.3	3.9	0.6	2.8	8.4	--	1.5	--
10	274-304	151.6	19.8	14.5	--	4.7	2.8	--	--	--
11	305-334	78.6	6.8	36.0	1.0	1.0	--	--	--	--
12	336-365	9.0	2.7	49.4	8.4	2.9	8.3	--	1.6	--
Mean:	24.4	3.5	17.1	1.6	3.2	7.0	0.2	1.2	0.6	

[a] Means over 1-3 yr; total of males and females for the listed month.

[b] No collection.

Table 5. Percentage of males within each of four *Dalbulus* species at three test sites in Mexico[a]

	Location		
Dalbulus species	Montecillos	Tlaltizapan	Poza Rica
maidis	75.9	65.4	60.5
elimatus	78.9	56.8	100.0
longulus	--	--	70.1
guevarai	--	56.2	33.3

[a] Averages for 1-3 yr.

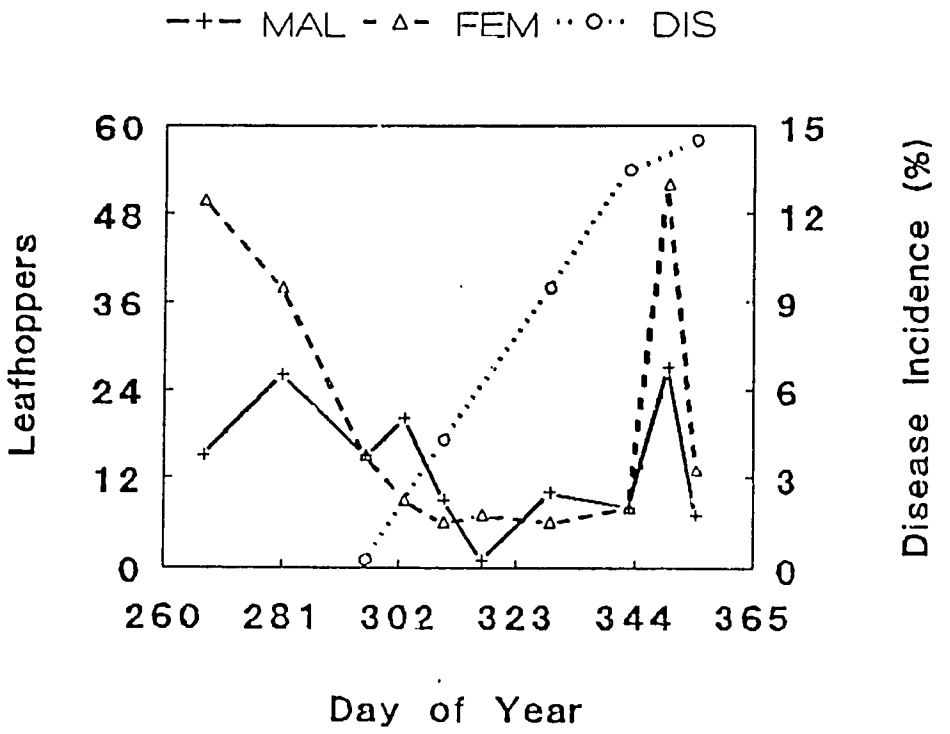
disease incidences recorded only after the growing season for the 11 plantings (last datum point shown in Fig. 5) versus the number of leafhoppers counted for the month prior to that of the final disease incidence rating showed no relationship between leafhopper numbers and disease incidence (Fig. 6).

Among the four *Dalbulus* species, *D. maidis* was recorded at the three locations and was the most abundant (Table 4). *D. elimatus* was also recorded at the three locations but was not abundant at any of them. The very low numbers of *D. elimatus* at Poza Rica are believed to have been misidentifications of species. *D. guevarai* was recorded at Tlaltizapan and Poza Rica, but in small numbers. *D. longulus* was recorded only at Poza Rica. The second most abundant species depended on the location. Specifically, the second most abundant species was *D. elimatus* at Montecillos, *D. guevarai* at Tlaltizapan and *D. longulus* at Poza Rica. The pattern of increase and decrease in numbers of *D. maidis* at Montecillos tended to parallel that for *D. elimatus*, although the latter were fewer. At Tlaltizapan, these patterns for *D. maidis* and *D. guevarai* were infrequently similar. Comparisons for the remaining species were not possible because of too many missing monthly numbers for the species other than *D. maidis*. Males of each *Dalbulus* species were recorded more frequently than females at the three locations, except for *D. guevarai* at Poza Rica (Table 5). This indicates a greater mobility for males than females of these *Dalbulus* species.

To monitor migrating *Dalbulus* species at the three test sites six suction traps were constructed and placed as described in the following. At Montecillos, four traps with different heights were placed in the field. The heights of the trap inlets were 30 ft (10 m), 15 ft (5 m) (two traps), and 6 ft (2 m). Traps were placed linearly at 300 m from test maize plots. Two other traps with the inlet at 15 ft (5 m) were placed at the CIMMYT weather stations. The Tlaltizapan trap was 200 m from maize plots and the Poza Rica trap 60 m from maize plot. Each trap had a collecting jar (200 ml), and an electric fan which "sucked" leafhoppers into the jar. Collections from jars were made at 9:00 am daily. Collection were begun March 1989. Collected insects were stored in alcohol 70%.

Only one migrating leafhopper (*D. elimatus*) was collected in the suction traps. Since other kinds of insects, e.g. different aphid species, were collected in these traps at each trap height, *Dalbulus* species

Alajuela, planted 10/1/87



Tabago, planted 10/13/87

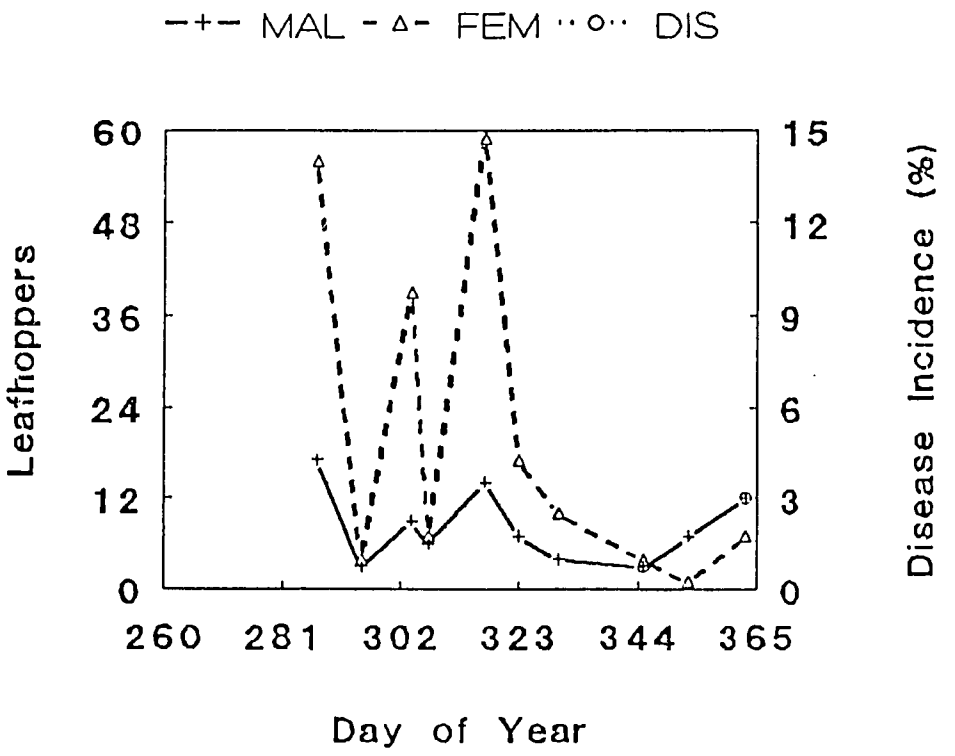


Fig. 1. Population density of Dalbulus maidis on four yellow sticky cards (male [MAL] and female [FEM]) and the percentage of plants showing disease symptoms (DIS) for Experiment 1.

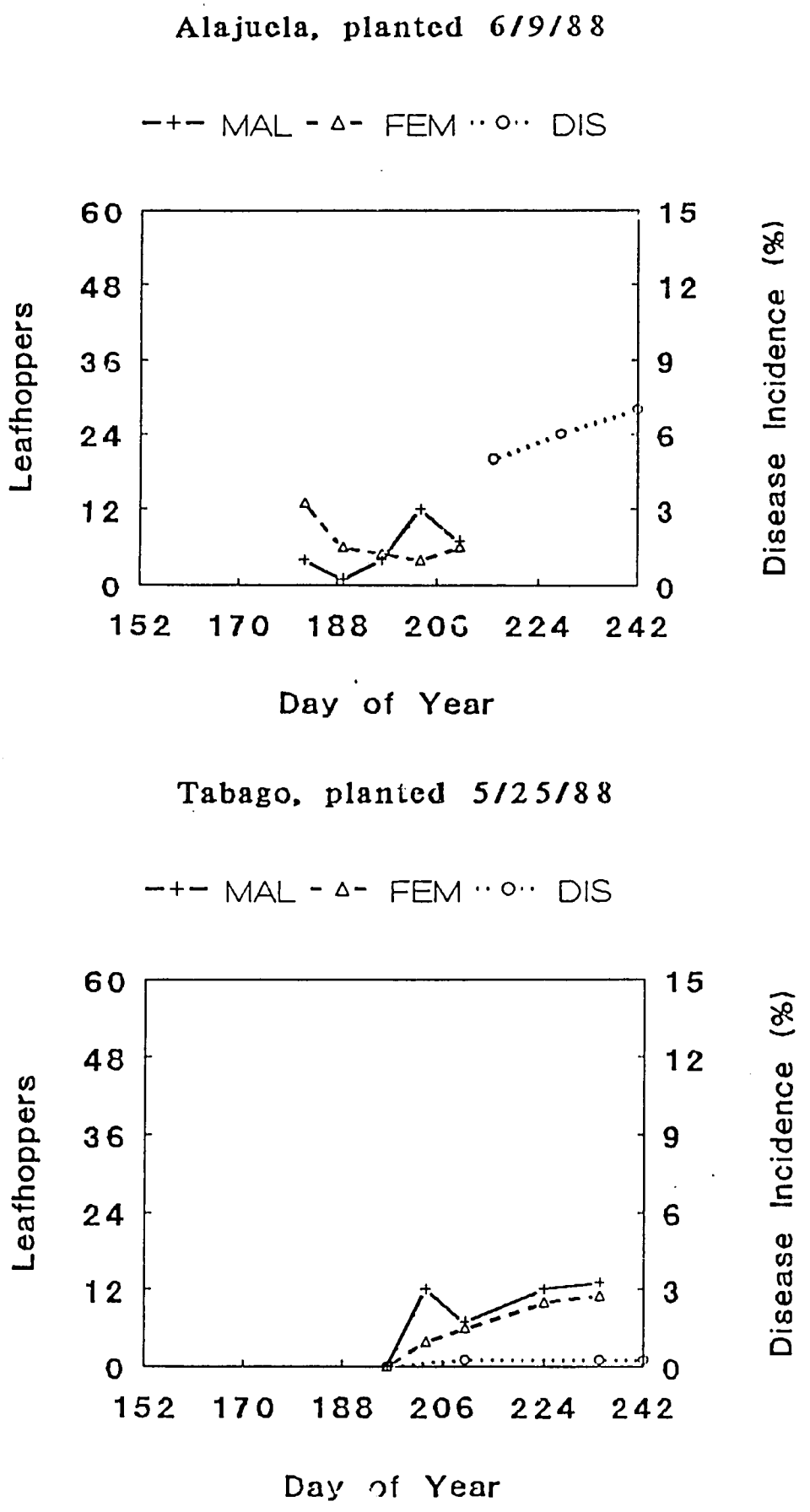
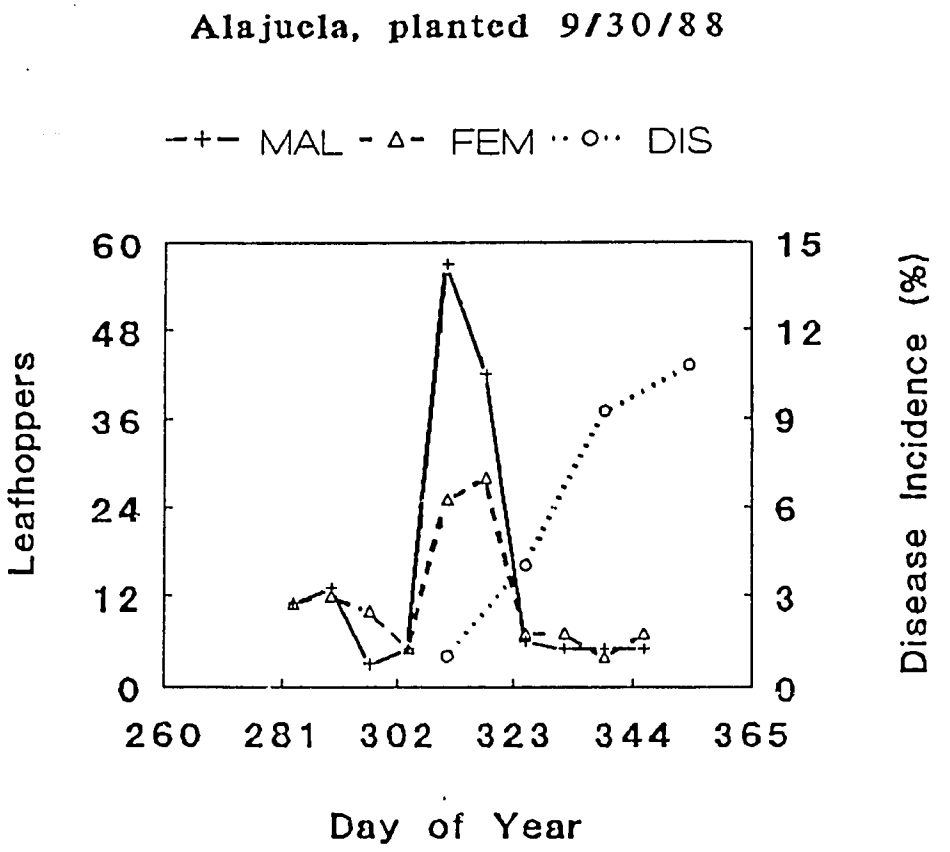


Fig. 2. Population density of Dalbulus maidis on four yellow sticky cards (male [MAL] and female [FEM]) and the percentage of plants showing disease symptoms (DIS) for Experiment 2.



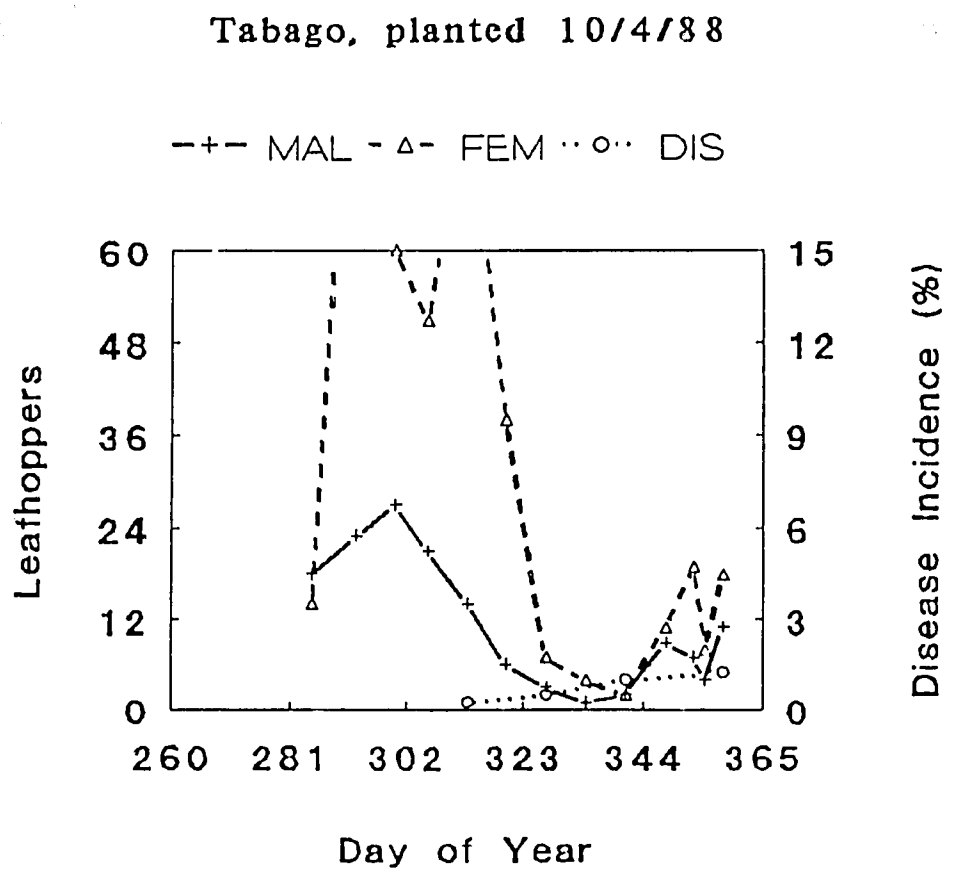
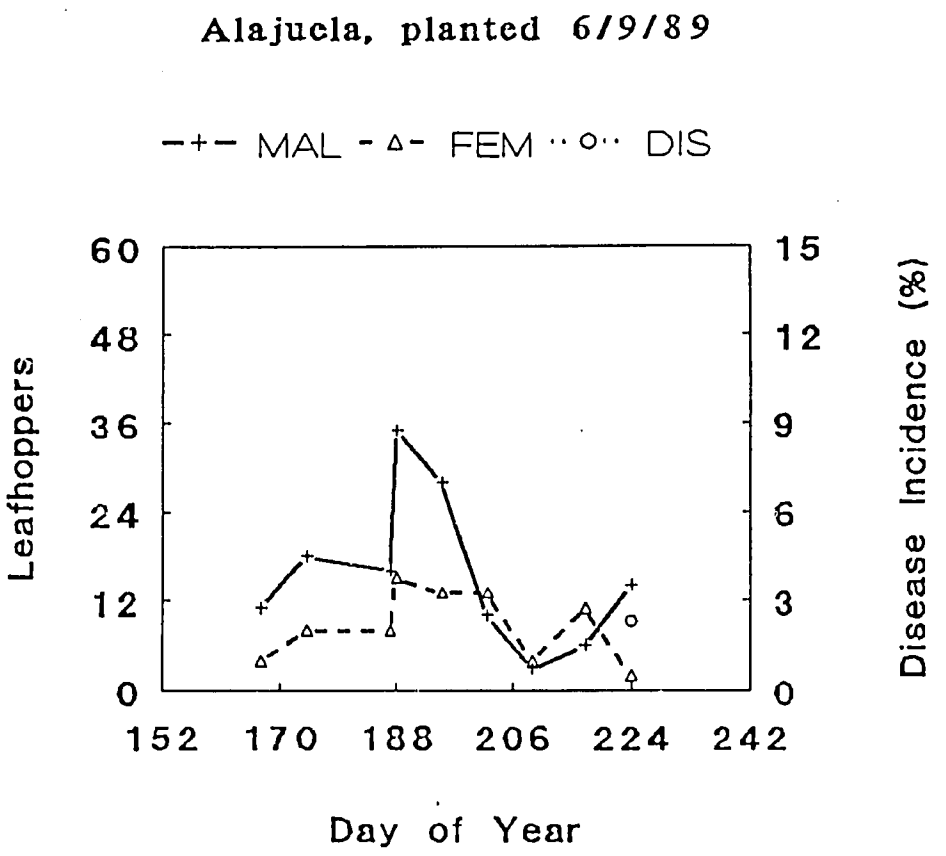


Fig. 3. Population density of Dalbulus maidis on four yellow sticky cards (male [MAL] and female [FEM]) and the percentage of plants showing disease symptoms (DIS) for Experiment 3.



