Identification of Maize Rayado Fino Virus in the United States

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ABSTRACT

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Maize rayado fino virus (MRFV) was identified in maize leaf samples from Texas and Florida. Identification was based on leaf symptoms, particle morphology, reactivity of virus with anti-MRFV serum in serologically specific electron microscopy and immune rate-zonal centrifugation, and persistent transmission by *Dalbulus maidis* leafhoppers. This is the first evidence for occurrences of MRFV in the United States. Samples from Texas were also coinfected with maize dwarf mosaic virus or corn stunt spiroplasma. The leafhopper *Graminella nigrifrons* was shown to be a new MRFV vector and provides a potential for spread of MRFV to major U.S. corn-growing regions.

Rayado fino disease of maize (*Zea mays*) was first reported in Central America by Ancalmo and Davis in 1961 (1). The pathogen is transmitted by the leafhopper *Dalbulus maidis* (DeLong & Wolcott) (1). Its viral nature was demonstrated in transmission studies by Gamez (10,11).

In Central America, yield losses of early infected local maize cultivars may be up to 40–50% of infected plants with field incidences of up to 20%. For introduced foreign or newly developed maize cultivars, losses and incidence may reach 100%. Incidence of the virus appears to be generally increasing in many areas of Central and South America (12).

Maize rayado fino has been found in Costa Rica (10), in El Salvador, Guatemala, Honduras, Nicaragua, Panama, and Mexico (12), and in Peru (20). Brazilian corn streak virus (15) and maize rayado Colombiano virus (MRCV) (16) are related symptomatologically and serologically to maize rayado fino virus (MRFV) (12).

Several corn-stunting, *D. maidis*-transmitted pathogens have been reported from the southern United States (18), but MRFV had not been detected in this country. We report the isolation and identification of MRFV from diseased maize collected from the Rio Grande valley of Texas and the Homestead area of Florida. Preliminary reports of this work appeared previously (3,7,17,18).

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MATERIALS AND METHODS

Source of diseased samples. In June 1976, leaf samples from dent maize plants with viruslike symptoms were collected at two sites near Harlingen in the Rio Grande valley of Texas by two of us (OEB and RWT) and assayed at the Ohio Agricultural Research and Development Center, Wooster. In January 1977, leaf samples with similar symptoms were collected by one of us (CWB) and sent to Wooster from sweet-corn breeding plots near Homestead, FL.

Electron microscopy. Expressed leaf sap or virus fractions from centrifuged sucrose gradients were negatively stained on Formvar-coated grids with 2% phosphotungstic acid neutralized to pH 7.0 with potassium hydroxide (KPT) and examined in Phillips 201 electron microscope.

Serologically specific electron microscopy (SSEM) as described by Derrick and Bolansky (9) was modified to include 1) removing the parlodion from the carbon film with amyl acetate before antiserum adsorption and 2) staining with KPT in place of uranyl acetate. Antiserum to Costa Rican MRFV was used. Controls included grids with adsorbed maize chlorotic dwarf virus (MCDV) antiserum and grids adsorbed with normal rabbit serum.

Centrifugation assays. Field-collected leaves were assayed by rate-zonal centrifugation as described previously (2). For immune rate-zonal centrifugation, partially purified virus was treated with normal rabbit serum (diluted 1:8 or 1:10 with phosphate-buffered saline) or with antiserum to MRFV (diluted 1:8) or to MRCV (diluted 1:10). The assay was performed as described previously (14). Virus identification was based on the reduction of virus peak area, as measured from UV absorbance profiles of centrifuged gradients, for antiserum treatments compared with normal rabbit serum controls.

