Involvement of Maize Chlorotic Dwarf Virus and Other Agents in Stunting Diseases of Zea mays in the United States

D. T. Gordon and L. R. Nault

Associate Professor, Department of Plant Pathology, and Professor, Department of Entomology, respectively, Ohio Agricultural Research and Development Center, Wooster, OH 44691.

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ABSTRACT

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Maize chlorotic dwarf virus (MCDV) was identified in 76% of 522 diseased corn *(Zea mays)* samples from 16 of 23 states during 1973-1975. The region of MCDV occurrence was from the Gulf of Mexico northward to southern Ohio, Indiana, Illinois, and Missouri and from southern Atlantic Coast states to Texas. Maize dwarf mosaic virus strain A (MDMV-A) was identified in 35% of 520 diseased samples from 16 of 21 states, MDMV strain B in 4% of diseased samples, and wheat streak mosaic virus in two diseased samples. Two (both from Texas) of 168 tested samples from 15 states were infected with a *Dalbulus maidis*-transmitted corn stunt spiroplasma. In immune density-gradient centrifugation, MCDV reacted with homologous antiserum but not with antiserum to MDMV-A. Maize chlorotic dwarf virus infections were correlated with plant stunting and leaf chlorosis or reddening, symptoms that frequently are attributed to corn stunt and maize dwarf mosaic diseases in the USA. Only chlorotic striping of tertiary veins was diagnostic for MCDV infections. This investigation implicates MCDV as the most frequent incitant of the major corn stunting disease in southern and adjacent corn belt states of the USA.

Additional key word: maize.

Corn stunt and maize dwarf mosaic have been reported to be important diseases of corn (*Zea mays* L.) in southern and adjacent corn belt states of the USA since the early to mid-1960's (20, 21, 50). (The "southern states" as used in this paper extend from Texas eastward to the Atlantic coast and from Maryland, Virginia, Kentucky, southern Missouri, and Oklahoma southward to the Gulf of Mexico.) The incitant of corn stunt is transmitted by leafhopper species, *Dalbulus maidis* (DeLong & Wolcott) (36) and *Graminella nigrifrons* (Forbes) (24), and it has a persistent relationship with these vectors. Corn stunt has been associated with mycoplasmalike bodies (MLB) in the USA (8, 23) and with spiroplasma in Mexico (15). The former association was made by electron microscopy of ultrathin tissue sections and the latter by phase-contrast microscopy of expressed juice from diseased plants. The differences in the techniques for visualizing the agents may account for apparent differences in morphology. Maize dwarf mosaic virus (MDMV), which incites maize dwarf mosaic (2, 33), is an aphid- and mechanically transmissible virus belonging to the potyvirus group (44). Strain A of MDMV (MDMV-A) infects Johnsongrass

[*Sorghum halepense* (L.) Pers.], whereas strain B (MDMV-B) does not (60).

Another virus disease of corn, maize chlorotic dwarf, was discovered in the USA in the early 1970's (6, 7, 45). The maize chlorotic dwarf virus (MCDV) is transmitted by *G. nigrifrons* and has a semipersistent relationship with this vector (43). The virus has a small isometric particle with a relatively fast sedimentation rate relative to other isometric plant viruses (6). Maize chlorotic dwarf virus has been reported to occur in corn in several southern states (6, 19, 35, 45), Illinois (40), and Ohio (37), but information on its relative importance in the USA has not been published.

Other viruses identified from naturally infected corn in the continental USA during recent years include sugarcane mosaic virus (50), wheat streak mosaic virus (WSMV) (29, 41, 63), brome mosaic virus (41), and a rhabdovirus that resembles both the maize mosaic (9) and wheat striate mosaic viruses (65). None of these viruses is known to be economically important over extensive corn-growing areas in the continental USA.

This paper reports on an immune density-gradient centrifugation assay for MCDV and on the distribution and incidence of viruses and other stunting agents of corn in the USA. Infection of corn by these pathogens is related to various symptoms. Preliminary accounts have been published (20, 21, 35, 40).