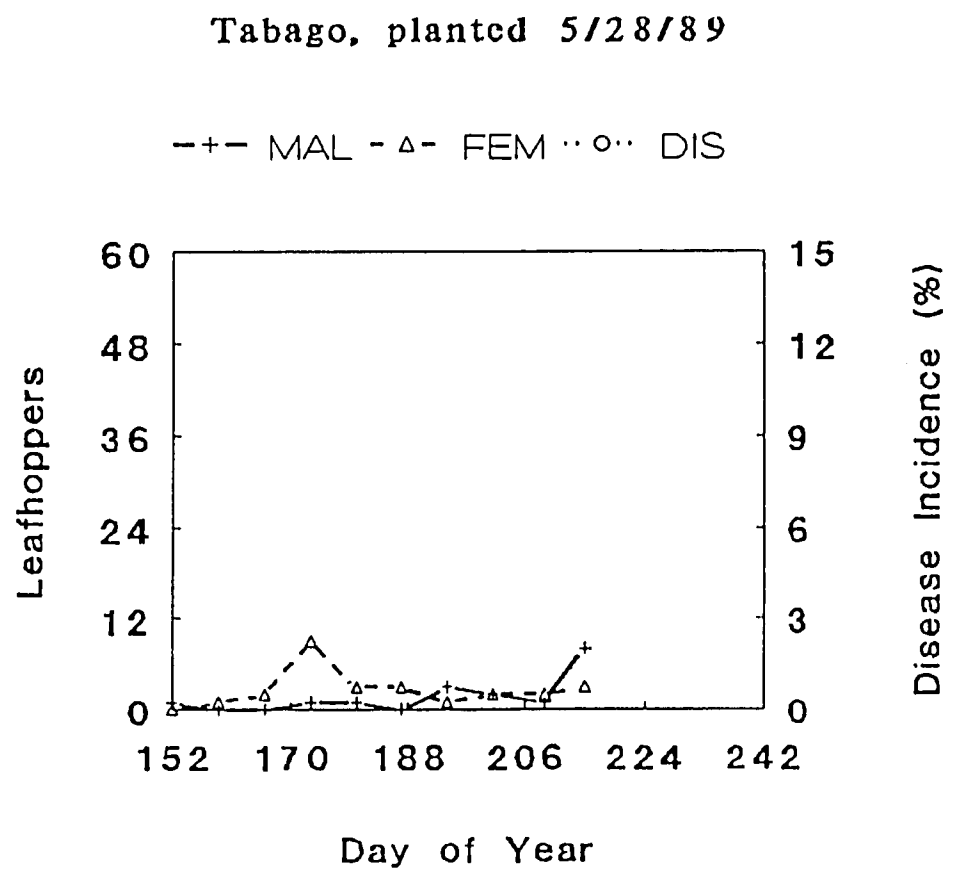


Fig. 4. Population density of Dalbulus maidis on four yellow sticky cards (male [MAL] and female [FEM]) and the percentage of plants showing disease symptoms (DIS) for Experiment 4.

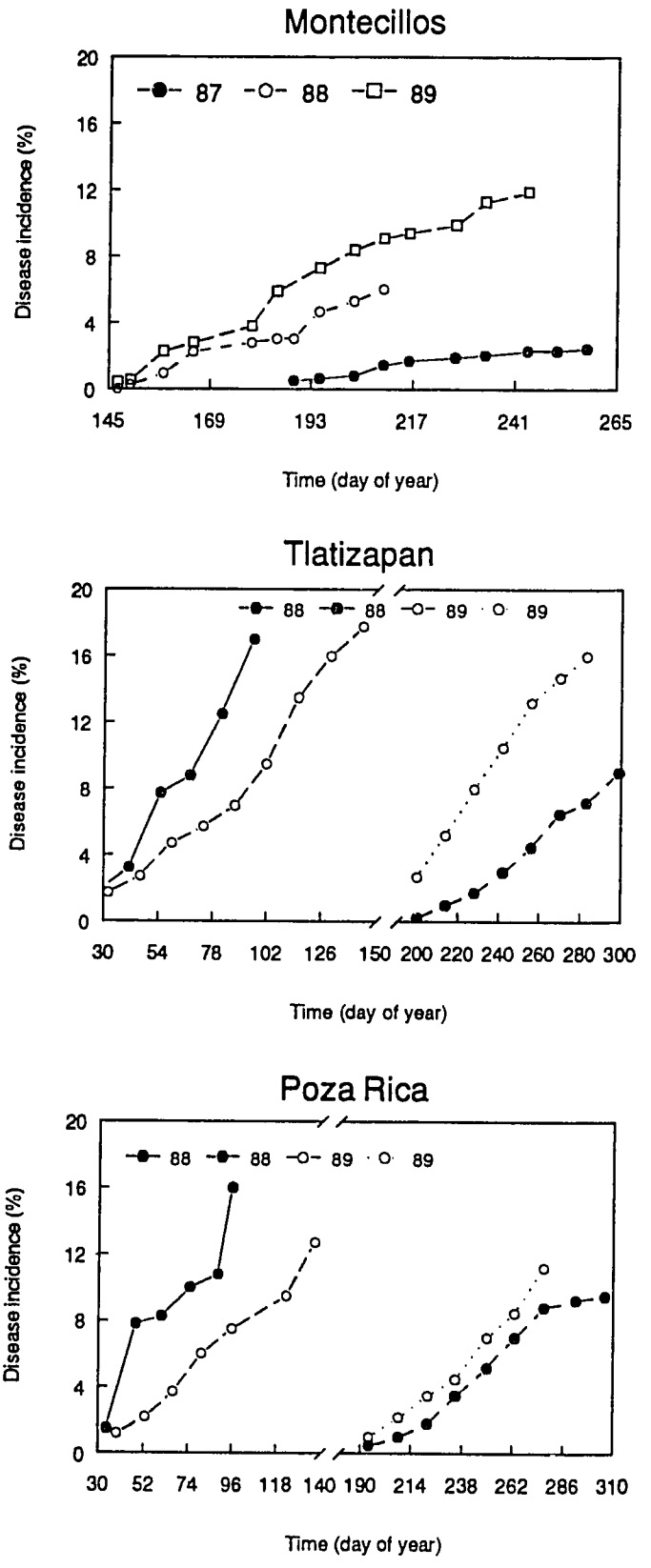


Fig. 5. Percentage of plants showing symptoms of MRFV at three locations in Mexico. Data are for 2-3 yr (marked in the figure [e.g., 89 for 1989])and one or two plantings per year.

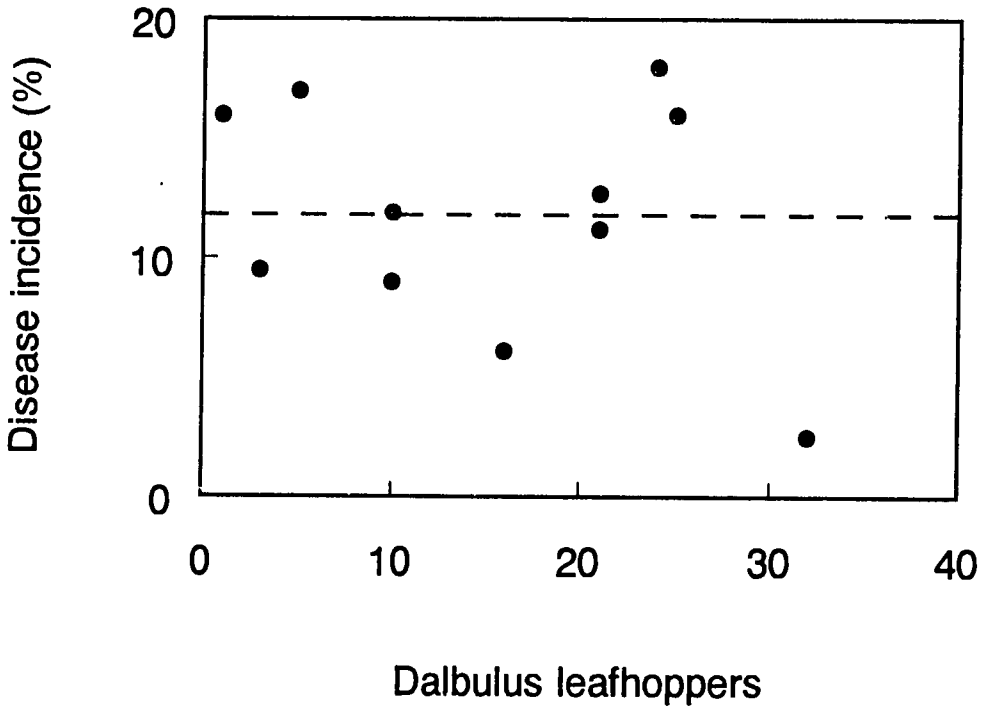


Fig. 6. Example of the lack of relationship between virus disease incidence and density of Dalbulus leafhoppers. Data represent final incidence from the 11 plantings (last point of the 11 curves in Fig. 5) and total number of leafhoppers trapped during the month before final disease assessment.

may not have been flying at the levels at which the inlet openings were placed. Alternatively, these traps may not have been suitable for collecting migrating *Dalbulus* leafhoppers.

Surveys for Vectors and Pathogens within Various Vegetational Zones

Costa Rica

Stunting disease incidences within maize fields within the 10 vegetational zones were very low (Table 1). The 10 vegetative zones varied in elevations from 10 m to 1821 m; type of forest from tropical dry to tropical rain forests; and terrain from lowland, premontane and lower montane forests. The highest disease incidence was 12.5% and only two other fields had disease incidences greater than 5%. Nine fields had no stunting disease out of the 33 for which records were made. The percent stunting diseased plants in the two fields sampled at a given location were frequently different. Commonly, one field had no stunting diseased plants while the second had a low percent.

Maize rayado fino incidences within maize fields within the 10 zones were also very low (Table 1). Incidences ranged from 0% (17 fields) to a maximum of 15% (one field). One field had an incidence of 13% while the remaining 13 fields where the disease was found had incidences of 0.5 to 2.5%.

Stunting disease and maize rayado fino incidences did not correlate with elevation, type of forest (dry to moist) or terrain (lowlands, premontane or lower montane). Further these incidences were not consistent from year to year. For example, the tropical moist forest had no stunting disease in 1988 but about 4% in 1989. The generally low disease incidences indicated that neither the stunting diseases nor maize rayado fino caused serious damage to maize production in 1988 and 1989.

Mexico

Surveys for vectors and pathogens were conducted within various vegetational zones. Disease incidences were assessed on maize plots in two vegetational zones. These sites were: Depresiòn del Balsas (Michoacan and Morelos) and Planicie Costera Nor-oriental (San Luis Potosi and Tamaulipas). Maize leaves showing MRFV symptoms as well as leafhoppers were collected and assayed by PAS-ELISA. Results of surveys for *Dalbulus* vectors within various vegetation zones and PAS-ELISA results for the pathogens infecting *Dalbulus* are presented in (Table 2). Samples assayed by PAS-ELISA included *Dalbulus* spp. collected in San Luis Potosi, 1989, in Michoacan, 1989; in Tamaulipas, 1989; in Cd. Victoria, Tamaulipas, 1989; and in Taltizapan, Morelos, 1989. Maize samples collected from "El Zapote", San Luis Potosi were also assayed by PAS-ELISA. As stated in the previous section, PAS-ELISA results were generally not satisfactory because of problems with the MRFV and MBSM antisera and only selected results, the most representative and best, are presented. The best PAS-ELISA results were obtained for assays of *Dalbulus* species. Disease incidence in maize in the two vegetational zones appear in Table 3.

Among the *Dalbulus* species tested by PAS-ELISA, the majority (95%) were *D. maidis* with the remaining species (*D. elimatus, D. guevarai,* and *D. longulus*) at 1% or less (Table 2). The majority (71%) of all *Dabulus* were female and 69% of all species tested positive for CSS and 51% for MRFV. These relatively high percentages of pathogen positive samples contrasts with the majority of results for PAS-ELISA of plant species within maize plots (see Tables 1-4 in "Tests of alternate sources of the three pathogens") where samples positive for the two pathogens were less than 1%.

Table 1. Percentage of plants with stunting disease and maize rayado fino virus (MRFV) symptoms in vegetational zones in Costa Rica

	Vegetational zone	Location	Elevation (m)	1988 Disease Incidence (%)	1988 MRF Incidence (%)	1989 Disease Incidence (%)	1989 MRF Incidence (%)
1.	Tropical premontane moist forest 41105	Alajuela	693	4	2.5	2	15
				4	1	1	0.5
2.	Tropical dry forest	Tabago	10	1	0	2	1
				-[a]	-	0	0
3.	Tropical dry forest moist province transition	Carrillo	23	0	0	0	0
				2	1	0	0
4.	Tropical wet forest	Cariario-Sagrada Familia	50	3	0.5	5.5	1.5
				0	0	11	1.5
5.	Tropical moist forest	Orotina	229	0	0	4	0
				0	0	4.5	0
6.	Tropical premontane rain forest	San Juan Lajas	1380	2.5	0	-	-
				4.5	1.5	-	-
7.	Tropical premontane wet forest	Palmares	1017	0	0	13	13
				4	0.5	4	1
8.	Tropical lower montane moist forest	Zarcero	1736	0.5	0	-	-
				2.5	0	-	-
9.	Tropical lower montane wet forest	Tapezco	1821	1.5	0	2	0.5
				2.5	0	0	0
10.	Tropical moist forest premontane belt transition	San Isidro	702	-	-	-	-
				-	-	-	-
11.	Tropical premontane wet forest, basal	Quepos	20	-	-	3.5	2
				-	-	3.5	1.5

[a] No data collected.

Table 2. PAS-ELISA of *Dalbulus* species collected from four locations in Mexico for CSS and MRFV.

Location	*Dalbulus* species	Number of samples	Number of Sex: Female	Male	Undetermined	Number PAS-ELISA positive samples: CSS	MRFV
San Luis	*D. elimatus*	2	2	0	0	0	0
	D. maidis	12	3	2	7	7	11
Michoacan	*D. maidis*	35	28	7	0	25	13
Tamaulipas	*D. maidis*	35	29	6	0	22	16
cd Victoria	*D. longulus*	2	1	1	0	1	0
	D. maidis	16	11	5	0	13	7
Tlaltizapan	*D. guevarai*	1	0	1	0	1	1
	D. maidis	23	16	7	0	17	16

Table 3. Disease (maize rayado fino) incidence in one or two maize fields in two vegetative zones in Mexico, 1989.

Vegetative zone and location within zone	Disease incidence (%)[a]
1. Depresion del Balsas	
Michoacan	4.5
Morelos (Tlaltizapan)	16
2. Planicie Costera Nor-oriental	
San Luis Potosi	12
Tamaulipas Field 1	6
Field 2	11.5

[a] 200 plants were examined by disease symptoms at both sites.

These results support the view that the source of these two pathogens is infected *Dalbulus* species, principally *D. maidis*, and not alternate plant hosts.

Incidence of maize rayado fino in maize fields in the two vegetational zones, Depresion del Balsas and Planecie Costa Nor-oriental, ranged from 4.5 to 16%. These incidences corresponded with those recorded for maize plots at the three test locations, Montecillos, Tlaltizapan and Poza Rica for 1987-1989. (See "Montoring *Dalbulus* vectors and disease incidence"). Surprizingly, PAS-ELISA of nine

maize plants, each with symptoms of maize rayado fino, were negative for MRFV (data not shown). These samples were also negative for CSS in PAS-ELISA. These results are in contrast to those for *Dalbulus* collected at the same site (Table 2) in which 50% tested positive for CSS and 79% for MRFV. Most surprizing is the absence of reported corn stunt symptoms in maize at this site, although vector leafhoppers in the vicinity were apparently infective.

Hibernation of *Dalbulus* leafhoppers

Insofar as is known, there are no perennial plant hosts of CSS or MBSM. Thus, these mollicutes likely overseason only in their *Dalbulus* vectors and their survival depends on survival of the leafhopper.

In the initial test to monitor the hibernation of *Dalbulus* leafhoppers, cages containing young maize plants (1.5 X 0.5 m) were placed in fields near test plots during the winter season of 1988-89 at the Montecillos site. *Dalbulus* leafhoppers were collected two days per week.

Dalbulus leafhoppers remained in hibernation as long as cages had maize plants. However, when temperatures dropped so that maize plants failed to grow, *D. elimatus* remained for a longer time than *D. maidis*. No other *Dalbulus* species was observed at Montecillos.

In a test of whether *D. maidis* overseasons in maize stubble left from the previous growing season, no leafhoppers emerged from screen cages placed over soil and residue at Chapingo [Rodriguez, *unpublished* or Corralitos, Jalisco, Mexico (Nault, *unpublished*)]. Yellow sticky cards placed near cages in the Corralitos field trapped large numbers of *D. maidis* in January and February, long after maize had matured and dried (Larsen et al., 1992). This suggested that leafhoppers may be overseasoning in the weeds and wild plants surrounding maize fields, or perhaps wooded areas nearby.

In a later study, we tested whether the first substantial rainfall in the spring brings *D. maidis* out of its overseasoning habitats in search of newly planted maize fields. An experiment was established at the Fabio Baudrit Experiment Station in Alejuela, Costa Rica, during the spring of 1990. Five areas were selected from fields planted with maize the previous year. Two plots (3 x 3 m each) were established in each field (10 m apart). The weedy grasses in the plot were watered 3 times per week with 10 gal of water, whereas the second plot was not. Leafhoppers were monitored in each plot with yellow sticky cards or with sweep nets. The experiment was started in mid-April before the first significant rainfall which occurred in late April (Table 1).

Compared to other leafhopper species, very few *D. maidis* were collected with sweep nets (net trap) or with yellow sticky cards (data not shown). A single UV light trap collected many more leafhoppers, including *D. maidis*. The results were inconclusive. Watered plots did not produce more *D. maidis* than unwatered ones. When the whorls of 100 maize seedlings were sampled in early, mid and late May and early June, large numbers of leafhoppers were counted (Table 2). This suggests that *D. maidis* adults were immigrating from some other source than weedy areas surrounding these fields (Villalobos and Nault, *unpublished*).

Table 1. Precipitation at Fabio Baudrit Experiment Station, Alajuela, Costa Rica, 1990.

	Precipitation in mm		
Date	April	May	June
1	-	-	20.0
2	-	-	19.8
3	-	-	0.3
4	-	-	0.9
5	-	-	-
6	-	1.5	2.0
7	-	-	-
8	-	-	37.8
9	-	10.2	38.4
10	-	-	-
11	-	-	45.2
12	-	-	13.1
13	-	6.7	6.0
14	-	14.3	-
15	-	6.1	3.0
16	-	-	18.9
17	-	2.0	6.3
18	-	-	2.8
19	-	-	-
20	-	-	0.1
21	-	26.3	-
22	-	9.6	-
23	-	26.5	2.0
24	0.7	0.6	15.0
25	-	3.7	-
26	17.6	6.2	4.7
27	0.8	14.7	6.7
28	0.5	-	0.8
29	9.1	10.7	-
30	42.3	6.2	2.5
31	-	0.4	-
TOTAL	71.00 mm in 6 days	104.9 mm in 16 days	246.3 mm in 21 days

Table 2. *D. maidis* in the whorls of 100 maize plants at the Fabio Baudrit Experiment Station, Alajuela, Costa Rica, 1990.

Date	Female	Male	Total	Young	Medium	Old	Ninfae
4-5-1990	4	3	7	2	4	1	-
11-5-1990 morning	29	13	42	11	12	19	1
26-5-1990 afternoon	11	15	26	10	2	3	16
1-6-1990 afternoon with rain	0	3	3	2	1	-	1

Tests of alternate sources of the three pathogens

Plant samples were collected during the dry and rainy seasons for 1988 and 1989 at Montecillos where maize is not grown continuously. Extracts from leaves of these plants were assayed for CSS and MRFV by PAS-ELISA. While the results of PAS-ELISA were problematic as discussed elsewhere in this report, the most reliable of these results are presented here (Tables 1-4).

The number of positive samples for CSS and MRFV were very low for both dry and rainy seasons of both years. The results suggest that the two pathogens were present at very low incidences in the vegetation of maize plots at Montecillos and that this vegetation was not the source of the two pathogens for infection of maize. MBSM incidence was higher than those of the other two pathogens for samples collected in the 1989 rainy season (Table 4) the only samples for which acceptable results were obtained are presented. However, in view of the generally poor performance of PAS-ELISA with MBSM antibodies, these results are of doubtful reliability and further tests of the positive species using the hybridization assay is needed for confirmation.