Vector transmission. Leafhoppers were reared in organdy-covered, aluminum-frame cages 18 × 38 × 38 cm, maintained at approximately 25 C with 18 hr of light per 24 hr. *D. maidis* was reared on corn and *Graminella nigrifrons* (Forbes) on oats. For isolation from field-collected maize samples, 10–15 cm (linear) of leaves showing symptoms was placed in a petri dish with 50 *D. maidis* for 24–48 hr. Leafhoppers were then isolated on caged maize plants for a 3-wk virus incubation period. Surviving leafhoppers were caged 10 per two plants on sweet corn (Aristogold Bantam Evergreen) and serially transferred at semiweekly intervals for 2 wk. Test plants were placed in the greenhouse and observed weekly for up to 6 wk after exposure to leafhoppers. Controls consisted of maize plants exposed to leafhoppers from stock colonies; none showed

virus symptoms during these studies.

Persistence of MRFV in *D. maidis* was tested by giving 75 late instar leafhoppers a 24-hr acquisition access period to MRFV-infected maize plants. Leafhoppers were then serially transferred at daily intervals for 42 days on maize test seedlings. This test was conducted at 25 C.

Vector specificity was tested by placing late instar leafhoppers on young infected seedlings for a 48-hr acquisition access period before transferring them to healthy host plants for completion of the virus incubation period. Leafhoppers were then transferred 10 per sweet corn test plant and serially transferred semiweekly for 2 wk.

Assays for mechanically transmissible viruses. Samples of field-collected leaves were ground in 0.01 M potassium phosphate buffer, pH 7.0, and extracts were rub-inoculated with 600-mesh Carborundum on seedlings of maize (inbred OH28), johnsongrass (*Sorghum halepense*), and wheat (*Triticum aestivum*, 'Monon' or 'Michigan Amber') (14). A systemic mosaic on maize and johnsongrass but not on wheat identified maize dwarf mosaic virus strain A (MDMV-A), whereas a systemic mosaic on maize only identified strain B.

Assays for spiroplasma. Dark-field light microscopy (8) was used to examine sap expressed from field-collected leaves for corn stunt spiroplasma. Spiroplasma maintained in greenhouse-grown maize by experimental transmission was used as a control.