MATERIALS AND METHODS

Sample collection and symptom rating.—Corn leaf samples were field-collected and promptly shipped to Wooster. Upon receipt, samples immediately were stored at 1-2 C until assayed. Maize chlorotic dwarf virus assays were performed within 10.6 days [σ(sigma) = 6.4 days] after sample collection, and mechanical inoculations within 8.4 days (σ = 5.7 days). Symptoms usually were rated by the collector, and frequently, leaf symptoms were rated by us. Ratings included information on the presence of mosaic, chlorotic banding of secondary veins, chlorotic striping of tertiary veins, chlorosis, reddening, leaf tearing, twisting or rolling of whorl leaves, plant tillering, shoot proliferation, ears and their condition, tassels and their condition, necrosis on leaves, and stunting of plants. Of 582 samples assayed, 525 had symptoms and 57 had no symptoms. The symptomless samples were collected for inclusion in the chi-square (χ^2) test for significance of association between pathogen and symptoms.

Leafhopper rearing.—*Graminella nigrifrons* were reared in organdy-covered aluminum frame cages 19 × 38 × 38 cm. A colony was started by infesting barley *(Hordeum vulgare* L.) plants in four 10-cm diameter pots with 200-250 adults for 3-4 days. Adults were removed after they had laid 500-600 eggs on barley plants in the four pots. This procedure provided leafhoppers of nearly uniform age. The temperature within the rearing room was 22-27 C and the relative humidity 40-60%. Corn stunt spiroplasma (CSS) and MCDV do not infect barley (42, 56). *Dalbulus maidis* was reared on corn by a similar procedure.

Leafhopper transmission assay for maize chlorotic dwarf virus.—Twenty-five adult *G. nigrifrons* were first given a 24-hour acquisition access period (AAP) on a 10- to 15-cm long corn leaf sample in a petri dish. Surviving leafhoppers then were caged on two inbred Oh28 or Oh43 corn seedlings for a 24-hour inoculation access period (IAP). Seedlings then were fumigated with dichlorvos and placed in a greenhouse. Final ratings for MCDV symptoms (chlorotic striping of tertiary veins) were made after 14 days.

Leafhopper transmission assays for corn stunt spiroplasma.—Twenty-five to 50 late-instar and adult *G. nigrifrons* and *D. maidis,* respectively, were given a 24-hour AAP on 10- to 15-cm long corn leaf samples in separate petri dishes according to leafhopper species. *Graminella nigrifrons* then was caged on barley and *D. maidis* on corn for a 21-day incubation period. Surviving leafhoppers were caged on two seedlings of Oh28 corn or Aristogold Bantam Evergreen sweet corn for a 7-day IAP. Following the IAP, test seedlings were fumigated with dichlorvos and placed in a greenhouse for a minimum of 5 weeks before final symptom ratings were made. Diagnostic symptoms were prominent chlorotic spots and stripes on leaves. This assay method gave excellent transmission of the Rio Grande and the Mississippi corn stunt agent strains of CSS (Nault, *unpublished*).

Immune density-gradient centrifugation assay.—Virus from corn leaf samples collected in 1973 was concentrated and partially purified as described previously (37). In 1974, samples were extracted by grinding in 0.1 M potassium phosphate buffer, pH 7.0, containing 0.5% 2-mercaptoethanol (6 g tissue and approximately 10 ml of buffer). Extracts were clarified with $CHCl_3$ (one-half volume). Virus was precipitated from the clarified extract by incubation for 1 hour at 3-4 C with 6% polyethylene glycol (mol. wt. 6,000) plus 4% KCl. Precipitated material was collected by centrifugation at 10,000 rpm for 10 min in a Beckman JA-20 rotor with the J-21 centrifuge (Beckman Instruments, Inc., Palo Alto, California). Both in 1973 and 1974, material in pellets was suspended in 0.5 M potassium phosphate buffer, pH 7.0 (0.44 - 0.5 ml/5 g of leaf tissue). A 0.2-ml aliquot of the suspension and 0.7 ml of buffer were layered successively onto a linear sucrose density gradient in a 1.3 × 5.1 cm cellulose nitrate tube. A second 0.2-ml aliquot was mixed with 0.2 ml of MCDV antiserum diluted 1/100 with physiologically buffered saline (PBS: 0.85% NaCl + 0.01 M phosphate, pH 7.0). The mixture was layered promptly onto another density gradient and 0.7 ml of buffer layered over this mixture. Gradients were prepared in tubes by successively layering 1.0, 1.3, 1.3, and 0.65 ml of 35, 25, 15, and 5% sucrose in 0.5 M potassium phosphate, pH 7.0 (w/w), respectively. Layers were allowed to diffuse overnight or longer at 2-4 C. Gradients were allowed to warm to room temperature (approximately 20 C) before preparations were layered. Centrifugation was in a Beckman SW 50.1 rotor at 45,000 rpm and 20 C for 5.36×10^{10} rad^2/second (approximately 40 minutes). Centrifuged gradients were scanned photometrically at 254 nm with an ISCO Model 640 Density Gradient Fractionator and a Model UA-2 Ultraviolet Analyzer (Instrumentation Specialties Co., Lincoln, Nebraska). The assay was modified from the procedure of Ball and Brakke (1).