Table 1. PAS-ELISA of plant samples collected in the dry season at Montecillos, April 1988, for CSS and MRFV

Plant genus and species	Number of samples	Number of pathogen positive samples	
		CSS	MRFV
Aegopogon cenchroides	3	0	0
Agrostis semiverticillata	3	1	0
Chenopodium ambrosiodes	1	0	0
C. mexcanum	1	0	0
Digitaria sanguinalis	3	0	0
Malva neglecta	16	0	0
Taraxacum officinale	4	0	0
Zea mays	2	0	0

Table 2. PAS-ELISA of plant samples collected in the dry season at Montecillos, April, 1989, for CSS and MRFV

Plant genus and species	Number of samples	Number of pathogen positive samples	
		CSS	MRFV
Agropogon cenchroides	1	0	0
Agrostis semiverticillata	2	0	0
Chenopodium album	3	0	0
Chenopoduim spp.	1	0	0
Digitaria sanguinalis	2	0	0
Graminea bromus	1	1	1
Malva neglecta	10	0	0
Taraxacum officinale	3	0	0
Trifoluim repens	2	0	0

Table 3. PAS-ELISA of plant samples collected in the rainy season at Montecillos, April, 1988, for CSS and MRFV[a]

Plant genus and species	Number of samples	Number of pathogen positive samples	
		CSS	MRFV
Aegopogon cenchroides	2	0	0
Agrostis semiverticillata	1	0	1
Bromus laciniatus	1	0	0
Camomella sp.	1	0	0
Chenopodium ambrosiodes	4	0	0
Digitaria sanguinalis	1	0	0
Malva neglecta	4	0	0
Sicyos angulatus	1	0	0
Taraxacum officinale	3	0	0
Trifoluim repens	2	0	0

[a] Antiserum to MBSM was included in assay but every sample showed a positive reaction with the antiserum indicating that results were unreliable and thus were not presented in table.

Table 4. PAS-ELISA of plant samples collected in the rainy season at Montecillos, April, 1989, for CSS, MBSM and MRFV

Plant genus and species	Number of samples	Number of pathogen positive samples		
		CSS	MBSM	MRFV
Aegopogon cenchroides	6	0	4	0
Agrostis semiverticillata	1	0	1	0
Chenopodium ambrosiodes	1	0	0	0
Digitaria sanguinalis	1	0	1	0
Malva neglecta	4	0	2	0
Sicyos angulatus	2	0	0	0
Taraxacum officinale	2	0	0	0
Trifoluim repens	1	0	0	0

Environmental measurements

Weather data from Montecillos, Tlaltizapan and Poza Rica, Mexico, were collected for the years0 1987-1989. The Montecillos data includes weekly averages of minimum and maximum temperatures, hours of sunlight, evaporation, wind speed at two elevations, precipitation and relative humidity. These data for Tlaltizapan and Poza Rica include the same categories except relative humidity was not

measured at either location. These data are presented in Appendix A.

The low disease incidences and the lack of an association of leafhoppers numbers with disease incidence at all location in all years of the study precluded the planned use of these environmental measurements in developing regression models to relate environment to leafhopper migration within and to maize fields and then leafhopper numbers to disease incidence.

Development and Comparison of Serological Assays

The lack of diagnostic symptoms for distinguishing CSS and MBSM infections and the inability to recognize diagnostic symptoms of maize rayado in combined infections of MRFV and CSS and/or MBSM motivated studies to develop reliable, specific and sensitive assays for these pathogens. These studies focused on serological assays, principally various versions of the enzyme linked immunosorbent assay (ELISA). A concomitant development was the attempt to produce antisera to the three pathogens and monoclonal antibodies to MBSM.

At the time these experiments were initiated, Protein A sandwich (PAS-ELISA) was a new and promising version which purportedly had enhanced sensitivity and specificity over previously developed ELISAs. Accordingly, we conducted a series of experiments to adapt PAS-ELISA for reliable and sensitive detection of the three pathogens.

PAS-ELISA involves coating microtiter plate wells with a layer of Protein A. Trapping antibodies, i. e., antibodies to the pathogen, are then added to wells and allowed to attach to the Protein A. The unattached trapping antibodies are then washed from wells and the test antigen is added and allowed to interact with the trapping antibody. Antigen which does not interact with the trapping antibody is washed from wells and detecting antibodies, i.e., the pathogen antibodies, are added. The detecting antibodies are allowed to interact with bound test antigen and then the unattached antibodies are washed from wells. A Protein A-biotin conjugate is then added and allowed to attach to bound detecting antibody. Unattached Protein A conjugate is washed away and a streptavidin horse radish peroxidase conjugate is added to wells where it becomes bound to any attached Protein-A biotin conjugate. The unbound streptavidin conjugate is washed from wells and the peroxidase substrate is added to detect bound horse radish peroxidase. After an appropriate incubation, the reaction is stopped by the addition of 3 N NaOH. The absorbances of well contents are read with a spectrophotometer and the absorbance values analyzed by appropriate statistical procedures to identify pathogen positive and negative samples. When monoclonal antibody was used as the detecting antibody, goat anti-mouse IgG conjugated with horse radish peroxidase was substituted for the Protein A-biotin and streptavidin horse radish-peroxidase conjugates.

In the experimental work to develop PAS-ELISA, CSS and MRFV polyclonal antisera, produced by us, were used. MBSM antiserum was from several sources: a) MBSM polyclonal antiserum and a monoclonal antibody produced by us; b) MBSM polyclonal antiserum provided by L. L. McDaniel, University of Florida, Fort Lauderdale: and c) a monoclonal antibody provided by T. A. Chen, Rutgers University, New Jersey. We were unable to obtain a reliable PAS-ELISA which was specific and sensitive using any of these antisera or monoclonal antibodies (data not shown). The quality of the MBSM polyclonal antisera and our monoclonal antibody was so poor that development of a satisfactory PAS-ELISA was not possible. The polyclonal antisera to CSS and MRFV and the Chen

monoclonal antibody were of sufficient quality but PAS-ELISA did not give consistent results. Our evidence suggested that the inconsistency was due to an inability to fully saturate the Protein A coat with trapping antibody or for the Protein A to fully retain trapping antibody prior to the addition of the detecting antibody. Under either circumstance, added detecting antibody would bind to the Protein A coat, in the absence of bound antigen (pathogen), giving a false positive result. An alternate possibility was that the Protein A conjugate bound to the Fc portion of the trapping antibody, assuming that there was more than one binding site on the Fc for Protein A. This possibility would also yield false positives. After many experimental tries to solve the problem, we abandoned our efforts without success.

We next attempted to develop antigen coat ELISA (AC-ELISA) which involves initially coating microtiter wells with antigen (pathogen). The method eliminates the problem of attachment of detecting antibody to the Protein A coat and the requirement for trapping antibody. The remainder of AC-ELISA was similar to the PAS-ELISA procedure. The same polyclonal antisera and the Chen monoclonal antibody were used in AC-ELISA. Again the MBSM polyclonal antisera were of insufficient quality for satisfactory results (data not shown). The Chen monoclonal antibody and the CSS and MRFV polyclonal antisera were satisfactory (data not shown). While these antibodies were generally performed well in AC-ELISA, inconvenient exceptions prompted us to attempt development of a third ELISA. The problem with AC-ELISA appeared to be that the antigen coat was not always satisfactory, probably due to interfering or co-coating materials in infected and healthy plant extracts.

The third ELISA was $F(ab')_2$-ELISA in which $F(ab')_2$ units prepared from the IgG fraction of the antiserum are used to coat microtiter plate wells and function to trap homologous antigen. $F(ab')_2$ units do not bind Protein A and thus do not react with the Protein A conjugate. The remainder of $F(ab')_2$-ELISA was similar to PAS-ELISA. $F(ab')_2$ units were prepared from the CSS and MRFV antisera and the Chen monoclonal antibody. The $F(ab')_2$-ELISA for CSS and MRFV performed satisfactorily whereas that with the Chen monoclonal antibody while specific was too weak in reactivity for reliability, especially with low pathogen titers. Thus, $F(ab')_2$-ELISA was used to detect CSS and MRFV and as explained in the section on monoclonal antibodies a cDNA hybridization assay was developed using a probe obtained from M. J. Davis (University of Florida, Fort Lauderdale) to detect MBSM.

Production of Monoclonal Antibodies to MBSM

A companion Research Project of the USAID/CSRS Collaborative Research on Special Constraints at the International Agricultural Research Centers (IARCS) Program, entitled "Differentiating the Corn Stunt and Maize Bushy Stunt Diseases in Latin America", (Grant Number: 86-CRSR-2-2868) was conducted with D. T. Gordon as the Principal Investigator and R. E. Gingery and R. A. Simkins as cooperators. This project was for the period August 1, 1986 - March 31, 1989. The objectives of the project were to develop rapid, reliable assays for the corn stunt spiroplasma (CSS) and the maize bushy stunt mycoplasma (MBSM) and to determine their respective involvement in the corn stunt disease in developing countries of Latin America. A main effort of the project was to develop enzyme-linked immunosorbent assays (ELISA) for these pathogens. The following work was accomplished as part of this study and the companion Research Project.

For the assay of MBSM, mice were immunized with preparations from either MBSM-infected maize

or *D. maidis* leafhoppers in an attempt to produce monoclonal antibodies. Several hundred hybridomas were obtained from fusions of spleen cells from the immunized mice with SP2/0 murine myeloma cells. These hybridomas were screened against infected and healthy insect or maize-plant extracts for MBSM-specific monoclonal antibodies. [The screening was done with an extract from the host other than the one from which the MBSM preparation for immunization was obtained, since it was expected that extracts from the host not used to immunize the mice would contain fewer host antigens to react with monoclonal antibodies produced to contaminating host antigens in the immunization. We expected that this approach would simplify detecting MBSM-specific monoclonal antibodies.] The screening yielded no monoclonal antibodies that reacted specifically with extracts from MBSM-infected tissue. We concluded that no monoclonal antibodies to MBSM were produced and that the monoclonal antibodies obtained were all raised against host antigens that had contaminated the extracts used to immunize the mice.

Following these results and as an alternate approach for producing antibodies to MBSM, we sought to isolate MBSM-specific proteins for immunizing the mice. A polyclonal antiserum, produced in rabbits immunized with extracts from MBSM-infected maize or leafhoppers, was used to monitor different purification steps for their effect on MBSM concentrations. (The polyclonal antiserum was obtained from Dr. L. L. McDaniel, University of Florida, Fort Lauderdale and had been recently produced. This antiserum, while useful in monitoring MBSM purification, did not work satisfactorily in attempts to detect MBSM using ELISA in infected-plant extracts because of unacceptably high background (non-pathogen) reactions.) By selecting those steps which maintained MBSM concentration and at the same time reduced impurities, a procedure was developed in which MBSM was partially purified. The procedure involved the following principal steps: 1) clarification of the infected-plant extracts with diatomaceous earth (Celite) and 2) centrifugation of the clarified extract on a Percoll density gradient.

Analysis of the proteins from partially purified MBSM preparations by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) revealed a 25-kilodalton protein (P25) that reacted strongly with the MBSM polyclonal antiserum using Western blot assays. P25 was not detected in healthy preparations partially purified by the MBSM procedure in Western blot assays. Rabbits were immunized with P25 purified by SDS-PAGE. Sera from the immunized rabbits were recovered and tested for antibodies specific to P25 by the Western blot assay and ELISA. No antibodies specific to the P25 were detected, even from serum collected after several injections of P25 over a period of several months.

In another approach for the serological detection of MBSM, monoclonal antibodies to MBSM were obtained from Dr. T. A. Chen, Rutgers University, shortly after publication of his success in producing these antibodies (Chen and Jiang, 1988). A cooperative study was begun in which Dr. Chen supplied the monoclonal antibodies and we developed the ELISAs for their use in testing field-collected, infected samples. We developed an AC-ELISA for this test and attempted to develop a $F(ab')_2$-ELISA using $F(ab')_2$ units for trapping the antigen to microtiter plate wells. The AC-ELISA seemed to be the more reliable of the two ELISAs. Then, we conducted a preliminary test involving diseased maize samples from Costa Rica, Mexico and Nicaragua to assess the relative frequency of CSS, MBSM and MRFV. The ELISAs for MRFV and CSS employed polyclonal antisera that we had produced to both pathogens (see following section on "Production of antisera to CSS and MRFV") and which had proven to be reliable in the many tests of greenhouse infected maize. ELISAs conducted on these samples revealed the following occurrences of the three pathogens.

Country	Date of sample collection	No. of samples assayed	Percentage of samples positive for pathogen		
			CSS	MBSM	MRFV
CR[a]	Sept-Oct, 1988	52	4	21	85
MX	Feb, 1989	48	15	33	38
NI	Aug-Sept, 1988	60	88	38	93

[a]The countries were CR=Costa Rica; MX=Mexico; and NI=Nicaragua.

CSS occurrence fluctuated the most among countries and relative to the occurrence of the other pathogens. MBSM showed the least fluctuation and MRFV an intermediate level. Among all samples, MRFV occurred most frequently (74%), and CSS and MBSM less frequently (39% and 31%, respectively). In an analysis of the relation of disease diagnosis based on symptoms to pathogen identification by ELISA, symptom diagnosis of corn stunt was significantly less accurate for samples with MRFV infection but more accurate for those infected with MBSM whether MRFV was present as well or not. However, for samples without infection by MBSM or MRFV, there was appreciable misdiagnosis (36%), principally for those with putative corn stunt symptoms but without detectable CSS infection. Symptom diagnosis of maize bushy stunt was highly inaccurate (56% to 63% misdiagnosis) whether the sample was infected with the other pathogens or not, i. e., there was poor agreement between ELISA detection of MBSM and recognition of infection by symptoms. Symptom diagnosis of maize rayado fino was significantly less accurate when CSS infection was present whether MBSM was present or not. However, MBSM infection alone was associated with an improved correct symptom diagnosis of maize rayado fino. Thus, in this preliminary test overall symptom diagnosis was not very accurate for predicting infection by any of the three pathogens as detected by ELISA and symptom diagnosis was poorest for MBSM.

The latter observation reflected a problem with the ELISA for MBSM. Of the ELISAs for the three pathogens, the MBSM ELISA gave the lowest absorbances for positive samples. MRFV and CSS ELISAs gave high absorbances for such samples which indicated that these ELISAs were of better quality than the MBSM ELISA. Since symptoms appeared to be an unreliable indicator of MBSM infection, an alternate method of MBSM detection to confirm ELISA results was needed. Infectivity assays for these pathogens require transmission by the leafhopper, *D. maidis*, and these assays are too laborious and inaccurate to provide reliable data to confirm ELISA results for the numbers of samples that were assayed. As another alternative method, we tested a DNA probe for MBSM genomic DNA in a cooperative study with Dr. Mike Davis, University of Florida, Fort Lauderdale, who provided the initial probe (Davis et al, 1988). This probe clearly identified MBSM in known infections (data not shown). It was used in conjunction with the ELISAs for CSS and MRFV to assess the accuracy of symptom diagnosis for pathogen identification involving samples collected in Nicaragua and Mexico (see the following section "Symptom diagnosis and pathogen identification").

Thus, in summary, sensitive ELISAs were developed for CSS and MRFV. This assay is available for use by CIMMYT and other scientists working in countries where corn stunt is a major disease. The polyclonal antiserum used in this assay was developed in our laboratory (see section on "Production of

Antisera to CSS and MRFV"). An AC ELISA was developed for MBSM. This ELISA involved the use of a monoclonal antibody to MBSM. The monoclonal antibody was specific for MBSM but did not provide a high level of reactivity and thus were not judged to be satisfactory for routine detection from field-collected samples. ELISAs for CSS, MBSM and MRFV infection were conducted on field-collected maize showing disease symptoms from Costa Rica, Mexico and Nicaragua. The results from these assays indicated that MRFV is the most prevalent of the three pathogens, that the levels of occurrence of CSS and MRFV vary considerably among the three countries and that symptoms are a poor indicator of the identity of the infecting pathogen. The latter finding suggests that previous diagnoses based on disease symptoms were inaccurate. Thus, results from previous resistance evaluation and selection studies may have been compromised and progress in breeding resistance impeded. Attempts to develop a polyclonal antiserum to a disease-specific protein isolated from MBSM-infected maize were unsuccessful. The purpose of this effort was to obtain an antibody preparation that produced a higher level of reactivity in ELISA than that produced by the monoclonal antibody, while at the same time retaining specificity.

Production of antiserum to CSS and MRFV

Preparation of CSS antiserum

Preparation of CSS antigen. CSS was cultured in a medium containing L-arginine, L-asparagine, methionine, ketoglutaric acid, pyruvic acid, fructose, sucrose, PPLO broth, lactalbumin, hydrolysate, HEPES buffer, palmitic acid, linoleic acid, phoshphatauic acid (egg yolk source), phosphatidylcholine (egg yolk source), lysophosphatedylocholine (egg yolk source), lyosophosphatedylcholine (soybean source), cholesterol and Tween 80 (Davis et al., 1987; Lee and Davis, 1984). The pH was adjusted of to 7.5 with 2 N NaOH and the solution was autoclaved (121°C) for 10 min. Fetal bovine serum (sterile) was added after the solution had cooled.

CSS for inoculating the culture medium was prepared as follows. Stalks from CSS- infected maize were sterilized in 20% (v/v) chlorox for 5 min and then rinsed three times with sterilized high purity water. Finely chopped stalk pieces were placed into 10 ml of the sterile culture medium and incubated for 10 min at room temperature. The contents of the stalk pieces were squeezed through sterile, double-thickness, fine mesh cheesecloth and then passed through a 0.45 micron pore diameter Acrodisc (Gelman Sciences, Inc., Ann Arbor, MI) membrane. 800 μl of the filtrate was diluted to 8 ml with sterile culture medium to yield the primary broth culture. The primary culture was diluted 1:10 with sterile culture medium and from the 1:10 dilution nine 10 fold dilutions were prepared in series. The third dilution of the series was found to have a suitable spiroplasma concentration and 10ml was added to 500 ml of culture medium and incubated at 30°C for 20 days

CSS was harvested from the culture as follows. CSS was pelleted from the culture medium by centrifugation at 15,000 rpm for 30 min at 15°C. The pellet was suspended in HBS (24 mM HEPES, 0.15 M NaCl, pH 7.4).

The agar plates were prepared as follows. 10 g agar was added to 100 ml distilled water. The mixture was autoclaved at 121°C for 15 min and 10 ml of the autoclaved agar was pipetted into 90 ml CSS culture medium. The mixture was heated in a microwave oven until the ingredients were completely dissolved. 10 ml of the dissolved mixture was pipetted into each of 10 sterile petri plates.

CSS was pelleted from the resuspension as above and the pelleted CSS was resuspended in 47 ml of HBS. CSS was again pelleted from the resuspension as above and the pelleted CSS was resuspended in 10 ml HBS. The resuspended pellet was centrifuged at 4000 rpm for 10 min. The supernatant fraction was recovered.

The CSS supernate was pipetted in 1 ml aliquots into 10 microfuge tubes to determine CSS protein concentration by the BioRad protein assay. Absorbances at 595 nm were measured and linearly plotted against dilutions to obtain the protein concentration of the CSS preparation. The concentration was 194 μg/ml and the preparation was stored in 1 ml aliquots at -20°C.

Immunization of rabbits for CSS antisera preparation. Two white New Zealand rabbits were used for immunizations. These rabbits were first bled to collect the premune sera. Injections of 194 μg rabbit were done at intervals of 2 weeks. A total of four injections were given. The rabbits were bled at 7 day intervals following each injection and the antiserum titer determined. When a titer of 1:800 and 1:1600 was obtained for the two rabbits, respectively, a large volume of blood was collected from each (10 days after the last injection) and the final antiserum titer was determined to be 1:1600 and 1:800.