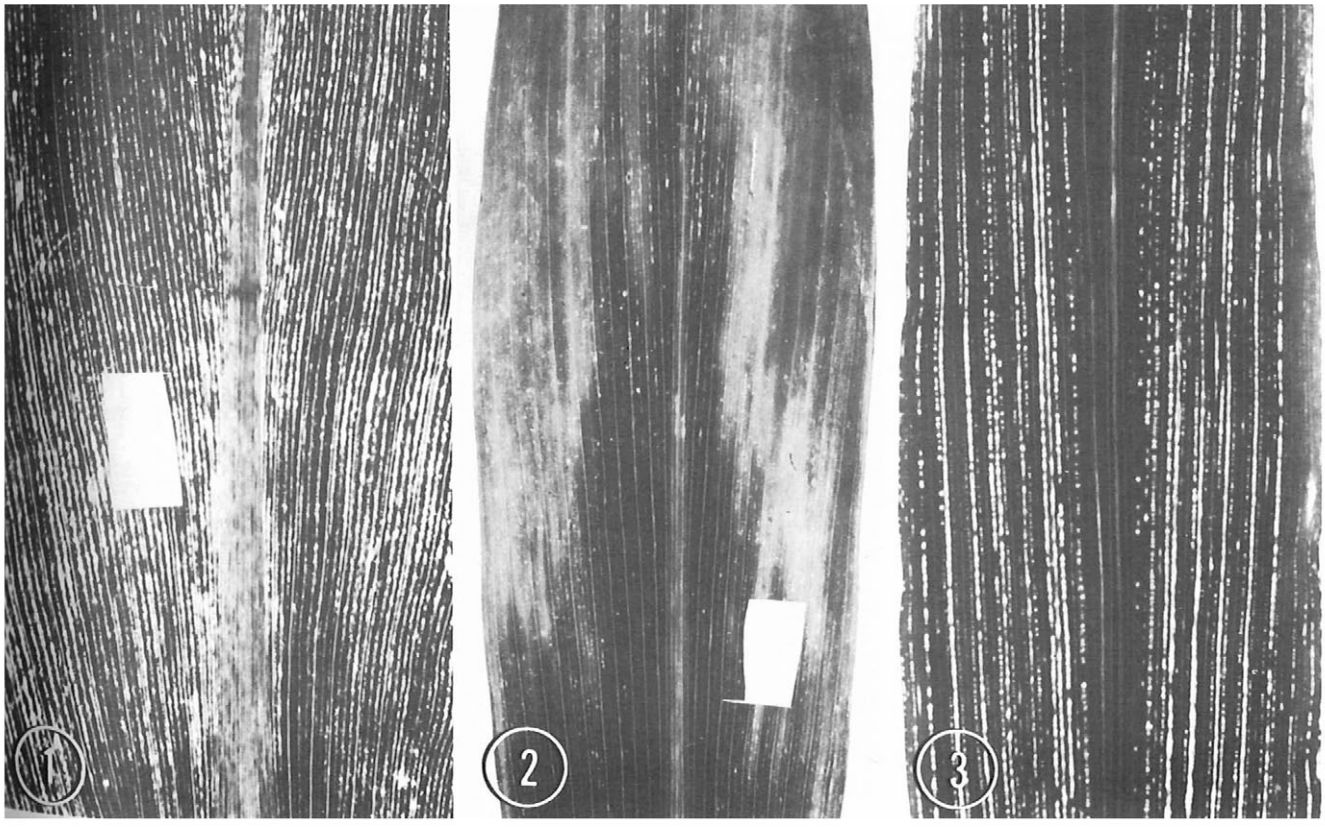
RESULTS

Field symptoms. Maize plants at the first site in the Rio Grande valley of Texas were in the silk stage and were proportionally stunted to approximately two-thirds the height of surrounding, unaffected plants. Leaf symptoms (Fig. 1) consisted of rows of fine, unevenly spaced dots and streaks of discoloration along second, third, and fourth order veins (in this terminology the midrib is the first order vein). The discolorations ranged from chlorosis to complete bleaching that vividly contrasted with normally green intervening leaf areas. Maize plants at the second site in the Rio Grande valley were in late dough stage and had some symptoms of corn stunt spiroplasma (CSS) disease. Affected plants were proportionally stunted, 30–50 cm shorter than surrounding unaffected plants. Leaf symptoms (Fig. 2) consisted of diffuse discoloration, with both chlorotic and reddened streaks at leaf tips and margins. The reddening was more extensive on younger leaves. Other symptoms usually associated with CSS—vivid chlorotic streaking on leaves and development of ear shoots at each node up to the main ear node—were not present. Leaf samples from diseased maize in Florida had symptoms similar to those found at the first collection site in Texas (Fig. 1).

Electron microscopy. Small isometric, viruslike particles appeared in KPT negatively stained preparations from 1) leaf dips of fine-streaked leaves from field collections (Fig. 4), 2) leaf dips of fine-streaked leaves from experimental transmissions (Fig. 5), 3) SSEM preparations of diseased leaves on grids with adsorbed MRFV-antiserum (Figs. 6 and 7), and 4) fractions from centrifuged gradients containing preparations from diseased leaves. Virus particles in these preparations were approximately 22–27 nm in diameter, similar to the range of diameters reported for MRFV (12). Some particles were penetrated with negative stain, while others displayed the morphological units of the virion capsid (Figs. 5–7).

Flexuous rods (Fig. 4), typical of MDMV particles, were also found in leaf-dip preparations from some field-collected, fine-streaked leaves from Texas. Preparations from experimentally vector-inoculated seedlings displayed large numbers of the small isometric particles but no other viruslike particles (Fig. 5).