Antiserum production.—Rabbits were immunized with purified MCDV (22) (0.42 - 6.0 A_{260} units per injection) suspended in 0.1 M phosphate, pH 7.0, or in PBS. Two initial intravenous injections (1.0 - 1.5 ml) were

followed by seven intramuscular injections (2.0 ml) given over a 6-month period. For intramuscular injections, virus suspensions were emulsified with an equal volume of Freund's adjuvant. Serum was diluted twofold with glycerol and stored at −10 to −20 C.

Assays for mechanically transmissible viruses.—Inoculum for mechanical transmission was prepared by grinding approximately 1 g of leaf tissue in 9 ml of 0.01 M potassium phosphate buffer, pH 7.0, in a mortar with a pestle. Leaves of seedling (two- to four-leaf stage) Oh28 corn (three or four plants), Johnsongrass (12 plants), and Monon or Michigan Amber wheat (*Triticum aestivum* L.) (eight or nine plants) were rubbed with cotton soaked with inoculum which contained 0.22 μm (600-mesh) Carborundum. An additional set of assay hosts (controls) was rubbed with buffer containing Carborundum. Assay plants grew in a steam-sterilized mixture of muck, Wooster silt loam, and peat (5:5:1) in 10-cm diameter plastic pots. Plants were kept in a

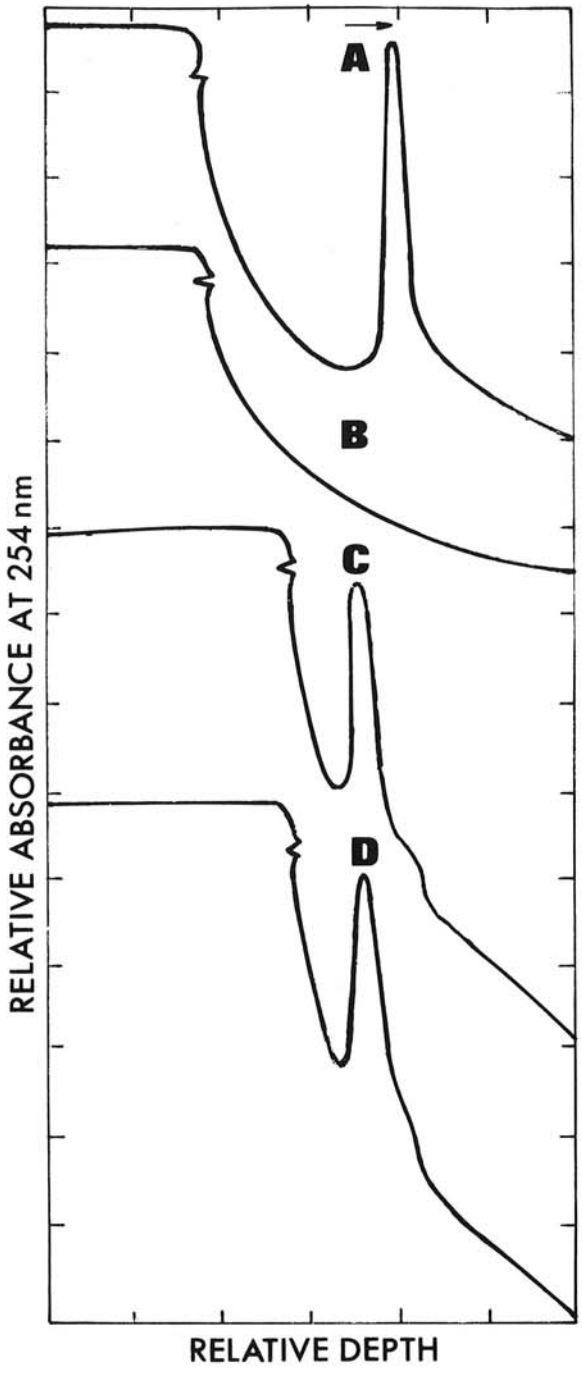

TABLE 1. Percentage of area under virus peaks from ultraviolet absorbance profiles of centrifuged sucrose density gradients containing maize chlorotic dwarf virus (MCDV) or maize dwarf mosaic virus strain A (MDMV-A) mixed with different sera at various dilutions