Preparation of MRFV antiserum

Purification of MRFV. MRFV was purified by the following protocol to obtain the immunogen. MRFV infected maize leaves (75 g) were homogenized in four volumes (w/v) of 0.5 M potassium phosphate buffer, pH 7.0, containing 0.1% thioglycollic acid. The homogenate was pressed through two layers of fine-mesh cheesecloth, and one third volume (w/v) of chloroform was added to the filtrate. The mixture was emulsified in a blender and the emulsion centrifuged at 7500 rpm for 18 min in the GSA rotor of the Sorvall RC5B centrifuge. The aqueous layer was recovered and filtered through a polyester filter. The clarified extract (filtrate) was centrifuged at 30,000 rpm for 163 min at 10°C in the Beckman Ti35 rotor in the L-70 ultracentrifuge. The pellets were recovered and resuspended in 0.5 M potassium phosphate buffer, pH 7.0. If further clarification was needed, the preparation was centrifuged at 7400 rpm for 10 min. The resuspended virus was layered onto linear 10-40% sucrose density gradients in 0.5 M potassium phosphate buffer, pH 7.0, and centrifuged at 40,000 rpm for 210 min in the Beckman SW41Ti rotor at 10°C. The virus band was collected from centrifuged gradients, diluted 75-fold in 0.05 M potassium phosphate buffer, pH 7.0, and centrifuged at 35,000 rpm for 120 min in the Beckman Ti42.1 rotor at 10°C. The pellets were recovered and resuspended in 0.5 M potassium phosphate buffer, pH 7.0. The resuspended virus was given a second cycle of centrifugation on linear 10-40% sucrose density gradients in 0.5 M potassium phosphate, pH 7.0, and the banded virus recovered and pelleted as described above. The latter was resuspended in 3 ml of PBS (0.15 M NaCl, 0.01 M potassium phosphate, pH 7.0). The MRFV concentration was determined by the Bio Rad protein assay. This virus preparation was used to immunize the rabbits.

Immunization of rabbits for MRFV antisera preparation. Two white New Zealand rabbits were immunized twice with 120 and 150 μg of purified virus per rabbit for the two injections, respectively. Preimmune sera were collected prior to the first infection and at weekly intervals until a titer of 1:200 and 1:400, respectively, was obtained in ELISA. Then a large amount of antiserum was collected from each rabbit. These antisera had ELISA titers of 1:200 and 1:400, respectively.

Serological relationship between MBSM and CSS

Antiserum to CSS failed to reacted with extracts from maize plants experimentally infected with cultures of MBSM (data not shown). Also, the MBSM probe did not reach with extracts from maize plants experimentally infected cultures of CSS (data not shown). In both tests the antiserum and probe reacted with the corresponding pathogen. Thus, there was no serological or nucleotide relationship between the two pathogens.

Symptom diagnosis and pathogen identification

Nicaraguan Study

A cooperative study was conducted to test whether symptoms could reliably diagnose the three diseases, corn stunt caused by CSS, maize bushy stunt caused by MBSM, and maize rayado fino caused by MRFV. Reliability was assessed by associating symptoms with infecting pathogens identified by specific assays. The first of two studies was conducted in field plots in Nicaragua from June to September 1990. Cooperators in the study were Ase Leirgulen and Ingrid Myrstad, Department of Plant Protection, Agricultural University of Norway, who made the plantings, inoculated plants and recorded symptoms in Nicaragua and assayed collected samples for CSS and MRFV by $F(ab')_2$-ELISA at Wooster (Leirgulen and Myrstad, 1991). We conducted the dot blot DNA hybridization assay for MBSM and the statistical analysis of the association of symptoms with pathogen infection was performed by Dr. N. H. Gordon, Department of Epidemiology and Biostatistics, School of Medicine, Case Western Reserve University, Cleveland, Ohio.

In the study, four maize varieties which varied in susceptibility to CSS and MBSM were tested at three sites, each located in a different climatic zone. Each variety was subjected to three treatments, [Treatment I: experimental infestation with *D. maidis* (experimental inoculation); Treatment II: protection from infestation (no experimental or natural inoculation); and Treatment III: natural infection. Symptoms were recorded weekly for all plants at the three locations from 40 days after plant emergence to harvesting. Following harvest, leaf samples were collected from plants showing different symptoms and transported to Wooster were the assays were performed.

The four maize varieties were: Tuza Morada, very susceptible to CSS and MBSM; NB-12, the most resistant to CSS and MBSM; H_s5, slightly less susceptible than Tuza Morada; and NB-6 (St. Rosa 8073), more resistant than H_s5 (hybrid) but less resistant than NB-12. H_s5 and the St. Rosa varieties are widely planted in Nicaragua because of their resistance to CSS and MBSM and high yielding potential.

Descriptions of the test sites and their climatic and topographic features follow. Site one, San Ramon, had a climatic zone classified as humid to dry with 4 to 6 dry months and life zones that are subtropical moist forest and lower montane forest with an annual precipitation of 1500-2000 mm. The monthly average precipitation for the three month experimental period was 251 mm. The plot was located on a slope surrounded by maize on three sides. The second site, the Sebaco Valley, was within a dry tropical climatic zone and its life zone is classified as a subtropical dry forest with annual precipitation of less than 1000 mm. The site received an average precipitation of 80.5 mm per month for the three month test period which was insufficient to prevent drought damage to the maize,

although the field was irrigated. Fields of tomato and maize were adjacent to the experimental plot. The third site, Masatepe, had a climatic zone within the humid to dry tropical zone with 4 to 6 dry months. The life zone is classified as tropical dry forest with an annual precipitation of 1500 mm. The area was hilly to steeply mountainous. The monthly average precipitation for the three month experimental period was 116 mm. The site was boarded by a fallow area on one side and beans on the other. The closest maize field was 500 m.

Results. The symptom most frequently observed in the field was reddening of leaf edges which is considered a typical symptom of both corn stunt and maize bushy stunt diseases. The degree of reddening and position of red leaves were recorded weekly. The initial reddening usually occurred on the lower part of the plant. NB-12 was the only maize variety to show other types of reddening. Reddening was evenly distributed or in diffuse spots on leaves. Disease development in Treatment I (experimental inoculation) was evaluated based on percentage of plants showing reddening at different developmental stages. The rating scale used was: 0 (no reddening) to 5 (most severe reddening). At all three locations, variety Tuza Morada had highest percentage of plants showing reddening followed by H_s5 which showed significantly more reddening than the other varieties. ANOVA revealed a significant difference in San Ramon at 64 days (p % = 0.05) and all later dates (p % = 0.01), in Sebaco at 59 days and 67 days (p % = 0.05) and later (p % = 0.01) and at Masatepe at 59 days (p % = 0.05) and later (p % = 0.01). For all maize varieties, a remarkably similar disease development was found for the three locations. The different treatments were compared on basis of percentage of reddening at ca. 100 days after planting at the three locations. The results of these comparisons were: Sebaco - percent red plants was same for Treatments I and II (89%); Masatepe - percent red plants was slightly greater for Treatments I than II (93/70%); and San Ramon - percent difference for red plants was as great as 65% between Treatments I and II. For all locations: percent red plants varied but was always lower in experimentally non-inoculated replicates (Treatments II and III) than in experimentally inoculated (Treatment I). Reddening was significantly more severe in the inoculated replicates than in the other Treatments and Treatment III always had the lowest intensity of reddening. The variety Tuza Morada had the highest score (reddening) of all varieties for all treatments.

Chlorotic striping, presumably a specific symptom of CSS infection, was not frequently seen in field plots. Tuza Morada (57%) and H_s5 (8%) were the only varieties showing the symptom and Sebaco was the only location where the symptom was seen. Stunting, assessed as the difference in the number of leaves between plants with and without stunting, was not demonstrated as no significant difference was found between the two groups of plants. Tillering, a symptom considered typical for corn stunt and maize bushy stunt infections, was observed only occasionally, but was probably not due to CSS or MBSM. For date of tasseling and female flowering, there was no significant correlation between these two characteristics and other symptoms. An increase in the number of flowers and ears is considered a symptom of both corn stunt and maize bushy stunt. For Tuza Morada, there was an increase in the number of female flowers (p % = 1.0) and ears (p % = 0.001) at all locations. Early infected H_s5 and NB-6 also had significant larger numbers of flowers and ears at Sebaco. For NB-12 there was no significant effect on either.

ELISA and DNA hybridization assays for pathogens in relation to symptoms. Leaves from plants showing the most commonly reported symptoms of corn stunt and maize bushy stunt were selected and assayed by $F(ab')_2$ ELISA for CSS and MRFV and by the dot-blot hybridization assay using a DNA probe for MBSM. The results of these assays with respect to the different categories of

symptoms are summarized in Table 1 and discussed in the following.

Only two plants with the chlorotic striping symptom from corn line H_s5 at one location were selected for assay. Assays revealed dual infection (CSS and MBSM) of both plants (data not shown). Assays of plants showing reddening revealed MBSM, CSS, CSS+MBSM and MRFV+MBSM infection among the 50 samples from plants with this symptom. For NB-12, 32% of the samples were healthy (no pathogen detected). For Tuza Morada, 25% of the samples were infected with CSS, 5-9% were healthy and 52.2% had dual infections. For H_s5, 47.8% of the samples had dual infections and for NB-6 40.0% had dual infections. For NB-12, 27% of samples were infected with MBSM. Samples with chlorosis and reddening (combination of two symptoms) were collected from San Ramon and Masatepe for variety Tuza Morada only. Of six samples assayed, four were dually infected and the remainder were infected with CSS and "others" (combinations of these pathogens). For plants with stunting, 12 samples were assayed and both CSS and the combination of CSS + MRFV were detected. MBSM was not detected. For Tuza Morada, none of the samples from stunted plants were infected with any of the pathogens. For NB-12, 66.7% of the samples were infected with CSS. For H_s5, 33.3% of samples were infected with CSS. For NB-6, 50% of samples were infected with CSS. Dual infections were detected in the following percentages for H_s5 - 33.3% and NB-6 - 25%.

For plants with no symptoms (healthy), 55 samples were assayed. Assays revealed infection by all combinations of pathogens. The highest percentage of healthy plants were not infected with any of the three pathogens. Most samples from all varieties, with exception of H_s5, had the highest percentage of healthy (no pathogen) plants. Dual infections were evenly distributed among varieties and ranged from 12 to 36% as follows: Tuza Morada - 36.4%: and H_s5, NB-6 and NB-12 - 12.5-20%. For H_s5, 33.3% of samples were infected with "others". Finally, NB-6 was the only variety infected with CSS (12.5%).

For plants with chlorosis and stunting, only one Tuza Morada sample was collected and it was infected with "others" (data not shown).

Fourteen samples with reddening and stunting were assayed. All pathogens, except MBSM, were detected. For NB-12, all samples were infected with other pathogens; for H_s5, 100% of samples were infected with both CSS and MBSM. For Tuza Morada, 50% of the samples were infected with both CSS and MBSM. For NB-6, 75% were infected with both CSS and MBSM; and Tuza Morada was the only variety infected with CSS alone.

Ten samples with chlorosis, reddening and stunting were assayed. For Tuza Morada and H_s5, 80% had dual infection (CSS + MBSM) and 20% were infected with "others". Tuza Morada had 35.7% dual infection and H_s5, 66.7%. No samples of NB-6 and NB-12 were collected.

Eight samples with multiple ears were assayed. No pathogen was detected in 37.5% of the samples and for those infected, only CSS and MRFV were detected. For H_s5, all samples were healthy (no pathogen). For Tuza Morada, 33.3% of the samples were healthy. For NB-12, 50% of the samples were healthy. For NB-6, 50% of the samples were infected with CSS. For Tuza Morada, 33.3% of the samples were infected with CSS. For NB-6, 50% of the samples were infected with MBSM, and for NB-12, 50% of the samples infected with "others".

In the univariate analysis of the association of symptoms with pathogen, MBSM showed correlations

with location (0.28[a]) (0.00[b]),[1] treatment (-0.14) (0.04), CSS (0.20) (0.01), chlorotic striping (0.18) (0.01) and multiple ears (-0.05) (0.05). CSS showed correlations with variety (-0.29) (0.00), treatment (-0.34) (0.00), MBSM (0.20) (0.01), MRFV (0.16) (0.02), chlorotic striping (0.26) (0.00), reddening (0.34) (0.00), stunting (0.33) (0.00), and symptom (0.38) (0.00). MRFV showed correlations with CSS (0.16) (0.02) and stunting (0.04) (0.04) only. Chlorotic striping showed correlations with (other than pathogens) variety (-0.29) (0.00), treatment (-0.14) (0.04), reddening (0.17) (0.02), stunting (0.30) (0.00) and symptom (0.21) (0.00). Reddening showed correlations with (other than pathogens) treatment (-0.25) (0.00), chlorotic striping (0.17) (0.00) and multiple ear (-0.25) (0.00). Stunting showed correlations with (other than pathogens) variety (-0.14) (0.04), treatment (-0.20) (0.00), chlorotic striping (0.30) (0.00) and symptom (0.33) (0.00). Multiple ear showed correlations with (other than pathogens) reddening (-0.25) (0.00). Symptom (chlorotic striping, reddening, stunting or multiple ear) showed correlations with (other than pathogens) treatment (-0.25) (0.00), chlorotic striping (0.21) (0.00), reddening (0.78) (0.00) and stunting (0.33) (0.00).

Multivariate analysis showed that chlorotic striping was significantly related only to variety (0.006); reddening to CSS (0.0003); stunting to treatment (0.0006)and location (0.002); multiple ears to none of the other variables; and symptom to CSS (0.0001).

Discussion. Disease development for plots not covered by cages indicated that few if any *D. maidis* carrying the pathogens were present until the leafhoppers from the inoculated treatment were released. Disease incidence was higher in treatment II (control) than in treatment III (natural infection). It seems as if the reason for this was that the control was located closer to the inoculated replicates and the leafhoppers migrated to this part of the field before migrating to plants of treatment III.

Tuza Morada, the most highly susceptible variety to CSS and MBSM, showed more reddening both in percentage and intensity. NB-12 considered more resistant to CSS and MBSM than the other varieties showed more reddening than expected. Assays revealed that a high percentage of plants with reddening (group B) were uninfected. Reddening had no negative effect on yield of NB-12. Reddening in NB-12 may have been caused by other factors, e.g., insect damage, P-deficiency and/or mechanical damage. Thus, using reddening as an indication of CSS and/or MBSM infection might be misleading in some varieties. Chlorotic striping was rarely seen for unknown reasons. Little stunting was observed. This may have been due to relatively late inoculation of plants (ca. 15 days after emergence). Plants sowed 1 wk after the rest were more affected. Increased wilting of leaves of plants expressing symptoms of CSS and/or MBSM was expected since reddening is usually followed by necrosis. The absence of tillering may have been due to the varieties used. Reduced kernel yield was associated with corn stunt and maize bushy stunt. Loss is partly due to poor pollination, resulting in few kernels and to poor filling of kernels. Increased number of female flowers reduced the size of each ear and thus the yield.

Results of assays of samples showing typical symptoms of corn stunt and maize bushy stunt lead to the conclusion that distinguishing the two disease in the field is an impossible task (Table 1). CSS and MBSM produce similar or identical symptoms. This similarity creates uncertainty of field diagnosis of disease based on symptoms. The typical symptoms for CSS and MBSM were observed on the very

---

[1][a]Ken Tau b correlation coefficient (R). [b]Probability > $|R|$ under Ho:R=0

susceptible varieties. Maize varieties with some resistance (NB-6 and NB-12) very seldom showed typical symptoms. Important factors which influenced specific symptom expression appeared to be variety, time of infection and dose of inoculum.

The relatively late inoculation (ca. 15 days after emergence) may have reduced pathogen effects on maize varieties even on the susceptible Tuza Morada. Earlier infection may have incited more symptoms and caused greater yield reduction. Treatments I and II (inoculated and control) were covered with a cage for about 40 days. The temperature within the cages was about 5° C warmer than outside. The higher temperature and the windshield effect of the cage may have affected symptom expression.

Reddening was the predominant symptom followed by stunting, chlorotic striping and finally multiple ears, which was least common (Table 1). Among the pathogens, MBSM was the most prevalent, CSS slightly less prevalent and MRFV the least common.

Conclusions from statistical analysis of data involving the univariate analysis were chlorotic striping was an indicator of both MBSM and CSS infection. The expectation was that chlorotic striping was an indicator of CSS infection only. Reddening was an indicator of CSS infection. The expectation was that reddening was an indicator of MBSM infection only. Stunting was an indicator of both CSS and MRFV infection. The expectation was that stunting was an indicator of MBSM, CSS and MRFV infection. Multiple ears was an indicator of MBSM infection. The expectation was that multiple ears was an indicator of MBSM and CSS infection. For the multivariate analysis chlorotic striping, stunting and multiple ears were not indicators of infection by MBSM, CSS or MRFV. The expectations were as presented for the univariate analysis. Reddening was an indicator of CSS infection. The expectation was that reddening was an indicator of MBSM infection.

Table 1. The association of pathogen (maize bushy stunt mycoplasma, MBSM; corn stunt spiroplasma, CSS; maize rayado fino virus, MRFV; or no pathogen, none) with symptoms (chlorotic striping; reddening; stunting; multiple ears; or no symptoms, none) observed on diseased or healthy maize plants in Nicaragua

Pathogen	None	Chlorotic striping	None	Reddening	None	Stunting	None	Multiple ears	Totals No Symptoms
None	60	1	31	30	50	11	56	5	197/47
MBSM	118	18	50	86	106	30	133	3	407/137
None	76	0	47	29	73	3	71	5	267/37
CSS	103	19	34	88	84	38	119	3	340/148
None	173	17	77	113	153	37	182	8	585/175
MRFV	6	2	4	4	4	4	8	0	22/10
Totals									1818/554
None	309	18	155	172	276	51	309	18	1308
Pathogen	227	39	88	178	194	72	260	6	1064

The following tables show the number of samples involved in the test for correlations betweer pathogen and symptoms and state whether the a correlation was found or not.

Association of MBSM infection with symptoms.

Pathogen/Symptom	None	Chlorotic Striping
None	60	1
MBSM	118	18

Total = 197

MBSM was correlated with chlorotic striping of leaves.

Pathogen/Symptom	None	Reddening
None	31	30
MBSM	50	86

Total = 197

MBSM was correlated with leaf reddening.

Pathogen/Symptom	None	Stunting
None	50	11
MBSM	106	30

Total = 197

MBSM was correlated with plant stunting.

Pathogen/Symptom	None	Multiple Ear
None	56	5
MBSM	133	3

Total = 197

MBSM was not correlated with multiple ear formation.

Association of CSS infection with symptoms

Pathogen/Symptom	None	Chlorotic Striping
None	76	0
CSS	103	19

Total = 198

CSS was correlated with chlorotic striping of leaves.

Pathogen/Symptom	None	Reddening
None	47	29
CSS	34	88

Total = 198

CSS was correlated with leaf reddening.

Pathogen/Symptom	None	Stunting
None	73	3
CSS	84	38

Total = 198

SS was correlate with plant stunting.

Pathogen/Symptom	None	Multiple Ear
None	71	5
CSS	119	3

Total = 198

CSS was not correlated with multiple ears.

Association of MRFV infection with symptoms

Pathogen/Symptom	None	Chlorotic Striping
None	173	17
MRFV	6	2

Total = 198

MRFV was not correlated with chlorotic striping of leaves.

Pathogen/Symptom	None	Reddening
None	77	113
MRFV	4	4

Total = 198

MRFV was not correlated with leaf reddening.

Pathogen/Symptom	None	Stunting
None	153	37
MRFV	4	4

Total = 198

MRFV was not correlated with plant stunting.

Pathogen/Symptom	None	Multiple Ear
None	182	8
MRFV	8	0

Total = 198

MRFV was not correlated with multiple ear.

Mexican Study

In a second cooperative study, we tested whether symptom diagnoses of corn stunt, maize bushy stunt and maize rayado fino accurately predicted infection by the pathogen of the respective diseases. Symptom diagnoses were made by CIMMYT scientists, Drs. Bobby Renfro and Dan Jeffers, who are expert maize pathologists and very familiar with symptoms of the three diseases.

Leaf samples from each of 10 maize plants showing characteristic symptoms of corn stunt, maize bushy stunt or maize rayado fino and from 10 maize plants showing no symptoms were collected from CIMMYT test plots at Tlaltizapan and Poza Rica and shipped to Wooster for pathogen assays. Symptoms of the respective diseases were evaluated by Drs. Bobby Renfro and Dan Jeffers.

CSS and MRFV were assayed by ELISA using antisera to each pathogen prepared in our laboratory to isolates of the pathogens both isolated from the vicinity of El Batan, Mexico. MBSM was assayed by cDNA dot blot hybridization assay using a MBSM specific probe obtained from Dr. Michael Davis. University of Florida, Fort Lauderdale.

Among samples (20) from maize plants diagnosed with corn stunt from Poza Rica and Tlaltizapan, 20% tested positive for CSS infection, 85% for MBSM, 5% for MRFV and 5% for no pathogen (Table 1). Among these, two samples tested positive for both CSS and MBSM and one for MBSM and MRFV (data not shown). All three of these samples were from Tlaltizapan.