Preparations of SSEM grids with MRFV antiserum consistently displayed large numbers of individual and clumped isometric particles (Figs. 6 and 7), while a



Figs. 1–3. Close-up photographs of maize leaves infected with a Texas isolate of maize rayado fino virus (MRFV): **(1)** Field-collected leaf with MRFV and maize dwarf mosaic virus. **(2)** Field-collected leaf with MRFV and corn stunt spiroplasma. (Location of sample removed for microscopic examination shown in Figs. 1 and 2.) **(3)** Leaf from seedling experimentally infected with MRFV. (Fig. 3 about ×2 magnification of Figs. 1 and 2.)

few dispersed particles were seen on control SSEM grids treated with MCDV antiserum or normal rabbit serum. Removing the parlodion and using KPT negative stain rather than uranyl acetate improved the image of virus particles on SSEM grids.

Centrifugation assays. The rate-zonal centrifugation assay revealed virus that sedimented to about 0.86 of the depth for the MCDV and 0.93 of that for MDMV. This slower sedimenting virus was identified from 10 maize plants with fine-streaked leaves (seven from Texas and three from Florida) and from four Texas maize plants with symptoms similar to those shown in Fig. 2. Immune rate-zonal centrifugation assays of partially purified virus were performed for five of the Texas samples and for the three Florida samples with antiserum to MRFV. Antiserum treatment reduced peak area in centrifuged gradients by 31 to 65% for the Texas samples and by 10 to 43% for the Florida samples. In the controls, antiserum treatment increased peak area for MCDV by 0.14% and for MDMV by 18%. Immune rate-zonal centrifugation assay involving antiserum to MRCV eliminated the virus peak for one Texas isolate maintained in greenhouse-grown maize and for the three Florida isolates. Treatment of virus preparations with antiserum to MCDV (14) had no effect on peak area for the seven Texas samples. In the control, the MCDV peak was reduced in area by 50% with this antiserum treatment.

Vector transmission. Maize rayado fino virus was transmitted from three of six samples from Texas and from one of three from Florida. First symptoms in test seedlings, seen 4–10 days after inoculation with *D. maidis*, were small chlorotic spots at the base of whorl leaves. The spots became more numerous and some eventually fused to form stripes on later developing leaves (Fig. 3). Symptoms were most conspicuous during the first 3–4 wk after inoculation and then became less distinct.

Of 75 single *D. maidis* leafhoppers tested, five transmitted MRFV. The minimum, maximum, and mean latent periods were 10, 23, and 15 days, respectively. A 12.5-day mean latent period at 25 C was reported by Gonzalez and Gamez for MRFV (13). Initially, three of five leafhoppers transmitted virus to nearly all test plants in the serial series before patterns became intermittent, whereas transmission by the remaining two vectors was brief or intermittent throughout the transmission period (Table 1).

G. nigrifrons also transmitted MRFV. Groups of 10 *G. nigrifrons* transmitted virus to four of 45 test plants, whereas groups of 10 *D. maidis* transmitted virus to 31 of 45 test plants.