Serum	Reciprocal of serum dilution	Peak area[a] (%) MCDV	MDMV-A
Anti-MCDV	10	0	106
	100	0	104
	1,000	4	108
	10,000	25	110
	100,000	36	112
Anti-MDMV-A	10	120	2
	100	112	20
	1,000	104	54
	10,000	105	67
	100,000		89
Normal	10	120	96
	100	99	115
	1,000	113	115
	10,000	109	105
	100,000	122	
SE[b]		7.92	6.04
N[c]		79	88

[a]Percent peak areas are averages from three to 12 determinations and were calculated with the PBS (0.85% NaCl + 0.01 M phosphate, pH 7.0) control serving as the 100% reference. Peak areas were determined by an area integration method.

[b]The letters SE refer to the standard error determined from percent values used to calculate the averages which appear in the column above the SE value. The SE was determined by a least-squares analysis of variance.

[c]The letter N refers to the number of observations in the analysis of variance.

Fig. 1. Ultraviolet absorbance profiles (A to D) of centrifuged sucrose density gradients containing: (A) maize chlorotic dwarf virus (MCDV); (B) MCDV mixed prior to centrifugation with an equal volume of anti-MCDV serum diluted 1:100; (C) maize dwarf mosaic virus strain A (MDMV-A); and (D) MDMV-A mixed prior to centrifugation with anti-MCDV serum diluted 1:100. The MCDV and MDMV-A were partially purified, concentrated 10- and 20-fold, respectively, and suspended in 0.5 M phosphate, pH 7.0. Virus suspensions (0.2 ml) and virus-antiserum mixtures (0.4 ml) were layered on 5-35% sucrose density gradients in 0.5 M phosphate, pH 7.0. Centrifugation was in the Beckman SW 50.1 rotor at 20 C and 45,000 rpm for about 45 minutes. Gradients were scanned at 254 nm with an ISCO ultraviolet analyzer. Arrow indicates direction of sedimentation.

greenhouse and each week were fertilized and sprayed with insecticide. Ratings for infection were made 10-30 days after inoculation.

Maize dwarf mosaic virus purification.—For the reciprocal serological tests in the immune density-gradient centrifugation assay, MDMV-A was partially purified from Oh28 corn or sorghum [*S. bicolor* (L.) Moench.] cultivar Sart. Leaves with symptoms were harvested 21-45 days after inoculation of seedling plants and were extracted in 0.5 M phosphate buffer, pH 7.0, containing either 0.1% or 0.5% thioglycollic acid (1 g tissue/1 ml buffer) with a Waring Blendor at room temperature. The extract was clarified and the virus concentrated and suspended as described for MCDV, except in several cases 1.0 M urea + 0.025 M phosphate, pH 7.4, was the suspension buffer. Antiserum to MDMV-A was produced as described previously (66).

TABLE 2. Occurrence of maize chlorotic dwarf virus (MCDV) and maize dwarf mosaic virus (MDMV) in samples from diseased[a] (525 samples) and symptomless (57 samples) *Zea mays* (L.) plants in the USA, 1973-1974

State	Number of samples infected with MCDV		
	Immune density gradient centrifugation assay[b]	Leafhopper assay[c]	Infected with MDMV[d]
Alabama	17/19[e]	7/8	5/19
Arkansas[f]	7/10	6/10	9/10
California[f]	0/3		2/3
Georgia	54/58	4/16	8/56
Illinois[f]	2/2		
Indiana	27/35	1/1	13/35
Iowa[f]	0/2		0/2
Kansas[f]	0/1	0/1	1/1
Kentucky[f]	6/10		3/10
Louisiana	10/12	2/6	1/12
Mississippi	44/51	8/21	14/51
Missouri	58/78	4/4	29/78
New Mexico	0/2	0/2	0/2
New York	0/15		11/15
North Carolina	4/10	4/5	2/10
Ohio	67/102	9/13	35/101
Oklahoma[f]	0/7	0/6	7/7
South Carolina[f]	7/7	5/7	2/7
South Dakota	0/26	0/8	0/26
Tennessee	46/61	14/20	35/61
Texas	40/56	3/8	29/52
Virginia	9/12	6/9	3/15

[a]Plants with symptoms of mosaic, chlorotic striping of tertiary veins, chlorosis or reddening on leaves, and/or stunting.