Among samples (20) from maize plants diagnosed with maize bushy stunt from Poza Rica and Tlaltizapan, 15% tested positive for CSS infection, 70% for MBSM, 20% for MRFV and 40% for no pathogen (Table 1). One sample tested positive for both CSS and MBSM and four samples for both MBSM and MRFV (data not shown). All five samples were from Tlaltizapan.

Among samples (20) from maize plants diagnosed with maize rayado fino, none tested positive for CSS infection, 5% for MBSM, 95% for MRFV and 5% for no pathogen (Table 1). One sample tested positive for both MBSM and MRFV (data not shown). This sample was from Tlaltizapan.

Among samples (20) from maize plants diagnosed as healthy, none tested positive for CSS infection, 5% for MBSM, 10% for MRFV and 90% for no pathogen (Table 1). The three pathogen positive samples were from Tlaltizapan.

Thus, symptoms correctly predicted CSS infection in 20% of the samples, MBSM in 70%, MRFV in 95% and healthy (no pathogen) plants in 90%. When MRFV was the only pathogen infecting maize, it was easily recognized by diagnostic symptoms. However, when in combined infections with CSS or MBSM, MRFV symptoms were frequently masked, presumably when the former pathogens had sufficient time to express characteristic symptoms. The high percent (85%) of plants with corn stunt symptoms which tested positive for MBSM illustrates the especially poor diagnostic value of these symptoms for predicting CSS infection, even when diagnoses were made by scientists most experienced in identifying the disease.

Table 1. Test of whether symptom diagnoses of corn stunt (CS), maize bushy stunt (MBS) or maize rayado fino (MRF) accurately predicted infection by their respective pathogens, corn stunt spiroplasma (CSS), maize bushy stunt mycoplasma (MBSM) and maize rayado fino virus (MRFV)

Disease		CSS	MBSM	MRFV	None
CS	PR[a]	1/10[b]	8/10	0/10	1/10
	TZ	3/20	9/10	1/10	6/10
	Total	4/20	17/20	1/20	7/20
	Percent	20%	85%	5%	35%
MBM	PR	2/10	5/10	0/10	3/10
	TZ	1/10	9/10	4/10	5/10
	Total	3/20	14/20	4/20	8/20
	Percent	15%	70%	20%	40%
MRF	PP	0/10	0/10	9/10	1/10
	TZ	0/10	1/10	10/10	0/10
	Total	0/20	1/20	19/20	1/20
	Percent	0%	5%	95%	5%
None	PP	0/10	0/10	0/10	10/10
	TZ	0/10	1/10	2/10	8/10
	Total	0/20	1/20	2/20	18/20
	Percent	0%	5%	10%	90%

[a] PR refers to samples collected from maize grown at the CIMMYT test site at Poza Rica, Mexico. TZ refers to samples collected from maize grown at the CIMMYT test site at Tlaltizapan, Mexico.

[b] Numerator indicates the number of samples which tested positive for the pathogen. Denominator indicates the number of samples assayed. CSS and MRFV were identified by ELISA and MBSM by a cDNA hybridization assay.

Investigators responsible for studies

Monitoring *Dalbulus* vectors and disease incidence at locations in
Costa Rica - Rodrigo Gamez
Mexico - Raefael Rodriguez-M.

Surveys for vectors and pathogens within various vegetational zones in
Costa Rica - Rodrigo Gamez
Mexico - Raefael Rodriguez-M.

Hibernation of *Dalbulus* leafhoppers
Costa Rica - Rodrigo Gamez
Mexico - Raefael Rodriguez-M.

Tests for alternate sources of the three pathogens in Mexico -
Raefael Rodriguez-M.

Environmental measurements
Mexico - Raefael Rodriquez-M.

Development and comparison of serological assays - D. T. Gordon.

Production of monoclonal antibodies to MBSM - R. E. Gingery and D. T. Gordon

Production of antisera to CSS and MRFV - D. T. Gordon

Serological relationship between MBSM and CSS - D. T. Gordon

Symptoms diagnosis and pathogen identification
Nicaraguan study - D. T. Gordon and L. R. Nault
Mexican study - D. T. Gordon

DISCUSSION OF EXPERIMENTAL RESULTS IN RELATION TO STUDY OBJECTIVES

(An Interpretative Summary)

We wished to gain insight into how MRFV, MBSM and CSS and thus *Dalbulus* vectors survived during the adverse dry season in the general absence of maize plantings. Alternate hosts for the three pathogens were unknown and it was assumed that the pathogens survived in *Dalbulus* in which all three are able to replicate. If the later assumption is correct, then survival of the pathogens would depend on the survival of the *Dalbulus* vector when infected by one or more of these pathogens. We considered survival within proximity to maize plantings following crop harvest as one likely possibility. A second was migration of infected *Dalbulus* from live maize plants growing under irrigation or in different ecological zones where continuing maize cultivation was possible due to ample-moisture. In the latter case, survival of the pathogens in maize would be possible freeing them from dependency on *Dalbulus* for survival. In experimental field tests of the two possible pathogen

sources (infective leafhoppers within proximity of current season maize plantings or immigrant infective *Dalbulus* from distant sources) for infection of maize, no evidence to support either hypothesis was obtained. The test for immigrant *Dalbulus* was conducted using suction traps positioned up to 5 m above ground. Only one *Dalbulus* leafhopper was trapped during the one season when suction traps were employed at the Montecillos location (high elevation) in Mexico. Proximate or nearby sources were tested by placing large cages over maize stubble from the previous growing season and monitoring *Dalbulus* within the cage. No *Dalbulus* were trapped within the cages, whereas relative large numbers were trapped in the vicinity of the cages, long after the maize had matured and dried. This result suggested that *Dalbulus* leafhoppers may overseason in weeds and noncultivated plants surrounding maize fields or wooded areas. We tested the hypotheses that the first substantial rainfall in the Spring at the high elevation in Alajuela, Costa Rica brings *D. maidis* out of its overwintering habitats in search of newly planted maize. The result of this test were inconclusive in that watered plots did not produce more *D. maidis* than unwatered ones. The presence of large numbers of *D. maidis* in the whorls of adjacent maize seedlings suggested that adult *D. maidis* were immigrating from sources other than weedy areas surrounding these fields.

Since we were unable to identify where *Dalbulus* survived during the dry season in the absence of maize, we are unable to speculate on the conditions that would favor or be disadvantageous to such survival and thus to pathogen survival.

The failure to obtain evidence for migratory *Dalbulus* indicates that the source of infective *Dalbulus* was local rather a distant region of Mexico where maize cultivation was continuous. Until we know the location of this source, we will be unable to advise CIMMYT and national scientists on measures they might follow to curtail or combat outbreaks of the three diseases.

A second hypothesis tested in the study was whether differential ecological requirements for survival of CSS, MBSM and MRFV resulted in different patterns of altitudinal distributions of the three pathogens. Based on previous information, we predicted that MRFV would be prevalent in maize grown at locations with higher moisture, irrespective of the temperature. Locations in this study with this climatic feature were Alajuela and Tabago in Costa Rica and Poza Rica in Mexico. (It is noted that although Tabago is considered a tropical dry forest zone, it receives an annual precipitation of about 1400 mm, which is greater than that received at Poza Rica, which is considered humid, tropical lowlands. However, Tabago has a longer dry season.) Further, previous information led us to predict that CSS would be prevalent at locations with relatively high temperature. These locations were Tabago and Poza Rica. In contrast, the third pathogen, MBSM, was predicted to be prevalent at cooler locations, i.e., at higher elevations, and at mid elevations during cooler periods. Locations with this characteristic were Montecillos (high elevation) and Tlaltizapan (mid elevation) in Mexico and Alajuela in Costa Rica.

In the experimental test of these predictions, disease incidences were recorded periodically during growing seasons in maize test plots at the five study locations (Alajuela and Tabago in Costa Rica and Montecillos, Tlaltizapan and Poza Rica in Mexico). These test showed that for the two or three years of the study recorded incidences frequently did not match predicted incidences. Further, disease incidences were low in both the Costa Rican and Mexican locations. In Costa Rica, the highest final incidence of the stunting diseases (corn stunt and/or maize bushy stunt) was 14.5% observed at Alajuela in 1987, and in Mexico stunting diseases were not reported. The highest final incidence of maize rayado fino in Costa Rica was 10% observed in Alajuela in 1988. The latter disease was

virtually absent from Tabago for all three years. In Mexico, the final highest incidence for maize rayado fino was less than 20% for all plantings at the three locations. Tlaltizapan among these locations most often had the highest incidence of the disease and Montecillos consistently the lowest. At both Tlaltizapan and Poza Rica, the highest disease incidence occurred unexpectedly during the dry season rather than the rainy season.

Thus, our prediction of prevalence of maize rayado fino at Alajuela was realized but not at Tabago. Further, our prediction of a greater prevalence of corn stunt at Tabago, a warm low elevation, was apparently not realized. Stunting disease (corn stunt and/or maize bushy stunt) were considerably more prevalent at Alajuela than Tabago. It is noted that CSS and MBSM infected plants could not be distinguished because we did not know of diagnostic symptoms for the two pathogens. For the Mexican locations, our predictions for the occurrence of corn stunt and maize bushy stunt were not fulfilled. For maize rayado fino, the higher prevalence at Tlaltizapan (less rainfall) rather than Poza Rica (more rainfall) was not predicted. Thus, the predicted adaptations of these pathogens to ecological zones in the two countries were not verified in this study. Further study will be needed to identify the environmental and perhaps other factors that determined the unexpected occurrences of these pathogens in the study.

Since disease incidences and presumably the survival of CSS, MBSM and MRFV are dependent on the *Dalbulus* leafhopper vectors, the low disease incidences found in this study may have been related to the size and phenology of leafhopper populations in fields where disease incidences were recorded. Movement of *Dalbulus* within these maize fields was monitored using yellow sticky cards to trap moving leafhoppers during periods when disease incidences were recorded. For the two Costa Rican locations, *D. maidis* was the only *Dalbulus* species trapped and the numbers were similar for some but not all plantings at both locations. Generally, *D. maidis* numbers were lower for plantings made at the beginning of the rainy season than for those made during the rainy season. Female *D. maidis* were more abundant than males in the majority of plantings. Of most importance, disease incidences and leafhopper numbers were not consistently nor directly related, i. e., lowest or highest disease incidence did not correspond with the lowest or highest *D. maidis* numbers, respectively.

For the three Mexican locations, four *Dalbulus* species were trapped. *D. maidis* was the most abundant and was trapped at all three locations. *D. elimatus* was also identified at the three locations but at much lower numbers. *D. guevarai* was trapped at Tlaltizapan and Poza Rica but in small numbers. *D. longulus* was recorded only at Poza Rica and at low numbers. The numbers of *Dalbulus* leafhoppers varied considerably with location, month of year and year and higher numbers were not consistently recovered for either dry or rainy season plantings. Larger numbers of male than female *Dalbulus* were trapped at the three locations. Similar to findings for Costa Rica, numbers of trapped *Dalbulus* at the three Mexican locations showed no relationship with disease incidence. Thus, the size of the *Dalbulus* leafhopper population was not directly related to disease incidence for any location in this study and did not suggest an explanation for the low disease incidences nor for their variation.

A further test of our second hypothesis concerning differential ecological requirements for survival of CSS, MBSM and MRFV creating different patterns of attitudinal distributions of the three pathogens was conducted in Costa Rica during two years. This involved surveys for *Dalbulus* vectors and maize with symptoms of stunting disease and/or maize rayado fino within 10 of the 11 vegetational zones of Costa Rica. The vegetational zones varied in elevation from 10 to 1821 m; type of ecological forest

from tropical dry to tropical rain forest; and terrain from lowland to premontane and lower montane. Based on our predictions on the prevalence of the three pathogens at the five test locations in Costa Rica and Mexico, we expected to find CSS prevalent within lower, warmer vegetational zones, MBSM at cooler, higher elevations and MRFV in the more moist zones. The data showed that the stunting diseases (corn stunt and/or maize bushy stunt) incidences within maize fields within the 10 zones were very low. The highest stunting disease incidence was 12.5% and nine of 33 fields had no stunting disease. Incidences of maize rayado fino within these maize fields were also very low with a maximum of 15% to 17 fields with no disease. Because of these low to absent disease incidences, stunting disease and maize rayado fino incidences did not correlated with elevation (high or low temperature), type of forest (dry to moist) or terrain. Thus, we were unable to confirm our hypothesis concerning the ecological adaptation of the three pathogens. Again the lack of diagnostic symptoms for distinguishing CSS and MBSM infections handicapped the study.

In view of this handicap and problems of recognizing diagnostic symptoms of MRFV infection when in combination with infection by CSS and /or MBSM, development of assays to reliably and sensitively detect these pathogens became essential for testing our hypotheses. This development proved to be one of the most difficult portions of the study. The difficulty had two sources. One was the quality of the antisera for detecting these pathogens and the second was the serological assay in which the antisera were used.

Attempts by us to produce a polyclonal antiserum and monoclonal antibodies to MBSM were unsuccessful. A polyclonal antiserum to MBSM produced in another laboratory was similarly unsatisfactory. A monoclonal antibody to MBSM also produced in another laboratory possessed specificity for MBSM but had low reactivity for routine detection of MBSM in field-collected samples with widely variable pathogen titers. Thus, after considerable attempts to develop a serological assay for MBSM, the attempt was ended and development of a cDNA hybridization assay using a newly available MBSM specific DNA probe, also developed in another laboratory, was made. This attempt resulted in an assay with sufficient specificity and sensitivity. We produced polyclonal antisera to cultured CSS and purified MRFV which were used to develop a satisfactory enzyme-linked immunosorbent assay (ELISA).

Our initial efforts to develop a satisfactory ELISA involved attempts to adapt Protein A sandwich ELISA (PAS-ELISA), a relatively new ELISA which at the time was purported to have greater sensitivity and specificity than other types of ELISA available at the time. PAS-ELISA, after a considerable number of experiments, proved to be unreliable although it had specificity and sensitivity. PAS-ELISA was used as the assay to identify these pathogens in Mexico and experience there and in our laboratory lead to the realization that the assay was not reliable. Unfortunately, the PAS-ELISA results for field-collected samples in Mexico had to be mostly discarded because the assay could not be trusted. The second ELISA we attempted to adapt was the antigen (pathogen) coat ELISA. It too was not dependable and efforts to adapt it were also abandoned. The third ELISA investigated was the $F(ab')_2$-ELISA for detecting CSS and MRFV. $F(ab')_2$-ELISA proved satisfactory. $F(ab')_2$-ELISA for CSS and MRFV along with the cDNA probe hybridization assay for MBSM provided sensitive, specific and reliable assays for the three pathogens and allowed us to continue attempts to test some our hypotheses, although the development satisfactory assays came very late in the study after most of the field studies had been completed.

With the development of the reliable assays, we conducted a cooperative study with other researchers

to examine critically whether symptoms could reliably diagnose infections by each of the pathogens. The first of the two studies was conducted in Nicaragua where higher incidences of plants showing symptoms of corn stunt and maize bushy stunt occurred than we had observed in Costa Rica or Mexico. Maize rayado fino was present as well. The Nicaraguan study was conducted at three sites located in different climatic zones and involved four maize varieties with differing susceptibilities to corn stunt and maize bushy stunt. Reliability of symptoms for disease diagnosis was assessed by statistical analysis of the association of symptoms with pathogens identified by serological or hybridization assay. The principal symptoms were reddening, chlorotic striping, stunting and multiple ears, symptoms previously attributed to infection by CSS or MBSM, as well as no symptoms. Univariate and multivariate analyses of the symptom and pathogen data confirmed our previous observation that symptoms do not allow distinguishing between corn stunt, incited by CSS, and maize bushy stunt, incited by MBSM. Important factors which seemed to influence specific symptom expression were variety, time of infection and inoculum dose.

The second phase of the study tested whether the CIMMYT scientists experienced in identifying corn stunt and maize bushy stunt could correctly identify maize plants infected with CSS, MBSM or MRFV by symptoms. Test samples were from Poza Rica and Tlaltizapan, Mexico. Symptoms correctly identified CSS infection in 20% of samples, MBSM in 70%, MRFV in 95% and no pathogen (healthy) in 85%. However, MRFV symptoms were masked when infecting jointly with CSS and/or MBSM following sufficient time for the latter two to express characteristic symptoms.

Thus, the findings from the two studies strongly indicated the necessity of assays for accurate disease identification.

In summary, this study sought to accomplish three goals. The first was to obtain evidence on whether infective *Dalbulus* leafhoppers which introduce these pathogens into seedling maize at the beginning of the season in Costa Rica and Mexico are local, surviving on weed or non-cultivated plants, or immigrants from other areas where maize is grown continuously due to irrigation or rainfall. The second was to gain insight into how maize rayado fino virus (MRFV), corn stunt spiroplasma (CSS) and maize bushy stunt mycoplasma (MBSM), three important maize pathogens in Mexico and Central America, and their *Dalbulus* leafhopper vectors have become adapted to maize in various ecological zones in Costa Rica and Mexico. The third goal was to develop reliable, specific and sensitive assays for CSS, MBSM and MRFV for the purpose of obtaining accurate disease diagnosis. Studies of the first goal offered no clear evidence as to whether infective *Dalbulus* were from a local or distant source where the leafhopper vector survives over season. However, the evidence suggested a local rather than a distant source. In studies related to the second goal incidences of the three disease were so low within all test sites within the two countries for the three years of the study that it was not possible to show that there was ecological adaptation of the pathogens to particular ecological zones. The third goal was achieved by the development of $F(ab')_2$ - ELISA for detection of CSS and MRFV and a cDNA hybridization assay using a pathogen probe for detection of MBSM. These assays were used to show that disease identification by symptoms was not reliable and that assay identification was necessary. The development of these assays and the production of an abundant supply of high quality antisera to CSS and MRFV plus the availability of the MBSM DNA probe provide to scientists in Central America and Mexico, especially CIMMYT scientists, the means to identify accurately these three pathogens which previously was not possible. Further, these assays have strengthened more recent studies on the survival of the three pathogens and their ecological adaptation. We believe that the eventual understanding of the ecology of these pathogens and their vector will enhance efforts to

control their diseases. The ability to identify accurately these pathogens should enhance the efficiency and relevance of the CIMMYT program of breeding resistance to these pathogens. Realization of the ultimate goal of disease control will contribute to the stabilization of maize yields, providing more food and reduce the need to import maize into these countries.

IMPACT, RELEVANCE AND TECHNOLOGY TRANSFER

Usefulness of experimental findings in Mexico and the developing countries of Central America.

Corn stunt has been designated by CIMMYT as a major constraint in maize production in Mexico and the developing countries of Central and northern South America. Long-term efforts to breed resistance to this disease by CIMMYT have been unsuccessful. A reason for this failure may be that the disease is caused by more than CSS, most likely MBSM, thereby making selection of genotypes resistant to a specific pathogen problematical. Assessment of the involvement of CSS and MBSM in the disease is now possible by employment of serological and cDNA hybridzation assays, respectively. This assessment seems especially crucial for developing efficient field screening techniques to select maize plants resistant to the corn stunt pathogen(s). Thus, the development of these serological assays will likely greatly strengthen the CIMMYT program of breeding for corn stunt resistance. Our results also show that MRFV is prevalent in those regions. Considering that no MRFV resistant genotypes have been identified and that unrecognized MRFV infection may influence corn stunt resistance breeding efforts, it seems advisable that future assays for corn stunt breeding programs include means to detect MRFV as well. Detection of MRFV is possible using ELISA and an antiserum which was prepared during this project.

Impact of project on individuals, laboratories, department and institutions. Technical staff in Mexico and Costa Rica received special technical training.

Use of the experimental results. From this study, researchers throughout Latin America where stunting diseases occur should be aware that symptomatology will not provide accurate identification of disease in field breeding nurseries where resistance evaluations are made. Resistance programs which seek to identify and test for genes for tolerance or immunity to the specific stunting disease, viz, corn stunt and maize bushy stunt, will need to make extensive use of pathogen assays to identity the pathogen to which tolerance or resistance genes are effective.