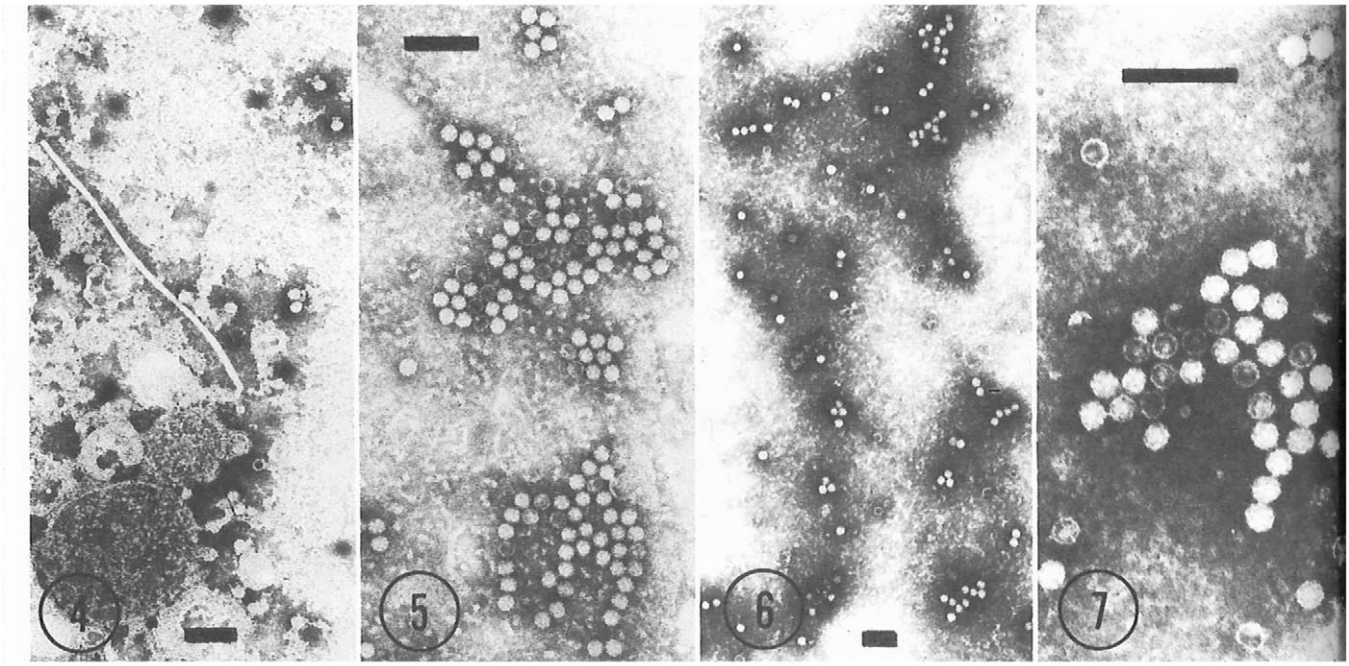
Mechanical transmission. Maize dwarf mosaic virus was transmitted from seven Texas samples that showed fine streaking on leaves. Strain A was found in six samples and strain B in one.

Light microscopy. Spiroplasma was found in four Texas samples with symptoms similar to those shown in Fig. 2.

DISCUSSION

Field symptoms, particle morphology, reactivity with MRFV and MRCV antisera, persistent transmission by *D. maidis*, and symptom development in experimentally inoculated plants indicate that diseased maize samples from Texas and Florida were infected with isolates or strains of the Costa Rican MRFV or the related MRCV. This is the first evidence of MRFV in the United States and the first evidence of MRFV transmission by *G. nigrifrons*.

Leaf symptoms of MRFV disease could be confused with those associated with an unidentified rhabdovirus presumed to be maize mosaic virus and recently discovered in the southern United States (6,7), except the MRFV-infected maize does not show the severe stunting associated with rhabdovirus infections. Leaf symptoms of MCDV (14) are significantly less vivid, except for a severe strain known to occur only in experimental transmissions in the greenhouse (19). Also, isometric particles in negatively stained leaf-dip preparations occur at much greater frequency than for MCDV, are smaller, and show greater internal structure (18). Persistent transmission of MRFV by *D. maidis* is a characteristic shared with CSS and maize bushy stunt mycoplasma (4), but latent periods and disease symptoms associated



Figs. 4–7. Electron micrographs of KPT negatively stained preparations of a Texas isolate of maize rayado fino virus (MRFV) particles: **(4)** Leaf-dip preparation from field-collected leaf with symptoms shown in Fig. 1. Note flexuous rod particle of MDMV in addition to isometric particles of MRFV. **(5)** Leaf-dip preparation from experimentally inoculated maize seedling with symptoms similar to those shown in Fig. 3. **(6 and 7)** Serologically specific electron microscopy preparations of diseased leaves on grids with adsorbed MRFV antiserum. Note isometric MRFV particles showing morphological units of the virion capsid and some particles penetrated with negative stain in Figs. 5 and 7. (Magnification scales about 100 nm long.)