[b]Assays were performed in sucrose density gradients with concentrated leaf extract preparations treated or untreated with anti-MCDV serum. Maize chlorotic dwarf virus was identified by the presence of virus in centrifuged gradients containing preparations without antiserum and its absence in gradients with antiserum treatments.

[c]Assays were performed with *Graminella nigrifrons* for semipersistently transmitted virus.

[d]Maize dwarf mosaic virus (MDMV) strains and percentage (in parentheses) of strain recovery from infected samples were: MDMV strain A (MDMV-A) (86%) and MDMV strain B (MDMV-B) (9%). The remaining MDMV isolates were not sufficiently tested to determine whether they were MDMV-A or MDMV-B.

[e]The numerator of ratios refers to the number of samples which tested positive for virus and the denominator to the number of samples assayed.

[f]Assays for virus were made only on samples received in 1973.

RESULTS

Immune density-gradient centrifugation assay.—In the centrifugation assay, MCDV banded about 1.10 times farther down centrifuged gradients than MDMV-A (Fig. 1). Treatment with a 1:100 dilution of antiserum to MCDV eliminated the MCDV-absorbance peak but had no effect on the size of the MDMV-A peak. Although treatment with greater dilutions of MCDV antiserum did not eliminate the MCDV peak, even a 1:100,000 dilution significantly reduced peak size (Table 1). None of the MCDV-antiserum dilutions significantly affected the size of the MDMV-A peaks. In reciprocal tests, treatment with MDMV-A antiserum dilutions to 1:10,000 significantly reduced MDMV-A peak size, but had no effect on the size of MCDV peaks. Normal serum had no effect on peak size of either virus. Thus, the immune-centrifugation assay with MCDV antiserum was specific for MCDV.

The virus-antiserum mixtures used in the above tests were layered onto gradients without the incubation recommended by Ball and Brakke (1). A test of the effect of incubation showed that the peak area for a 1-hour-incubated MCDV and MCDV-antiserum mixture was 41% ($\sigma = 7\%$, $N = 6$) less than the PBS control, whereas the area for a mixture incubated 48 hours was 43% ($\sigma = 3\%$, $N = 6$) less than the control (data not in Table 1). In this test the antiserum was diluted 1:500. Thus, incubation of the virus and antiserum mixture had no effect on the amount of MCDV in the centrifuged gradient.

In assays of preparations from field-collected samples, the MCDV absorbance peaks appeared after about 3.5 ml had been displaced from centrifuged gradient tubes. These peaks were removed or appreciably reduced in size by treatment with MCDV antiserum diluted 1:100.

Occurrence of maize chlorotic dwarf virus and mechanically transmissible viruses.—Maize chlorotic dwarf virus was identified by the immune density-gradient centrifugation assay in diseased corn samples from 15 of 22 states during 1973 and 1974 (Table 2). Infectivity assays employing *G. nigrifrons* identified MCDV in samples from 13 of these 15 states in 1973. Maize chlorotic dwarf virus was identified from about 76% of diseased samples by the immune-centrifugation assay. In assays performed in 1975 (results not in Table 2), MCDV was identified from four corn samples from Maryland. Thus, MCDV was recovered from diseased corn from a total of 16 states.

Mechanically transmissible virus isolates were recovered from samples from 19 of 21 states and were identified by host range and symptomatology. Isolates that produced a systemic mosaic on corn and Johnsongrass were identified as MDMV-A, those that produced a systemic mosaic only on corn as MDMV-B (60), and those that produced a systemic mosaic only on corn and wheat as WSMV (63). Among isolates identified as MDMV-A, 72% infected corn and Johnsongrass and the remainder only corn on the first assay. The identification

Fig. 2-(A to D). Symptoms on plants and leaf of corn (*Zea mays* L.) infected with maize chlorotic dwarf virus. **A)** Severely shortened upper internodes and reddened and yellowed upper leaves; **B)** Proportionately shortened internodes and reduced leaf size; **C)** Elongation reduced more in upper than lower internodes and upper leaves yellowed; **D)** (Upper) Infected greenhouse-grown inbred Oh43 leaf showing fine chlorotic striping of tertiary veins; (Lower) Healthy Oh43 leaf. Numbered cards on plants in photographs A and B were for identification.