Need for further experimental trials. Renewed effort must be made to find the overseasoning sites for *D. maidis*. To suggest how difficult finding these sites might be, it has recently been reported that the potato leafhopper, a pest of alfalfa, beans and potatoes and other herbaceous and woody ornamentals trees in the midwestern U. S., migrates to the southern U. S. at the end of the growing season. There it survives in the tops of southern pine trees where it feeds but does not breed.

The difference the project had made in Costa Rica and Mexico. This study has clearly shown that corn stunting and maize rayado fino diseases may be sporadic in importance and that as long as they continue to be of minor importance as found in this study, these diseases will require minor attention for their control. However, they have been important in previous years and we do not understand why they were not important in our study. This lack of understanding creates a sense of disquietude

which can be relieved by a better understanding of the ecology of the *Dalbulus* vectors and the pathogens and the availability of disease resistant cultivars. Thus, it is advisable to continue breeding for tolerance or resistance to these diseases in the event that they again become a major limiting factor in maize production in these countries.

New capacities, equipment and expertise remaining in Costa Rica and Mexico. Technical staff in Mexico and Costa Rica received special technical training. Also, antisera to CSS and MRFV and the MBSM probe are available for use by researchers in these two countries.

Improvement of the scientific capabilities of Costa Rica and Mexico Scientists involved in project. During the course of these studies, the co-investigators in Costa Rica and Mexico both accepted new responsibilities which diminished their active participation in the project and in any potential subsequent studies. With the principal participants from these countries only marginally involved, the opportunities for the improvement of the scientific capabilities and strengthening research capacity of LDC institutions as a result of the research activities of this project were significantly reduced in these two countries. However, in Mexico CIMMYT scientist, Dr. Dan Jeffers, step into the breech and has cooperated with Dr. Nault in several subsequent studies which has improved the scientific capacity in Mexico.

Strengthening Research Capacity of LDC Institutions. In the previous statement, we present one reason why we believe that this project was handicapped in strengthening the research capacity of the LDC institutions involved. We believe another reason was that USAID declined our request for funding to hold meetings in each country with all investigators present in order to discuss planned research and research findings and at the end of the project to interpret results and plan publication of research findings. Without the ability for all the researchers involved to have "face-to-face" interaction, the project lacked a sense of cohesiveness in that we were never certain what investigators in the other two countries were doing nor of their results. It proved very difficult to maintain meaningful contact by mail, in the period before FAX and E-mail transmission. Even with the latter two means for easier communication, "face-to-face" interactions in joint meetings, in our experience, generate interest and enthusiasm unobtainable through the mail or electronic communication. The absence of this opportunity was especially serious because of the diminished involvement of the co-investigators from Costa Rica and Mexico. The lack of this group interest and enthusiasm diminished the potential impact of the project in strengthening research capacity and scientific capabilities in the two countries.

PROJECT ACTIVITIES/OUTPUTS

Meetings attended and held during the project. The PI visited with CIMMYT scientists Drs. B. Renfro and N. Bajet at CIMMYT Headquarters from March 30 to April 3, 1987, concerning the detection of CSS and MBSM and other matters. At that time, polyclonal antisera to CSS and MRFV were delivered by the PI. Permission for the trip to Mexico was requested from the U. S. Embassy-Mexico and granted by Mr. Sam Taylor.

Training of personnel involved in the project. Barbara Hernandez, B. S. degree, of the Colegio de Postgradudos, Montecillos, Mexico was trained at the OARDC to perform various ELISAs for CSS, MBSM and MRFV from July to September, 1987.

William Villalobos, B. S. degree, CIBCM, University of Costa Rica was trained at the OARDC to identify *Dalbulus* leafhoppers and corn stunting pathogens as well as conduct biological studies with vectors during 2 weeks in the summer of 1989.

Finally, during a two week period in January and February, 1987 Dr. R. A Simkins, a collaborator in this project, visited the Virology Laboratory of the USDA in Beltsville, MD, to learn spiroplasma culture techniques from Dr. Ing-Ming Lee. This training covered the methodologies for extracting CSS from infected corn leaf and stem tissues, preparing both defined and undefined (serum-containing) medium, examining the differences in spiroplasma growth characteristics on both media, growing spiroplasma on agar-supported medium, and cloning primary cultures of CSS.

Primary Publications: None

The following publications were a follow up to the studies of this project and addressed issues raised in this study but which were unanswered. These later studies were funded by subsequent grants to L. R. Nault *et al.*

Ebbert, M. A., and Nault, L. R. 1994. Improved overwintering Ability in *Dalbulus maidis* (Homoptera: Cicadellidae) Vectors Infected with *Spiroplasma kunkelli* (Mycoplasmatales: Spiroplasmataceae). Environ. Entomol. 23(3):634-644.

Larsen, K. J., Lee, Jr., R. E., and Nault, L. R. 1993. Influence of developmental conditions on cold-hardiness of adult *Dalbulus* leafhoppers: implications for overwintering. Entomol. exp. appl. 67:99-108.

Larsen, K.J., Nault, L. R., and Moya-Raygoza, G. 1992. Overwintering Biology of *Dalbulus* Leafhoppers (Homoptera: Cicadellidae): Adult Populations and Drought Hardiness. Environ. Entomol. 21(3):566-577.

Taylor, R. A. J., Nault, L. R., and Styer, W. E. 1993. Experimental analysis of flight activity of three *Dalbulus* leafhoppers (Homoptera: Auchenorrhyncha) in relation to migration. Ann. Entomol. Soc. Am. 86(5):655-667.

M. Sc. Thesis

Leirgulen, A., and Myrstad, I. 1991. Corn stunt and maize bushy stunt disease; Study of symptom expression in four varieties of maize, at the locations in Nicaragua. M. Sc. thesis Agricultural University of Norway. 94 pp.

PROJECT PRODUCTS AND OUTPUTS

Several planned analyses of leafhopper and disease incidence data ("Analysis of data from vector and pathogen surveys in relation to environment") were not conducted because of the lack of a correlation between the two and the low disease incidences. More specifically, the plan to correlate the number of leafhoppers trapped with the environmental variables was not carried out since change in disease incidence was not directly proportional to the change in numbers of vectors, a prerequisite for the

correlation. Consequently, regression models to relate the environment to leafhopper development and then the numbers of leafhoppers to disease incidence were not developed. Also, because numbers of *Dalbulus* found at the different locations were so low, life tables were not prepared for the *Dalbulus* species. Further, disease progress was not analyzed using the logistic equation or a more appropriate model since disease incidences were too low.

The planned experiment on the "Comparisons of serological and infectivity assays" was not conducted. The purpose of the experiment was to correlate the transmission rate by insects with the relative amount of the pathogen in the insect as estimated by ELISA. The difficulty and time required in developing ELISA for detecting the three pathogens precluded conducting this planned experiment. Further, satisfactory assays were not available until the end of field experiments where they were to have been used to estimate inoculativity of field-collected, infected vectors.

FUTURE WORK

The DNA-probe assay developed for MBSM could be expanded to include DNA probe assays for CSS and MRFV as well. Then it would be possible to have both serological and DNA-probe assays for the latter two very important pathogens. Such an accomplishment would greatly strengthen CIMMYT's disease resistance breeding program and modernize the assay aspect of the program. It appears that recent program developments at CIMMYT would allow their scientists to utilize both kinds of assays.

The results of this project left the status of several issues unresolved. First, our evidence did not resolve the question of the source (overseasoning sites) of infective *Dalbulus* for disease occurrences in maize grown in areas where cultivation was not continuous. Research on this issue continued following the completion of this project and we (L. R. Nault *et al*) gathered evidence from experimentally manipulated leafhoppers in growth rooms that support the migration hypothesis (Taylor et al., 1993). Nault *et al* found that leafhoppers which developed under late fall conditions could survive for long periods while feeding only on free water or non-developmental hosts (Larsen et al., 1992). Also, these leafhoppers were larger (Larsen and Nault, 1994) and more cold hardy than those that develop under summer temperatures (Larsen et al., 1993). Furthermore, they found that if *D. maidis* was infected with CSS, its overwintering ability was further improved (Ebbert and Nault, 1994). But other evidence suggested that at least some *D. maidis* at the three CIMMYT stations are morphologically distinct (Nault, *unpublished*). Currently, additional research is being conducted to locate the source(s) of *Dalbulus* which migrate to maize at the beginning of the season (L. R. Nault, *unpublished*).

A second issue concerned the infectivity of field-collected *Dalbulus* which we did not investigate, although it had been planned (see above). Currently, leafhoppers are being collected twice yearly by sweep net from maize plots at El Batan, Tlaltizapan and Poza Rica and shipped to Ohio where they are tested for CSS serologically and MBSM by hybridization assay and for both pathogens by bioassay. Data are still being collected and thus the success of these studies is yet unknown.

LITERATURE CITED

Chen. T. A., and Jiang, X. F. 1988. Monoclonal antibodies against the maize bushy stunt agent. Can. J. Microbiol. 34: 6-11.

Davis, R. E., Lee, I. M., Jordan, R., and Konai, M. 1987. Detection of corn stunt spiroplasma in plants by ELISA employing monoclonal antibodies and by isolation of the pathogen and its phages in serum-free medium. Curr. Plant Sci. Biotechnol. Agric. 4:306-312.

Davis, M. J., Tsai, J. H., Cox, R. L., McDaniel, L. L., and Harrison, N. A. 1988. Cloning of chromosomal and extrachromosomal DNA of the mycoplasmalike organism that causes maize bushy stunt disease. Mol. Plant-Microbe Interact. 1:295-302.

Ebbert, M. A., and Nault, L. R. 1994. Improved overwintering Ability in *Dalbulus maidis* (Homoptera: Cicadellidae) Vectors Infected with *Spiroplasma kunkelli* (Mycoplasmatales: Spiroplasmataceae). Environ. Entomol. 23(3):634-644.

Larsen, K. J., Lee, Jr., R. E., and Nault, L. R. 1993. Influence of developmental conditions on cold-hardiness of adult *Dalbulus* leafhoppers: implications for overwintering. Entomol. exp. appl. 67:99-108.

Larsen, K.J., Nault, L. R., and Moya-Raygoza, G. 1992. Overwintering Biology of *Dalbulus* Leafhoppers (Homoptera: Cicadellidae): Adult Populations and Drought Hardiness. Environ. Entomol. 21(3):566-577.

Lee, I.-M., and Davis, R. E. 1984. New media for rapid growth of *Spiroplasma citri* and corn stunt spiroplasma. Phytopathology 74:84-89.

Leirgulen, A., and Myrstad, I. 1991. Corn stunt and maize bushy stunt disease; Study of symptom expression in four varieties of maize, at the locations in Nicaragua. M. Sc. thesis Agricultural University of Norway. 94 pp.

Lin, C. P., and Chen, T. A. 1985. Monoclonal antibodies against corn stunt spiroplasma. Can. J. Microbiol. 31:900-904.

Taylor, R. A. J., Nault, L. R., and Styer, W. E. 1993. Experimental analysis of flight activity of three *Dalbulus* leafhoppers (Homoptera: Auchenorrhyncha) in relation to migration. Ann. Entomol. Soc. Am. 86(5):655-667.

APPENDIX A

Environmental Measurements

ENVIRONMENTAL MEASUREMENTS CP-MONTECILLO,1987.

WEEK AVERAGE

DATE	TEMPERATURE °C MAX	MIN	SUN HR	EVAPORA- TION	WIND Ø.5m	2.Øm	PRECIPI- TATION (mm)	RELATIVE HUMIDITY (%)
JANUARY								
29- 5	24.1	-7.5	8.8	3.5	1.16		Ø.Ø	43.8
6-12	27.2	-3.4	8.8	4.6	2.27		Ø.Ø	47.5
13-19	25.4	1.Ø	9.9	7.Ø	4.1		Ø.Ø	42.4
2Ø-26	24.8	-1.1	8.7	5.4	3.Ø5		Ø.Ø	44.1
FEBRUARY								
27- 2	24.8	-5.7	7.1	4.8	1.71		Ø.Ø	41.5
3- 9	22.6	-1.8	7.7	5.Ø	3.15		Ø.Ø	47.8
1Ø-16	26.3	-1.Ø	9.2	5.Ø	2.25		Ø.Ø	43.3
17-23	26.8	-1.8	8.3	7.8	4.49		Ø.Ø	43.4
MARCH								
24- 2	23.1	6.1	8.1	8.9	5.Ø6		Ø.Ø	42.6
3- 9	18.5	-Ø.1	6.4	5.1	3.Ø4		Ø.Ø	5Ø.7
1Ø-16	27.7	1.4	9.Ø	6.5	2.89		Ø.Ø	43.5
17-23	29.6	4.7	7.9	8.8	3.33		Ø.Ø	32.2
24-3Ø	24.3	7.1	5.9	6.4	2.57		1.3	5Ø.8
APRIL								
31- 6	25.7	2.8	9.5	7.8	3.2Ø		Ø.Ø	42.7
7-13	26.5	5.1	7.2	7.4	3.Ø1		Ø.Ø	44.5
14-2Ø	28.4	5.6	6.2	5.5	1.53		Ø.5	46.4
21-27	14.Ø	3.2	5.6	4.4	1.61		Ø.9	54.1

ENVIRONMENTAL MEASUREMENTS CP-MONTECILLO,1987.

WEEK AVERAGE

DATE	TEMPERATURE °C MAX	MIN	SUN HR	EVAPORA- TION	WIND Ø.5m	2.Øm	PRECIPI- TATION (mm)	RELATIVE HUMIDITY (%)
MAY								
28- 4	25.5	3.7	9.8	7.1		2.99	Ø.Ø7	35.3
5-11	25.Ø	4.6	7.6	6.7		1.95	3.1	51.Ø
12-18	25.7	5.7	7.3	5.2		1.64	1.4	46.3
19-25	35.Ø	4.3	9.7	6.5		2.Ø2	Ø.8	[illegible].8
JUNE								
26- 1	27.4	8.1	7.6	5.9		2.17	1.8	53.2
2- 8	22.5	8.7	3.7	3.2		2.1Ø	1.1	65.6
9-15	25.Ø	12.1	5.4	3.2		1.58	8.9	63.2
16-22	24.4	8.9	6.5	3.5		1.75	1.Ø	57.3
23-29	26.7	8.Ø	9.4	5.3		2.25	Ø.8	5Ø.4
JULY								
3Ø- 6	25.1	8.8	5.4	3.9		1.4Ø	4.4	53.9
7-13	25.3	8.6	6.4	4.Ø		1.4Ø	2.7	52.5
14-2Ø	25.5	6.6	7.6	4.5		2.Ø8	Ø.8	55.9
21-27	22.1	9.4	3.2	2.4		1.53	7.4	69.8
AUGUST								
28- 3	22.6	9.3	3.9	3.Ø		1.33	5.2	68.6
4-1Ø	23.6	8.1	6.4	3.6		1.4Ø	4.6	65.6
11-17	25.1	6.6	9.1	4.7		1.69	2.9	58.8
18- 4	24.5	7.2	7.2	3.2		1.41	6.9	63.8
25-31	23.7	8.2	5.8	3.9		1.54	2.8	63.9

ENVIRONMENTAL MEASUREMENTS CP-MONTECILLO,1987.

WEEK AVERAGE

DATE	TEMPERATURE °C MAX	MIN	SUN HR	EVAPORA- TION	WIND Ø.5m	2.Øm	PRECIPI- TATION (mm)	RELATIVE HUMIDITY (%)
SEPTEMBER								
1- 7	24.8	6.5	9.Ø	2.3		2.3Ø	Ø.Ø	53.7
8-14	25.9	4.1	9.2	2.1		2.19	1.1	54.1
15-21	26.2	9.Ø	6.4	1.8		8.85	2.9	61.2
22-28	24.1	9.1	6.6	2.1		2.18	7.4	65.5
OCTOBER								
29- 5	22.2	3.9	8.3	3.4		1.2Ø	Ø.Ø	55.7
6-12	22.5	Ø.15	9.1	4.6		3.11	Ø.Ø	49.1
13-19	25.3	-4.8	1Ø.3	4.1		8.8	Ø.Ø	42.7
2Ø-26	26.7	1.2	9.7	5.5		1.71	Ø.Ø	46
NOVEMBER								
27- 2	23.6	-2.9	9.5	4.5		2.58	Ø.Ø	42.8
3- 9	24.6	-5.1	9.2	4.5		2.19	Ø.Ø	42.4
1Ø-16	21.5	2.3	7.Ø	4.1		2.82	Ø.Ø	55.7
17-23	24.1	Ø.3	.6	3.6		1.95	Ø.Ø	5Ø.5
24-3Ø	24.5	1.7	7.1	3.5		2.53	Ø.Ø7	49.7
DECEMBER								
1- 7	23.6	-3.4	8.3	3.4		1.53	Ø.Ø	44.1
8-14	22.7	Ø.6	6.3	3.6		2.32	Ø.Ø	46.9
15-21	24.6	-Ø.8	7.2	5.8		2.43	Ø.Ø	47.6
22-28	24.4	-1.5	8.2	5.8		2.Ø9	Ø.Ø	49.Ø

ENVIRONMENTAL MEASUREMENTS CP-MONTECILLO,1988.

WEEK AVERAGE

DATE	TEMPERATURE °C MAX	MIN	SUN HR	EVAPORA-TION	WIND Ø.5m	2.Øm	PRECIPI-TATION (mm)	RELATIVE HUMIDITY (%)
JANUARY								
29- 4	19.2	-3.Ø	6.3Ø	3.Ø		1.48	Ø.Ø	53.5
5-11	22.8	-4.Ø	9.Ø	Ø.Ø		3.17	Ø.Ø	46.3
12-18	22.5	-2.8	8.5Ø	Ø.Ø		2.45	Ø.Ø	44.9
19-25	21.Ø	-2.1	7.5Ø	1.8		2.51	Ø.Ø	41.3
FEBRUARY								
26- 1	21.9	-8.8	11.Ø	4.Ø		1.89	Ø.Ø	45.1
2- 8	24.5	-1.7	8.52	4.2		1.77	Ø.Ø	25.5
9-15	23.1	Ø.5	7.3Ø	3.9		2.Ø6	2.Ø8	4Ø.3
16-22	23.9	-Ø.Ø4	9.21	7.7		3.91	Ø.Ø	3Ø.4
23-29	25.9	-Ø.98	8.1	4.2		1.68	Ø.Ø2	35.7
MARCH								
1- 7	19.5	4.5	4.51	3.9		3.57	5.45	42.6
8-14	22.1	-Ø.24	8.51	5.2		3.23	Ø.Ø	35.9
15-21	23.6	1.1	9.33	6.4		3.Ø3	Ø.Ø	26.4
22-28	26.3	Ø.6	8.1Ø	5.8		2.Ø4	Ø.Ø	32.9
APRIL								
29- 4	24.7	-Ø.2	9.57	6.4		2.73	Ø.64	15.3
5-11	23.4	2.1	7.2Ø	4.3		2.14	Ø.99	Ø.Ø
12-18	24.Ø	2.7	8.12	5.5		2.37	Ø.36	4.Ø
19-25	29.4	1.3	1Ø.36	7.7		2.99	Ø.Ø	22.4

ENVIRONMENTAL MEASUREMENTS CP-MONTECILLO,1988.

WEEK AVERAGE

DATE	TEMPERATURE °C MAX	MIN	SUN HR	EVAPORA-TION	WIND Ø.5m	2.Øm	PRECIPI-TATION (mm)	RELATIVE HUMIDITY (%)
MAY								
26- 2	28.6	5.Ø	9.14	6.1	1.93		1.4Ø	24.4
3- 9	32.2	4.4	9.3Ø	5.6	1.84		Ø.2Ø	22.5
1Ø-16	31.4	4.6	8.22	5.3	2.12		3.31	28.3
17-23	31.7	5.2	8.4Ø	6.3	2.Ø6		Ø.58	27.6
23-3Ø	31.1	7.5	9.1Ø	5.7	1.92		3.84	37.3
JUNE								
31- 6	31.2	7.5	7.51	5.7	1.6Ø		Ø.86	25.1
7-13	29.7	6.Ø	7.32	5.9	2.48		Ø.98	36.1
14-2Ø	27.3	1Ø.2	4.51	3.4	2.69		3.93	27.8
21-27	27.2	1Ø.5	3.Ø5	3.4	1.41		7.21	52.6
JULY								
28- 4	28.8	1Ø.6	6.Ø7	3.8	1.61		1.86	5Ø.4
5-11	29.3	8.6	7.42	4.5	1.Ø7		5.5Ø	4Ø.7
12-19	28.5	9.Ø	5.15	3.3	1.23		4.36	56.3
19-25	27.2	7.8	5.Ø3	2.6	1.16		2.22	57.6
AUGUST								
26- 1	29.Ø	7.7	7.11	3.8	1.16		1.9Ø	52.Ø
2- 8	29.7	8.4	6.17	3.6	1.28		1.65	52.4
9-15	27.7	8.6	4.34	3.4	2.36		Ø.72	53.Ø
16-22	28.8	9.9	5.5Ø	4.1	2.24		Ø.41	51.7
23-29	31.8	6.2	7.48	4.4	1.78		Ø.Ø2	35.6

ENVIRONMENTAL MEASUREMENTS CP-MONTECILLO,1988.