Table 1. Daily transmission pattern of maize rayado fino virus (MRFV) by five *Dalbulus maidis* vectors[a]

Insect number	Days after MRFV acquisition																									
	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	−	+	−	−	−	−	−
2	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	−	+	−	−
3	−	−	−	−	−	−	+	+	−	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
4	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−	+	+	−	−	−	+	+	−	+	+
5	−	−	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+	−	−	+	−	−	−	−	−	−	−

[a]Insects were serially transferred to test plants at daily intervals from 2 to 44 days after acquisition. None transmitted before day 10 or after day 36.

with these mollicutes are distinct from those associated with MRFV (4,18).

Our finding of maize plants doubly infected with MRFV plus MDMV or CSS in addition to the apparent masking of MDMV symptoms by MRFV and of MRFV symptoms by CSS emphasizes the hazard in disease diagnosis and pathogen identification by symptomatology or limited assays. The MDMV-B detected in one sample was not differentiated from sugarcane mosaic virus previously reported by Viallalon (22) in Rio Grande valley sugarcane. Natural coinfection of MRFV plus CSS has been previously reported in Costa Rica (1) and suggests that individual *D. maidis* can transmit both pathogens together in the field as well as in the laboratory (11).

The origin of MRFV for infections in Texas and Florida is unknown. One possibility is that MRFV was introduced into the Rio Grande valley by vectors in flight from Mexico; southerly winds are common and there are no obvious natural barriers. Recurring hurricanes from the Caribbean Islands could assist long vector flights over water and introduction of MRFV into Florida.

Our understanding of what limits maize virus spread is not sufficient to allow us to predict the potential of MRFV to move into more northerly maize production areas. However, experimental transmission of MRFV by *G. nigrifrons* is significant because of the abundant occurrence of this oligophagous leafhopper throughout the eastern half of the United States (21). Relevantly, *G. nigrifrons* experimentally transmits CSS in a persistent manner, and yet this pathogen is not known to cause significant losses in the United States or to occur north of Texas, where it has been known since the early 1940s (5,14,18).

Additional surveys for virus-infected maize, further characterization of MRFV as to host range and vector relationships, and determination of the genetic vulnerability of maize genotypes to MRFV are needed to evaluate the potential importance of MRFV occurrences in the United States.