of the latter as isolates of MDMV-A was made in subsequent assays in which the originally infected test corn was used as the virus source and corn and Johnsongrass as assay hosts. A similar difficulty in identifying Johnsongrass-infecting isolates from diseased field corn has been reported (59). Isolates identified as MDMV-B were assayed a second time to verify results; the originally infected test corn served as the virus source. Fifty-four percent of these MDMV-B isolates were identified as MDMV-A on reassay. Thus, initial host-range assays of diseased corn from the field did not always correctly identify isolates. This inadequacy may have been related to the titer of virus, relative sensitivity of assay hosts, presence of infection inhibitors, or combinations of these. Among the mechanically transmissible isolates, 86% were identified as MDMV-A, 9% as MDMV-B, and 1% as WSMV. The remaining 4% were not tested sufficiently to determine whether they would systemically infect Johnsongrass.

Among the buffer-rubbed control plants, one of 200 corn plants developed mosaic symptoms. None of 805 Johnsongrass controls nor 556 wheat controls showed symptoms.

Maize dwarf mosaic virus strain A was identified in samples from Alabama, Arkansas, California, Georgia, Indiana, Kentucky, Louisiana, Mississippi, Missouri, New York, North Carolina, Ohio, Oklahoma, Tennessee, Texas, and Virginia. Maize dwarf mosaic virus strain B was identified in samples from Arkansas, Kansas, Mississippi, Missouri, New York, Ohio, Oklahoma, and Tennessee. Wheat streak mosaic virus was recovered from samples from Oklahoma and South Dakota.

Association of maize chlorotic dwarf virus with occurrence of corn-stuntlike symptoms.—To determine the relationship of MCDV, MDMV, and CSS to symptoms, leaves of diseased plants were collected by us on field trips and assayed for the three agents. Samples were collected in Louisiana, Mississippi, Texas, and Virginia. Plants with "corn-stuntlike" symptoms (Fig. 2); i.e., reddening or yellowing of leaves plus stunting of the plant, were selected by cooperators who were authorities (see acknowledgements) on corn stunt in the southern USA. Of the 33 samples with corn-stuntlike symptoms, 32 contained MCDV as determined by the immune-centrifugation assay. Results were inconclusive for the remaining sample. The two leafhopper species were used in tests of 23 of these samples and no persistently transmitted agent was detected. Three additional samples collected on this trip did not have corn-stuntlike symptoms, but did contain MCDV. Two of these showed a fine chlorotic striping of tertiary veins (Fig. 2) and the third showed a mosaic: the latter also was infected with MDMV-A. Maize chlorotic dwarf virus was not detected in a fourth sample without corn-stuntlike symptoms. Maize dwarf mosaic virus was recovered from nine of the 33 samples with corn-stuntlike symptoms, and in all cases these samples were also infected with MCDV.

Based on these results, MCDV appeared to be the principal pathogen involved in the corn-stuntlike disease. However, its presence in diseased plants without corn-stuntlike symptoms prompted an analysis of data from 575 samples assayed during the study in terms of the relationship between MCDV and MDMV and the principal symptoms (Table 3). This analysis indicates the degree of confidence possible in using symptoms to diagnose MCDV and MDMV infections.

Statistically significant interactions existed between MCDV and chlorotic striping of tertiary veins, chlorosis and reddening of leaves, and stunting of plants. In the relationship of tertiary-vein chlorotic striping to MCDV, 88% of samples with the symptom were infected with MCDV, whereas 65% without vein striping were not. For samples without vein striping but infected with MCDV, 83% had mosaic, chlorosis, or reddening (data not in Table 3) that may have obscured the vein striping. Thus, chlorotic vein striping was a good indicator of MCDV infection. In the relationship of chlorosis and reddening to MCDV, 84% and 86% of samples with yellow-chlorotic

TABLE 3. Association of maize chlorotic dwarf virus (MCDV) and maize dwarf mosaic virus (MDMV) infection with various symptoms on 575 field-collected leaf samples from *Zea mays* (L.) plants in 21 states[a]

			Symptom[c]									
			Mosaic		Chlorotic striping of tertiary veins		Leaf yellow-chlorosis		Leaf reddening		Plant stunting	
Virus		Statistical[b] parameter	−	+	−	+	−	+	−	+	−	+
MCDV[d]	−[e]		106[f]	70	127	44	132	43	145	32	65	74
	+		226	158	67	311	167	224	193	205	48	324
		X^2	NS		***		***		***		***	
MDMV[g]	−		280	70	116	227	184	172	211	150	77	248
	+		50	152	72	124	111	89	124	80	37	141
		X^2	***		NS		NS		NS		NS	

[a]States and numbers of samples are listed in Table 2. The California sample, one wheat streak mosaic virus infected South Dakota sample and three MDMV-infected Virginia samples were excluded.