WEEK AVERAGE

DATE	TEMPERATURE °C MAX	MIN	SUN HR	EVAPORA-TION	WIND Ø.5m	2.Øm	PRECIPI-TATION (mm)	RELATIVE HUMIDITY (%)
SEPTEMBER								
3Ø- 5	25.Ø	11.3	1.Ø	1.2	1.21		11.7Ø	47.9
6-12	27.9	3.9	8.5Ø	4.4	1.63		Ø.47	43.6
13-19	28.4	6.4	7.44	3.4	2.Ø3		1.69	26.3
2Ø-26	3Ø.5	7.Ø	8.32	4.6	1.52		Ø.Ø4	46.4
OCTOBER								
27- 3	29.5	5.2	8.35	3.5	1.23		1.ØØ	16.9
4-1Ø	28.2	3.6	5.57	3.6	1.95		Ø.Ø8	47.6
11-17	28.Ø	4.1	6.54	3.2	1.35		Ø.Ø	35.6
18-24	29.7	2.4	8.28	3.6	1.16		Ø.Ø	46.5
26-31	31.Ø	1.Ø	8.5Ø	4.Ø	1.32		Ø.23	36.5
NOVEMBER								
1- 7	27.3	-2.9	1Ø.3Ø	4.3	1.86		Ø.Ø	25.4
8-14	29.Ø	-4.7	1Ø.Ø2	4.2	1.63		Ø.Ø	3Ø.7
15-21	28.2	-1.Ø	1Ø.Ø3	3.8	1.71		Ø.Ø	34.1
22-28	27.4	2.2	8.33	4.Ø	1.57		Ø.Ø5	42.5
DECEMBER								
29- 5	21.4	-Ø.1	8.14	3.Ø	1.82		Ø.Ø	17.8
6-12	25.6	-1.3	8.44	4.2	2.48		Ø.Ø	3Ø.9
13-19	26.Ø	-1.5	9.14	3.Ø	1.43		Ø.Ø	35.6
2Ø-26	26.7	-5.Ø	1Ø.27	3.6	1.46		Ø.Ø	37.5
27- 1	24.4	-1.5	9.51	4.Ø	2.37		Ø.Ø	35.4

ENVIRONMENTAL MEASUREMENTS CP-MONTECILLO,1989.

WEEK AVERAGE

DATE	TEMPERATURE °C MAX	MIN	SUN HR	EVAPORA-TION	WIND Ø.5m	2.Øm	PRECIPI-TATION (mm)	RELATIVE HUMIDITY (%)
JANUARY								
4-1Ø	2Ø.Ø	-3.4	9.98	2.43	1.14		Ø.Ø	58.7
11-17	27.2	-1.7	9.33	Ø.Ø	1.Ø9		Ø.Ø	61.4
18-24	26.4	Ø.2	7.97	Ø.Ø	1.56		Ø.Ø	61.6
25-31	26.Ø	-1.2	8.58	Ø.Ø	1.35		Ø.Ø	6Ø.3
FEBRUARY								
1- 7	26.8	-Ø.8	8.53	Ø.Ø	1.37		Ø.Ø	58.2
8-14	24.5	-Ø.8	9.44	4.Ø	1.65		Ø.Ø	58.6
15-21	25.2	-2.Ø	1Ø.Ø2	4.5	1.47		Ø.Ø	53.7
22-28	24.7	-7.2	1Ø.51	5.2	1.74		Ø.Ø	5Ø.5
MARCH								
1- 7	26.2	-1.2	9.7	7.49	2.49		Ø.Ø	49.9
8-14	26.5	-8.2	1Ø.45	5.82	1.69		Ø.Ø	48.7
15-21	29.5	-1.Ø	9.29	5.51	1.42		Ø.Ø	54.6
22-28	27.Ø	2.2	8.83	5.93	2.55		Ø.4	54.3
APRIL								
29- 4	29.Ø	Ø.5	7.59	5.37	1.61		Ø.Ø	52.Ø
5-11	27.8	Ø.6	8.54	5.56	1.72		Ø.8	52.1
12-18	26.5	3.Ø	15.81	5.29	1.16		Ø.7	46.4
19-25	27.Ø	-Ø.4	11.53	5.84	1.85		Ø.2	58.8

ENVIRONMENTAL MEASUREMENTS CP-MONTECILLO,1989.

WEEK AVERAGE

DATE	TEMPERATURE °C MAX	MIN	SUN HR	EVAPORA-TION	WIND Ø.5m	2.Øm	PRECIPI-TATION (mm)	RELATIVE HUMIDITY (%)
MAY								
26- 2	28.4	2.2	7.34	5.34	1.56		Ø.1	52.1
3- 9	28.6	4.2	9.1	6.Ø8	1.58		1.Ø	64.3
1Ø-16	3Ø.2	5.7	9.57	6.43	1.66		Ø.Ø	53.6
17-23	26.4	7.2	9.77	6.9	1.69		5.Ø	53.3
24-3Ø	29.2	6.2	9.Ø1	5.5	1.49		2.2	55.Ø
JUNE								
31- 6	3Ø.2	6.2	9.Ø3	5.28	1.51		Ø.2	55.Ø
7-13	32.2	7.2	9.Ø7	6.39	1.66		2.2	53.3
14-2Ø	28.Ø	8.1	5.84	5.1	1.94		5.2	6Ø.4
21-27	26.7	9.5	4.63	3.75	1.85		7.8	56.4
JULY								
28- 4	26.7	8.2	6.25	3.56	1.56		3.3	63.3
5-11	26.8	8.2	6.97	3.56	1.18		2.8	6Ø.8
12-18	28.2	4.4	7.18	4.16	Ø.94		1.Ø	61.9
19-25	27.8	7.1	6.99	4.15	1.Ø2		5.5	68.1
AUGUST								
26- 1	25.4	8.8	6.31	4.57	1.64		4.4	69.8
2- 8	27.4	7.5	7.Ø8	3.6	1.14		2.3	9.7
9-15	26.5	7.3	8.49	4.2	1.4		1.9	19.2
16-22	28.4	7.1	6.88	3.69	Ø.82		2.Ø	77.4
23-29	27.4	1Ø.2	5.16	3.62	1.Ø9		5.4	69.5

ENVIRONMENTAL MEASUREMENTS CP-MONTECILLO,1989.

WEEK AVERAGE

DATE	TEMPERATURE °C MAX	MIN	SUN HR	EVAPORA-TION	WIND Ø.5m	2.Øm	PRECIPI-TATION (mm)	RELATIVE HUMIDITY (%)
SEPTEMBER								
3Ø- 5	27.5	9.1	6.84	3.57	1.2		1.1	66.7
6-12	28.Ø	9.7	6.13	3.58	Ø.8		4.6	68.3
13-19	24.7	9.8	5.Ø8	3.72	2.4		Ø.5	71.3
2Ø-26	23.4	8.3	4.42	3.23	1.8		3.5	7Ø.6
OCTOBER								
27- 3	24.7	3.1	4.9	2.7	Ø.91		Ø.1	67.7
4-1Ø	26.8	6.6	5.84	3.2	1.1		Ø.6	68.2
11-17	26.5	-Ø.7	9.58	4.41	1.27		Ø.Ø	59.9
18-24	24.3	Ø.6	7.97	4.63	1.56		Ø.Ø	61.7
25-31	25.7	-1.3	9.34	3.54	1.14		Ø.Ø	59.4
NOVEMBER								
1- 7	28.2	Ø.2	9.42	4.Ø1	1.12		Ø.Ø	59.1
8-14	27.7	1.7	8.23	3.4	Ø.9		1.Ø	61.3
15-21	26.7	1.4	8.83	4.17	1.82		Ø.Ø	59.8
22-28	25.9	Ø.2	8.43	3.5	1.24		Ø.Ø	59.6
DECEMBER								
29- 5	23.4	3.4	5.37	2.29	1.45		Ø.7	66.2
6-12	22.Ø	-Ø.4	6.23	2.84	1.76		1.2	62.8
13-19	25.2	-Ø.5	8.4	3.42	1.65		Ø.Ø	57.7
2Ø-26	23.5	-3.7	8.17	2.69	1.Ø4		Ø.Ø	58.3

ENVIRONMENTAL MEASUREMENTS TLALTIZAPAN, MOR., 1987.

WEEK AVERAGE

DATE	TEMPERATURE °C MAX	MIN	SUN HR	EVAPORA-TION	WIND Ø.5m	2.Øm	PRECIPI-TATION (mm)
JANUARY							
31- 6	29.8	7.8	8.3	4.18		5Ø.7	Ø.Ø
7-13	3Ø.7	9.7	9.8	4.64		67.2	Ø.Ø
14-2Ø	26.8	9.2	8.5	5.Ø8		39.1	Ø.Ø
21-27	31.1	1Ø.2	9.6	5.35		77.4	Ø.Ø
FEBRUARY							
28- 3	29.9	5.6	7.5	4.Ø5		62.4	Ø.1
4-1Ø	29.7	8.8	1Ø.Ø	6.Ø6		1Ø3.1	Ø.Ø
11-17	31.4	9.4	9.8	5.93		79.Ø	Ø.Ø
18-24	33.4	11.3	1Ø.4	5.82		76.4	Ø.Ø
25- 3	34.4	13.2	1Ø.Ø	7.6Ø		11Ø.1	Ø.Ø
MARCH							
4-1Ø	3Ø.8	11.9	1Ø.Ø	7.97		125.1	Ø.61
11-17	33.4	12.5	1Ø.2	7.Ø2		1Ø8.2	Ø.Ø
18-24	36.3	13.3	9.5	7.78		1Ø5.8	Ø.Ø
25-31	34.7	13.1	7.9	6.62		113.9	Ø.22
APRIL							
1- 7	33.6	13.1	1Ø.8	7.94		1Ø2.7	Ø.Ø
8-14	34.2	16.4	7.2	6.52		87.1	Ø.Ø
15-21	31.7	18.2	7.5	6.37		97.5	Ø.5Ø
22-28	33.5	17.3	1Ø.3	7.75		117.7	Ø.1Ø

ENVIRONMENTAL MEASUREMENTS TLALTIZAPAN,MOR.,1987.

WEEK AVERAGE

DATE	TEMPERATURE °C MAX	MIN	SUN HR	EVAPORA-TION	WIND Ø.5m	2.Øm	PRECIPI-TATION (mm)
MAY							
29- 5	34.9	14.Ø	1Ø.9	7.9Ø		1Ø1.2	Ø.Ø
6-12	34.8	16.7	8.3	8.54		119.3	2.17
13-19	35.9	17.9	8.8	9.45		1Ø1.7	Ø.Ø
2Ø-26	35.9	17.8	8.8	8.Ø5		133.5	Ø.Ø
JUNE							
27- 2	34.1	19.1	8.Ø	11.38		11Ø.Ø	11.26
3- 9	31.2	18.9	5.9	4.64		92.1	7.67
1Ø-16	32.4	19.8	6.6	5.12		77.1	4.18
17-23	31.7	19.2	9.2	5.69		76.1	6.22
24-3Ø	32.8	2Ø.4	9.3	5.65		9Ø.4	2.64
JULY							
1- 7	3Ø.9	19.1	7.4	3.88		87.9	13.7Ø
8-14	3Ø.9	19.7	6.5	4.91		75.3	9.37
15-21	31.3	25.6	9.6	5.81		82.6	Ø.95
22-28	29.Ø	18.7	3.9	3.41		35.1	4.61
AUGUST							
29- 4	3Ø.1	18.3	7.7	4.89		Ø.Ø	5.25
5-11	31.3	18.5	9.3	5.8Ø		Ø.Ø	4.41
12-18	31.2	18.1	1Ø.9	3.88		7Ø.9	3.51
19-25	31.4	18.2	1Ø.1	7.1Ø		69.5	7.87

ENVIRONMENTAL MEASUREMENTS TLALTIZAPAN,MOR.,1987.

WEEK AVERAGE

DATE	TEMPERATURE °C MAX	MIN	SUN HR	EVAPORA-TION	WIND Ø.5m	2.Øm	PRECIPI-TATION (mm)
SEPTEMBER							
26- 1	31.8	18.1	9.7	5.Ø8		81.1	2.12
2- 8	31.9	18.4	9.9	5.58		74.7	3.2Ø
9-15	32.Ø	18.6	9.7	5.29		87.7	1.91
16-22	31.1	19.6	8.9	5.13		96.8	6.72
23-29	3Ø.8	19.Ø	9.1	5.64		76.5	15.57
OCTOBER							
3Ø- 6	28.8	15.7	7.1	3.78		65.9	3.ØØ
7-13	2Ø.5	13.7	9.5	4.3Ø		74.Ø	Ø.Ø
14-2Ø	29.8	11.6	9.3	3.59		6Ø.7	Ø.Ø
21-27	32.1	14.6	9.1	4.23		67.Ø	Ø.Ø
NOVEMBER							
28- 3	3Ø.4	1Ø.1	1Ø.3	3.17		59.3	Ø.Ø
4-1Ø	3Ø.4	1Ø.4	9.4	4.Ø3		7Ø.7	2.62
11-17	3Ø.1	12.Ø	8.5	3.51		55.Ø	Ø.Ø
18-24	3Ø.9	12.1	8.9	3.52		65.9	Ø.Ø
DECEMBER							
25- 1	31.7	13.3	9.1	3.76		5Ø.1	Ø.Ø
2- 8	31.2	1Ø.7	9.6	3.27		43.5	Ø.Ø
9-15	29.7	11.1	6.2	2.64		44.6	Ø.Ø
16-22	3Ø.8	11.8	8.5	3.19		66.6	Ø.Ø
23-29	32.Ø	1Ø.3	9.5	3.56		72.1	Ø.Ø

ENVIRONMENTAL MEASUREMENTS TLALTIZAPAN,MOR.,1988.

WEEK AVERAGE

DATE	TEMPERATURE °C MAX	MIN	SUN HR	EVAPORA-TION	WIND Ø.5m	2.Øm	PRECIPI-TATION (mm)
JANUARY							
6-12	3Ø.5	8.1	9.6	3.54		54.8	Ø.Ø
13-19	31.Ø	9.6	9.8	3.77		54.2	Ø.Ø
2Ø-26	29.8	7.3	9.7	4.81		87.3	Ø.Ø
FEBRUARY							
27- 2	28.3	7.7	1Ø.5	4.39		93.5	Ø.Ø
3- 9	31.7	1Ø.9	9.3	5.2Ø		89.6	Ø.Ø
1Ø-16	32.Ø	13.6	9.8	5.91		88.Ø	Ø.Ø
17-23	33.1	1Ø.3	1Ø.4	5.66		78.9	Ø.Ø
MARCH							
24- 1	33.4	14.1	8.4	5.88		99.7	Ø.Ø
2- 8	29.8	14.3	6.Ø	4.49		67.3	2.38
9-15	33.7	11.6	9.4	6.34		85.9	Ø.Ø
16-22	33.7	15.5	1Ø.1	7.1		1Ø4.8	Ø.Ø
23-29	35.5	16.6	1Ø.1	7.22		1Ø1.Ø	Ø.Ø
APRIL							
3Ø- 5	36.Ø	13.7	1Ø.2	6.14		9Ø.9	Ø.Ø
6-12	35.6	16.7	9.5	8.14		84.5	Ø.Ø
13-19	34.4	14.4	9.7	7.94		62.8	Ø.Ø
2Ø-26	37.1	13.8	1Ø.6	7.94		56.4	Ø.Ø

ENVIRONMENTAL MEASUREMENTS TLALTIZAPAN,MOR.,1988.

WEEK AVERAGE

DATE	TEMPERATURE °C MAX	MIN	SUN HR	EVAPORA-TION	WIND Ø.5m	2.Øm	PRECIPI-TATION (mm)
MAY							
27- 3	37.3	17.3	1Ø.3	7.56		63.5	Ø.Ø
4-1Ø	37.1	16.2	9.9	7.63		64.3	Ø.Ø
11-17	36.1	15.3	8.8	6.97		51.6	Ø.Ø
18-24	37.4	17.1	9.3	7.22		7Ø.7	Ø.Ø
25-31	4Ø.Ø	18.Ø	9.8	7.73		69.5	3.68
JUNE							
1- 7	37.3	19.5	9.8	8.4		89.Ø	Ø.14
8-14	35.3	18.6	9.5	8.73		33.3	5.37
15-21	33.7	2Ø.Ø	5.8	4.66		14Ø.4	Ø.62
22-28	31.Ø	19.5	6.9	6.71		65.7	18.32
JULY							
29- 5	31.1	19.Ø	7.2	5.9		59.5	18.Ø
6-12	4Ø.Ø	18.9	1Ø.1	5.73		65.3	1Ø.41
13-19	3Ø.8	19.5	7.6	5.81		63.2	4.5
2Ø-26	3Ø.5	17.8	8.5	5.75		48.2	7.14
AUGUST							
27- 2	32.2	17.6	1Ø.Ø	5.15		59.8	Ø.Ø
3- 9	31.7	18.2	9.5	6.16		55.7	7.11
1Ø-16	3Ø.3	19.2	6.7	4.31		44.9	12.9
17-23	3Ø.7	19.Ø	7.1	4.29		45.2	1.15
24-3Ø	31.4	18.4	7.7	4.65		48.5	Ø.Ø

ENVIRONMENTAL MEASUREMENTS TLALTIZAPAN,MOR.,1988.

WEEK AVERAGE

DATE	TEMPERATURE °C MAX	MIN	SUN HR	EVAPORA-TION	WIND Ø.5m	2.Øm	PRECIPI-TATION (mm)
SEPTEMBER							
31- 6	28.5	19.1	3.Ø	2.91		41.2	9.84
7-13	3Ø.3	17.Ø	9.6	4.66		37.5	Ø.55
14-2Ø	31.3	17.2	8.8	5.Ø7		53.7	3.11
21-27	31.5	18.Ø	9.4	5.64		45.6	1Ø.Ø7
OCTOBER							
28- 4	31.1	17.2	7.9	4.33		7Ø.2	1.6
5-11	31.Ø	15.9	9.5	5.59		72.7	3.68
12-18	3Ø.3	15.Ø	1Ø.Ø	4.21		41.4	Ø.Ø1
19-25	31.8	15.3	8.7	4.72		32.8	Ø.72
NOVEMBER							
26- 1	32.4	15.4	9.3	4.36		49.9	Ø.35
2- 8	31.7	9.7	1Ø.1	4.2Ø		31.2	Ø.Ø
9-15	32.2	8.Ø	9.8	3.69		36.7	Ø.Ø
16-22	31.8	11.3	9.5	3.88		48.6	Ø.Ø
23-29	31.8	11.7	1Ø.2	4.35		62.Ø	Ø.Ø
DECEMBER							
3Ø- 6	29.9	1Ø.Ø	9.6	4.23		87.3	Ø.Ø
7-13	3Ø.5	1Ø.1	9.3	3.96		58.7	Ø.Ø
14-2Ø	3Ø.7	1Ø.2	9.8	4.1Ø		52.9	Ø.Ø
21-27	26.7	6.8	1Ø.Ø	3.58		48.2	Ø.Ø
28- 3	3Ø.4	9.Ø	9.7	3.81		54.6	Ø.Ø

ENVIRONMENTAL MEASUREMENTS TLALTIZAPAN,MOR.,1989.