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LITERATURE CITED

1. ANCALMO, O., and W. C. DAVIS. 1961. Achaparramiento (corn stunt). Plant Dis. Rep. 45:281.
2. AYERS, J. E., J. S. BOYLE, and D. T. GORDON. 1978. The occurrence of maize chlorotic dwarf and maize dwarf mosaic viruses in Pennsylvania in 1977. Plant Dis. Rep. 62:820-821.
3. BRADFUTE, O. E., L. R. NAULT, D. T. GORDON, D. C. ROBERTSON, R. W. TOLER, and C. W. BOOTHROYD. 1977. Maize rayado fino virus in the United States. Proc. Am. Phytopathol. Soc. 4:171. (Abstr.).
4. BRADFUTE, O. E., L. R. NAULT, D. C. ROBERTSON, and R. W. TOLER. 1977. Maize bushy stunt—a disease associated with a nonhelical mycoplasma-like organism. Proc. Am. Phytopathol. Soc. 4:171. (Abstr.).
5. BRADFUTE, O. E., and D. C. ROBERTSON. 1974. Identification of the Mississippi corn stunt agent by electron microscopy. Proc. Am. Phytopathol. Soc. 1:35-36. (Abstr.).
6. BRADFUTE, O. E., D. C. ROBERTSON, and R. W. TOLER. 1974. Possible occurrence of maize mosaic virus in the continental United States. Proc. Am. Phytopathol. Soc. 1:36. (Abstr.).
7. BRADFUTE, O. E., and D. C. ROBERTSON. 1976. Electron microscopy as a means for discovery of new maize viruses and virus-like pathogens. Pages 103-107 *in* L. E. Williams, D. T. Gordon, and L. R. Nault (eds.). Proc. Maize Virus Dis. Colloq. Workshop, 16-19 August. Ohio Agric. Res. Dev. Cent., Wooster. 145 pp.
8. DAVIS, R. E. 1974. Spiroplasma in corn stunt-infected individuals of the vector leafhopper, Dalbulus maidis. Plant Dis. Rep. 58:1109-1112.
9. DERRICK, K. S., and R. H. BOLANSKY. 1976. Assay for viruses and mycoplasmas using serologically specific electron microscopy. Phytopathology 66:815-820.
10. GAMEZ, R. 1969. A new leafhopper-borne virus of corn in Central America. Plant Dis. Rep. 53:929-932.
11. GAMEZ, R. 1973. Transmission of rayado fino virus of maize (Zea mays) by Dalbulus maidis. Ann. Appl. Biol. 73:285-292.
12. GAMEZ, R. 1976. Leafhopper-transmitted maize rayado fino virus in Central America. Pages 15-19 *in* L. E. Williams, D. T. Gordon, and L. R. Nault (eds.). Proc. Maize Virus Dis. Colloq. Workshop, 16-19 August. Ohio Agric. Res. Dev. Cent., Wooster. 145 pp.
13. GONZALEZ, V., and R. GAMEZ. 1974. Algunos factores que afectan la transmision del virus del rayado fino del maiz por Dalbulus maidis DeLong & Wolcott. Turrialba 24:51-57.
14. GORDON, D. T., and L. R. NAULT. 1977. Involvement of maize chlorotic dwarf virus and other agents in stunting diseases of Zea mays in the United States. Phytopathology 67:27-36.
15. KITAJIMA, E. W., T. YANO, and A. S. COSTA. 1976. Purification and intracellular localization of isometric viruslike particles associated with Brazilian corn streak virus infection. Cienc. Cult. (Brazil) 28:427-430.
16. MARTINEZ-LOPEZ, G., L. M. RICO DE CUJIA, and C. SANCHES DE LUQUE. 1974. Una nueva enfermedad del maiz en Colombia transmitida por el saltahojas Dalbulus maidis (DeLong & Wolcott). Fitopatologia 9:93-99.
17. NAULT, L. R., and O. E. BRADFUTE. 1977. Reevaluation of leafhopper vectors of corn stunting pathogens. Proc. Am. Phytopathol. Soc. 4:172. (Abstr.).
18. NAULT, L. R., and O. E. BRADFUTE. 1979. Corn stunt: Involvement of a complex of leafhopper-borne pathogens. Pages 561-586 *in* K. Maramorosh and K. Harris (eds.). Leafhopper Vectors and Plant Disease Agents. Academic Press: New York. 625 pp.
19. NAULT, L. R., D. T. GORDON, D. C. ROBERTSON, and O. E. BRADFUTE. 1976. Host range of maize chlorotic dwarf virus. Plant Dis. Rep. 60:374-377.
20. NAULT, L. R., D. T. GORDON, R. E. GINGERY, O. E. BRADFUTE, and J. CASTILLO L. 1979. Identification of maize viruses and mollicutes and their potential insect vectors from Peru. Phytopathology. (In press.)
21. STONER, W. N., and R. D. GUSTIN. 1967. Biology of Graminella nigrifrons (Homoptera: Cicadellidae), a vector of corn (maize) stunt virus. Ann. Entomol. Soc. Am. 60:496-505.
22. VIALLALON, B. 1977. Identification of sugarcane mosaic virus infecting cane in the lower Rio Grande valley of Texas. Plant Dis. Rep. 61:503-508.