[b]Statistical parameter: X^2 = chi square test for significance. Level of significance: NS = no significance; *** = significant at $P = 0.001$.

[c]The symbols − = absence of symptoms and + = presence of symptoms.

[d]Maize chlorotic dwarf virus was detected by immune density-gradient centrifugation.

[e]The symbols − = absence of virus and + = presence of virus.

[f]Number of samples within a category.

[g]Maize dwarf mosaic virus strains and percent of recovery from infected samples are listed in footnote "d" of Table 2.

and reddened leaves, respectively, were infected with MCDV. But, only 44% and 43% of samples without chlorosis and reddening, respectively, were not infected with MCDV. Thus, these symptoms were good indicators for MCDV infection, but their absence was not related to the absence of MCDV. For samples from stunted plants, most (81%) were infected with MCDV, whereas 58% of the nonstunted plants were not. Only chlorotic striping of tertiary veins appeared diagnostic for MCDV infections.

Of the samples evaluated for MCDV and symptom association, 290 came from plants with corn-stuntlike symptoms. Of these, 91% were infected with MCDV. Of 242 samples from plants without corn-stuntlike symptoms, 48% were infected with MCDV. Many of these, however, showed either stunting or leaf discoloration.

A statistically significant interaction existed between MDMV and mosaic, but not with the remaining symptoms. None of the other symptoms included in the rating information occurred on more than 10% of disease samples, and these symptoms were not correlated with the presence of the two viruses.

Occurrence of corn stunt spiroplasma.—Results from samples collected on field trips discussed above indicated the absence of *D. maidis* or *G. nigrifrons* persistently transmitted agents in corn with corn-stuntlike symptoms in the southern USA. Additional samples shipped by cooperators in 1973 and 1974 also were tested with the two leafhopper species. States from which samples were obtained on field trips or shipped by cooperators and the number tested (in parentheses) were: Alabama (8), Arkansas (10), Georgia (1), Indiana (5), Kansas (1), Lousiana (9), Mississippi (32), Missouri (4), New Mexico (2), North Carolina (6), Ohio (15), South Carolina (9), Tennessee (20), Texas (34), and Virginia (12). Corn stunt spiroplasma was recovered from only two samples, and these were from Texas in 1973. Recoveries were made with *D. maidis,* but not with *G. nigrifrons*. Spiroplasma were observed by dark-phase light microscopy in sap from test corn plants with symptoms produced by each isolate. Mycoplasmalike bodies were observed by electron microscopy in ultrathin tissue sections from the original field plants (8). Symptoms in greenhouse test plants were indistinguishable from those incited by the Rio Grande strain of CSS. Corn stunt spiroplasma was not transmitted by leafhoppers from one Texas and one Louisiana sample in which MLB were observed by electron microscopy (8). None of 500 *D. maidis* or 500 *G. nigrifrons* taken from stock cultures transmitted CSS to control corn plants during this study.

DISCUSSION

Occurrences of corn stunt in the USA have been reported from southern and southwestern states (10, 12, 13, 16, 17, 24, 27, 28, 32, 34, 36, 38, 39, 46, 50, 57, 64), several states bordering on the South (11, 31, 49), and California (36). Some initial reports of the occurrence of maize dwarf mosaic and MDMV indicated a similar disease in some of these same states and in several additional ones within this area (13, 30, 48, 59, 61, 62). The distribution of MCDV generally agrees with that of the diseases described in the above reports; exceptions are Florida, Arizona, and California. However, in this study, samples were not received from the first two states. In a 1973 survey of diseased field- and sweet corn in California, no plants with maize chlorotic dwarf or corn stunt symptoms were observed (R. Louie, *personal communication*). Tests of corn for a *D. maidis*-transmissible corn stunt virus in the area of California where the corn stunt disease was originally observed (36) were negative (18). Thus, there is reason to doubt that corn stunt or maize chlorotic dwarf occur in California.