WEEK AVERAGE

DATE	TEMPERATURE °C MAX	MIN	SUN HR	EVAPORA-TION	WIND Ø.5m	2.Øm	PRECIPI-TATION (mm)
JANUARY							
28- 3	32.Ø	8.8	1Ø.2	3.8		57.5	Ø.Ø
11-17	31.5	1Ø.2	1Ø.2	4.Ø		59.4	Ø.Ø
18-24	3Ø.9	11.Ø	9.7	4.1		81.7	Ø.Ø
25-31	31.7	9.6	9.9	4.4		68.5	Ø.Ø
FEBRUARY							
1- 7	31.7	1Ø.5	9.5	4.1		68.6	Ø.Ø
8-14	31.5	11.7	1Ø.5	6.Ø		117.6	Ø.Ø
15-21	32.2	11.3	9.2	6.Ø		1ØØ.6	Ø.Ø
22-28	3Ø.3	7.8	1Ø.4	6.Ø		1Ø7.8	Ø.Ø
MARCH							
1- 7	33.9	9.8	1Ø.5	6.5		77.5	Ø.Ø
8-14	26.8	8.8	1Ø.1	6.7		96.2	Ø.Ø
15-21	34.Ø	12.5	1Ø.2	6.9		81.6	Ø.Ø
22-28	33.5	12.8	11.Ø	7.5		82.4	Ø.Ø
APRIL							
29- 4	34.7	13.2	9.6	7.Ø		7Ø.3	Ø.Ø
5-11	35.1	14.8	9.7	7.1		1Ø2.8	Ø.Ø
12-18	34.6	14.2	1Ø.5	7.4		79.9	Ø.Ø1
19-25	35.3	15.3	1Ø.3	9.2		1Ø2.9	Ø.Ø

ENVIRONMENTAL MEASUREMENTS TLALTIZAPAN,MOR.,1989.

WEEK AVERAGE

DATE	TEMPERATURE °C MAX	MIN	SUN HR	EVAPORA-TION	WIND Ø.5m	2.Øm	PRECIPI-TATION (mm)
MAY							
26- 2	34.4	15.1	9.8	8.9		61.5	Ø.Ø
3- 9	34.1	16.7	9.3	7.8		34.5	Ø.Ø
1Ø-16	34.Ø	17.4	9.5	7.9		19.5	Ø.2
17-23	36.3	19.3	9.8	8.Ø		24.2	Ø.Ø
24-3Ø	34.3	19.1	1Ø.3	8.Ø		28.5	1.Ø
JUNE							
31- 6	39.6	2Ø.3	9.4	7.3		11.8	Ø.15
7-13	34.5	21.2	9.4	8.3		14.3	4.3
14-2Ø	3Ø.6	19.Ø	6.6	5.6		1Ø.8	4.8
21-27	29.9	17.6	6.1	5.1		7.8	9.Ø
JULY							
28- 4	29.9	17.9	7.9	5.1		7Ø.8	7.7
5-11	3Ø.2	17.3	9.1	6.7		1ØØ.1	3.3
12-18	31.1	15.3	8.9	5.2		78.1	2.3
19-25	3Ø.9	16.9	8.7	6.3		93.1	8.8
AUGUST							
26- 1	3Ø.5	17.7	7.5	4.5		87.3	Ø.6
2- 8	3Ø.2	17.Ø	9.5	6.7		9Ø.5	6.8
9-15	3Ø.1	16.6	9.8	5.9		77.Ø	1Ø.8
16-22	3Ø.1	17.1	8.7	5.3		88.3	5.5
23-29	29.7	17.5	7.5	5.1		82.6	7.2

ENVIRONMENTAL MEASUREMENTS TLALTIZAPAN,MOR.,1989.

WEEK AVERAGE

DATE	TEMPERATURE °C MAX	MIN	SUN HR	EVAPORA-TION	WIND Ø.5m	2.Øm	PRECIPI-TATION (mm)
SEPTEMBER							
3Ø- 5	3Ø.4	17.1	9.7	5.2		88.Ø	5.7
6-12	3Ø.6	17.7	9.2	5.6		79.2	5.5
13-17	3Ø.Ø	18.1	6.8	4.9		69.6	1.4
2Ø-26	28.6	17.Ø	4.8	4.2		7Ø.9	1.2
OCTOBER							
27- 3	29.5	16.3	6.8	4.7		69.3	1.5
4-1Ø	3Ø.Ø	17.9	8.5	4.7		82.8	8.4
18-24	29.1	12.5	1Ø.Ø	4.5		85.5	1.3
25-31	29.4	12.3	9.1	3.2		66.Ø	Ø.Ø
NOVEMBER							
1- 7	31.6	12.7	9.8	3.8		67.5	Ø.Ø
8-14	31.2	15.3	9.1	4.2		76.Ø	1.Ø5
15-21	31.4	12.6	1Ø.Ø	4.5		74.5	Ø.Ø
22-28	31.3	1Ø.6	1Ø.1	5.1		7Ø.8	Ø.Ø
DECEMBER							
29- 5	29.4	13.5	6.1	3.6		83.4	Ø.Ø
6-12	28.Ø	1Ø.1	9.Ø	2.9		78.6	Ø.6
13-19	3Ø.7	1Ø.7	9.1	3.6		58.2	Ø.Ø
2Ø-26	29.6	8.5	9.8	4.22		86.3	Ø.Ø

ENVIRONMENTAL MEASUREMENTS POZA RICA, VER.,1987.

WEEK AVERAGE

DATE	TEMPERATURE °C MAX	MIN	SUN HR	EVAPORA-TION	WIND Ø.5m	2.Øm	PRECIPI-TATION (mm)
JANUARY							
31- 6	23.6	9.1	7.7	3.12		27.4	Ø.Ø
7-13	22.1	13.1	3.9	2.21		24.1	Ø.42
14-2Ø	25.3	14.1	4.2	2.26		24.Ø	Ø.34
21-27	23.3	9.5	6.6	2.96		28.5	Ø.15
FEBRUARY							
28- 3	27.2	13.2	5.6	2.83		2Ø.1	Ø.Ø
4-1Ø	26.4	12.4	7.Ø	3.29		33.7	1.Ø1
11-17	28.2	13.4	6.5	3.91		36.6	Ø.Ø
18-24	21.2	14.8	1.3	1.58		25.2	2.Ø8
MARCH							
25- 3	26.2	16.8	1.9	2.79		32.5	Ø.Ø
4-1Ø	23.9	13.7	3.9	2.47		28.3	2.77
11-17	24.5	17.4	1.9	1.8Ø		27.8	6.64
18-24	29.3	18.8	4.2	3.45		3Ø.2	Ø.Ø
25-31	25.2	16.9	1 7	2.72		43.3	Ø.68
APRIL							
1- 7	26.3	12.9	4.3	3.17		29.4	1.62
8-14	26.5	17.1	2.Ø	2.59		24.7	1.24
15-21	3Ø.7	17.7	5.Ø	4.53		32.4	Ø.Ø
22-28	28.1	19.1	3.6	3.61		2Ø.5	Ø.8Ø

ENVIRONMENTAL MEASUREMENTS POZA RICA, VER.,1987.

WEEK AVERAGE

DATE	TEMPERATURE °C MAX	MIN	SUN HR	EVAPORA-TION	WIND Ø.5m	2.Øm	PRECIPI-TATION (mm)
MAY							
29- 5	32.9	2Ø.2	8.5	5.1Ø		1Ø.5	7.64
6-12	3Ø.Ø	21.6	4.9	4.23		7.1	1.57
13-19	32.2	22.9	6.2	5.17		26.6	Ø.28
2Ø-26	33.7	22.7	3.8	6.2Ø		44.1	Ø.17
JUNE							
27- 2	33.9	23.7	5.3	5.15		44.1	4.91
3- 9	29.9	21.9	4.Ø	2.95		34.4	1Ø.6Ø
1Ø-16	33.3	23.7	8.Ø	6.25		4Ø.Ø	Ø.14
17-23	34.9	23.6	9.3	6.31		41.Ø	Ø.64
24-3Ø	35.3	22.4	9.2	7.Ø1		46.2	Ø.21
JULY							
1- 7	31.6	23.1	3.6	4.5Ø		39.7	1Ø.32
8-14	32.7	23.1	6.1	5.5		43.2	6.6Ø
15-21	32.9	23.6	8.4	5.65		35.Ø	2.2Ø
22-28	29.4	22.3	1.1	3.18		34.7	9.41
AUGUST							
29- 4	31.8	22.9	6.1	4.85		24.7	3.35
5-11	32.7	2Ø.Ø	7.8	3.59		34.8	2.74
12-18	34.8	24.3	9.5	5.52		41.4	Ø.Ø
19-25	32.5	21.3	7.9	5.19		34.6	4.11

ENVIRONMENTAL MEASUREMENTS POZA RICA, VER.,1987.

WEEK AVERAGE

DATE	TEMPERATURE °C MAX	MIN	SUN HR	EVAPORA-TION	WIND Ø.5m	2.Øm	PRECIPI-TATION (mm)
SEPTEMBER							
26- 1	32.Ø	22.3	6.7	4.67		31.8	1.28
2- 8	32.8	21.8	6.4	5.15		32.4	Ø.37
9-15	35.Ø	22.9	8.8	6.Ø2		39.1	Ø.34
16-22	34.3	24.Ø	6.8	4.66		45.3	2.42
23-29	31.5	22.Ø	5.2	4.Ø3		25.4	21.55
OCTOBER							
3Ø- 6	29.8	18.8	6.4	4.Ø7		31.2	2.ØØ
7-13	3Ø.1	17.9	7.6	4.28		32.21	1.37
14-2Ø	31.4	17.7	8.6	4.95		32.4	Ø.Ø
21-27	31.1	18.9	8.2	3.2Ø		39.3	Ø.42
NOVEMBER							
28- 3	29.4	16.7	8.1	4.Ø3		4Ø.1	7.75
4-1Ø	28.4	17.3	6.3	3.2Ø		36.1	1.65
11-17	26.1	16.8	3.9	2.17		24.4	Ø.17
18-24	28.3	16.6	4.Ø	2.16		25.6	3.85
DECEMBER							
25- 1	24.4	17.4	2.6	1.74		28.2	2.6Ø
2- 8	28.7	16.8	7.6	2.98		24.7	1.27
3-15	26.9	15.1	4.4	1.65		23.9	Ø.14
16-22	22.8	15.5	1.9	1.21		18.7	2.8Ø
23-29	22.8	19.6	2.3	1.42		3Ø.9	2.ØØ

ENVIRONMENTAL MEASUREMENTS POZA RICA, VER.,1988.

WEEK AVERAGE

DATE	TEMPERATURE °C MAX	MIN	SUN HR	EVAPORA-TION	WIND Ø.5m	2.Øm	PRECIPI-TATION (mm)
JANUARY							
3Ø- 5	21.1	13.6	1.6	1.39		23.Ø	Ø.48
6-12	2Ø.6	13.4	1.8	Ø.96		27.5	1.Ø1
13-19	26.Ø	14.6	4.2	2.Ø6		18.9	1.Ø4
2Ø-26	2Ø.4	12.Ø	2.7	2.67		38.4	Ø.41
FEBRUARY							
27- 2	24.2	12.9	3.8	2.Ø8		26.Ø	Ø.44
3- 9	21.5	14.4	Ø.9	1.63		34.8	1.31
1Ø-16	22.8	13.9	2.9	2.Ø7		29.7	2.74
17-23	25.6	14.2	6.3	3.9		28.5	Ø.52
MARCH							
24- 1	26.1	17.Ø	3.4	2.46		24.4	Ø.67
2- 8	24.6	18.1	2.4	1.91		27.9	2.84
9-15	26.2	12.8	5.Ø	3.24		34.1	1.18
16-22	23.8	13.7	3.5	3.56		35.7	Ø.48
23-29	3Ø.5	19.8	5.6	4.93		35.6	Ø.11
APRIL							
3Ø- 5	3Ø.8	2Ø.2	5.3	4.Ø7		5.2	2.97
6-12	26.2	17.6	4.Ø	3.71		1Ø.Ø	6.81
13-19	31.7	17.7	7.8	5.32		6.6	Ø.ØØ
2Ø-26	34.1	2Ø.5	8.5	5.81		6.9	Ø.ØØ

ENVIRONMENTAL MEASUREMENTS POZA RICA, VER.,1988.

WEEK AVERAGE

DATE	TEMPERATURE °C MAX	MIN	SUN HR	EVAPORA-TION	WIND Ø.5m	2.Øm	PRECIPI-TATION (mm)
MAY							
27- 3	33.3	22.9	5.4	4.79		1Ø.1	Ø.ØØ
4-1Ø	32.5	22.1	5.6	4.95		12.9	Ø.ØØ
11-17	31.4	22.7	5.3	5.19		3Ø.9	5.44
18-24	33.6	23.8	4.9	4.74		4Ø.1	Ø.ØØ
25-31	28.4	23.3	6.4	5.Ø8		47.6	2.6Ø
JUNE							
1- 7	33.4	27.7	7.7	5.68		43.1	3.6
8-14	3Ø.2	23.4	8.6	5.72		43.3	Ø.62
15-21	3Ø.2	22.4	2.2	2.Ø3		28.2	35.95
22-28	32.4	23.3	6.2	4.89		32.9	9.17
JULY							
29- 5	32.8	23.3	6.8	6.15		36.8	6.74
6-12	33.9	23.5	9.Ø	6.26		39.4	Ø.25
13-19	32.4	23.Ø	5.4	4.67		3Ø.5	1.75
2Ø-26	31.9	22.3	7.Ø	4.5Ø		36.4	7.37
AUGUST							
27- 2	32.8	22.8	6.1	4.84		34.2	4.38
3- 9	33.3	22.5	6.5	4.72		26.4	12.3Ø
1Ø-16	31.1	22.7	4.1	4.35		24.4	6.9Ø
17-23	31.7	23.1	2.7	3.Ø		26.4	Ø.9Ø
24-3Ø	32.9	22.1	8.4	5.59		29.5	3.7Ø

ENVIRONMENTAL MEASUREMENTS POZA RICA, VER.,1988.

WEEK AVERAGE

DATE	TEMPERATURE °C MAX	MIN	SUN HR	EVAPORA-TION	WIND Ø.5m	2.Øm	PRECIPI-TATION (mm)
SEPTEMBER							
31- 6	29.Ø	21.8	3.4	2.36		42.1	42.22
7-13	31.6	19.4		7.8		4.85	24.6
14-2Ø	32.7	22.9	6.1	5.32		26.9	3.87
21-27	32.5	22.5	8.1	5.Ø2		5.Ø	2.48
OCTOBER							
28- 4	31.6	21.3	6.Ø	4.43		5.5	3.44
5-11	27.7	19.1	2.Ø	3.Ø3		5.5	1.84
12-18	29.1	18.4	5.6	3.46		15.6	Ø.31
19-25	31.2	21.5	6.Ø	4.28		29.7	Ø.Ø4
NOVEMBER							
26- 1	31.9	2Ø.8	8.4	4.5Ø		55.8	5.3
2- 8	32.1	16.5	9.5	4.1Ø		49.7	Ø.ØØ
9-15	31.1	19.3	8.Ø	3.8		52.Ø	Ø.ØØ
16-22	3Ø.4	18.2	3.3	2.51		Ø.Ø	Ø.38
23-29	31.Ø	17.6	7.3	4.28		66.5	Ø.ØØ
DECEMBER							
3Ø- 6	22.2	16.Ø	1.1	1.Ø2		4Ø.2	4.9
7-13	22.8	15.2	2.3	1.52		42.3	Ø.34
14-2Ø	23.2	14.6	3.5	1.39		42.3	1.77
21-27	28.8	17.Ø	7.2	2.91		51.5	Ø.ØØ

ENVIRONMENTAL MEASUREMENTS POZA RICA, VER.,1989.

WEEK AVERAGE

DATE	TEMPERATURE °C MAX	MIN	SUN HR	EVAPORA-TION	WIND 0.5m	2.0m	PRECIPI-TATION (mm)
JANUARY							
28- 3	26.7	16.7	4.8	2.32		44.3	0.0
4-10	22.6	17.6	5.0	2.47		46.3	0.27
11-17	24.0	15.6	2.8	1.49		49.4	1.11
18-24	24.4	16.6	2.6	2.00		39.7	1.51
25-31	28.9	18.5	5.8	2.90		46.9	0.42
FEBRUARY							
1- 7	22.7	16.4	2.8	2.07		55.8	1.77
8-14	22.5	14.8	3.2	1.74		39.3	4.64
15-21	24.4	19.3	5.4	3.20		53.8	1.07
22-28	26.9	13.1	8.1	4.07		58.4	0.08
MARCH							
1- 7	25.7	14.6	4.1	3.35		56.5	0.00
8-14	28.22	9.4	10.3	5.30		110.0	0.00
15-21	31.9	17.9	7.9	5.15		74.0	0.00
22-28	24.8	17.3	6.1	4.68		56.0	1.72
APRIL							
29- 4	33.0	18.8	8.4	6.29		59.2	0.00
5-11	30.7	17.5	7.0	5.82		73.3	1.61
12-18	30.3	18.9	5.1	4.20		60.2	1.71
19-25	32.8	19.4	9.1	6.48		72.0	0.05

ENVIRONMENTAL MEASUREMENTS POZA RICA, VER.,1989.

WEEK AVERAGE

DATE	TEMPERATURE °C MAX	MIN	SUN HR	EVAPORA-TION	WIND Ø.5m	2.Øm	PRECIPI-TATION (mm)
MAY							
26- 2	33.9	21.6	7.4	5.74		73.1	3.Ø7
3- 9	33.8	21.7	9.2	6.47		72.Ø	2.92
1Ø-16	35.7	23.1	9.Ø	6.72		74.4	Ø.1Ø
17-23	37.6	25.Ø	8.5	6.73		68.5	Ø.ØØ
24-3Ø	36.1	21.5	9.9	7.2Ø		89.Ø	Ø.ØØ
JUNE							
31- 6	36.9	23.9	1Ø.2	8.43		86.2	Ø.ØØ
7-13	38.5	24.9	1Ø.3	8.35		94.3	Ø.ØØ
14-2Ø	33.Ø	21.4	5.3	4.6Ø		64.9	16.31
21-27	33.2	22.6	6.5	5.Ø4		56.2	Ø.85
JULY							
28- 4	35.5	23.1	9.4	7.63		66.1	4.42
5-11	32.2	22.2	7.3	4.91		63.8	2.64
12-18	33.1	23.Ø	7.7	5.18		55.Ø	8.5Ø
19-25	33.4	22.8	7.9	6.1Ø		56.2	2.42
AUGUST							
26- 1	31.6	22.3	7.9	4.39		49.5	52.94
2- 8	33.1	23.2	6.8	3.8Ø		54.9	7.Ø4
9-15	32.9	21.9	8.6	5.2Ø		57.6	Ø.3Ø
16-22	33.2	22.1	8.7	5.87		6Ø.2	2.21
23-29	31.8	22.8	5.3	4.43		51.5	7.5Ø

ENVIRONMENTAL MEASUREMENTS POZA RICA, VER.,1989.

WEEK AVERAGE

DATE	TEMPERATURE °C MAX	MIN	SUN HR	EVAPORA-TION	WIND Ø.5m	2.Øm	PRECIPI-TATION (mm)
SEPTEMBER							
3Ø- 5	32.5	22.4	7.7	4.24		47.8	5.44
6-12	33.5	22.6	7.3	5.76		53.3	Ø.14
13-19	29.2	21.8	2.6	1.Ø8		41.5	13.75
2Ø-26	31.3	19.5	9.2	4.13		48.9	25.12
OCTOBER							
27- 3	31.1	17.7	7.1	4.72		44.2	Ø.Ø
4-1Ø	32.Ø	21.4	8.5	5.13		56.7	3.71
11-17	32.1	18.2	8.9	5.1Ø		52.1	Ø.Ø
18-24	22.6	15.6	5.6	3.Ø4		54.7	1.97
25-31	3Ø.7	18.9	6.7	3.88		53.7	Ø.Ø
NOVEMBER							
1- 7	27.5	19.Ø	3.3	2.2Ø		36.Ø	4.34
8-14	3Ø.6	21.3	6.Ø	3.69		47.2	1.Ø4
15-21	26.9	16.7	4.Ø	2.7		44.6	1.94
22-28	28.3	18.6	4.8	3.26		4Ø.Ø	3.26
DECEMBER							
29- 5	15.9	13.5	Ø.3	Ø.79		47.7	9.32
6-12	23.9	13.2	4.9	1.65		44.3	6.5
13-19	2Ø.3	12.5	1.5	1.Ø7		34.6	Ø.5
2Ø-26	18.4	7.6	4.4	1.52		54.3	Ø.64