The region of maize chlorotic dwarf occurrence generally is defined by the overlapping areas of frequent occurrence of Johnsongrass, [an overwintering host for MCDV (6, 40, 42, 43, 45)], and of *G. nigrifrons* (58). It generally is accepted that Johnsongrass occurs frequently in river bottom areas as far north as about the 40° N latitude. *Graminella nigrifrons* has been reported in most states east of 102° W longitude. Maize chlorotic dwarf virus was not detected in plants in states outside this area. Maize chlorotic dwarf virus also was not detected in samples from Kansas and Oklahoma; however, Johnsongrass (54, 64) and *G. nigrifrons* (58) are present in at least some areas of these two states, and thus MCDV occasionally might be found. For all states outside of the area of corn-stuntlike disease occurrence, the number of samples assayed was small, and MCDV occurrences could have been missed. However, the apparent absence of MCDV in these states appears to be correlated with the absence of the corn-stuntlike disease.

Occurrences of MDMV-A in Alabama (26), Arkansas (14), California (66), Georgia (34), Indiana (2), Kentucky (66), Louisiana (51), Mississippi (66), Missouri (53), New York (4), North Carolina (28), Ohio (33), Oklahoma (64), Tennessee (30), Texas (59), and Virginia (66) were confirmed in this study. The presence of MDMV-B in corn in Arkansas, Kansas, Mississippi, Missouri, Oklahoma, and Tennessee; and of WSMV in Oklahoma, to our knowledge, has not been reported previously. Maize dwarf mosaic virus strain B was identified previously from corn in New York (4) and Ohio (33). Wheat streak mosaic virus infection of South Dakota corn has been reported (41).

The type of plant stunting and discoloration of leaf blades associated with MCDV infections in this study closely corresponds to symptoms associated with field occurrences of the corn-stuntlike diseases mentioned in early reports (3, 10, 11, 13, 16, 17, 24, 25, 27, 28, 30, 31, 32, 34, 46, 49, 52, 56, 61, 62). Some of these same symptoms previously have been associated with MCDV infections (6, 8, 35, 37, 45). Although yellowing and reddening of leaves were associated with MCDV infection in the present study, it appears that certain factors are necessary for their expressions. To illustrate, a few corn lines (e. g., MP490 × T232) that are considered to be resistant to the corn-stuntlike disease in the southern USA, were susceptible to MCDV infection but developed no reddening or yellowing, although prominent chlorotic striping of tertiary veins was present. Maize chlorotic dwarf virus was detected readily in samples from these plants by the centrifugation assay. A similar observation was reported recently for corn line F23413 in tests in southern Ohio (37). Thus, corn line seemed to be a factor in the expression of leaf reddening or yellowing

associated with MCDV infections.

An earlier study indicated vein banding to be diagnostic for MCDV infections (37). The description of vein banding included a fine chlorotic striping over the smaller veins. Observations in the present study indicated that the fine chlorotic striping of tertiary veins alone (Fig. 2) also is diagnostic for MCDV infections.

The frequent association of MCDV with corn-stuntlike symptoms suggests that in most cases this disease is maize chlorotic dwarf and not corn stunt. Furthermore, the absence of agents persistently transmitted by *D. maidis* or *G. nigrifrons* in all but two of 168 assayed samples suggests that the stunting disease is not caused by CSS. Recent studies have indicated that the corn-stuntlike disease in Georgia (35), Virginia (19), and southern Ohio (37) involved MCDV, but these studies did not include tests for agents persistently transmitted by *D. maidis* or *G. nigrifrons*. Previous reports of corn stunt in the USA have based identifications on symptomatology or transmission by *D. maidis* or *G. nigrifrons* (3, 5, 10, 11, 12, 16, 17, 24, 28, 32, 34, 36, 38, 39, 46, 47, 49, 55, 64). Attempts by others to identify CSS or MLB in corn-stuntlike diseased corn plants from several southern states and Ohio have been unsuccessful (7, 45) except for several plants in Louisiana and Texas (8). In contrast, CSS invariably was associated with corn stunt occurrences in Mexico (15). Since symptoms used to identify corn stunt in earlier studies in the USA do not differentiate corn stunt from maize chlorotic dwarf, these identifications are to be questioned. Likewise, transmission of corn stunting pathogens by *G. nigrifrons* does not differentiate CSS from MCDV, unless persistence of the pathogen in the vector is determined. Thus, the only evidence in previous reports that a stunting pathogen other than MCDV was involved in a corn-stuntlike disease in the USA was the transmission of stunting agents by *D. maidis*. Since the present study confirmed the existence of such an agent only in Texas, the CSS appears involved only to a minor extent in the stunting disease. Consequently, maize chlorotic dwarf appears to be the principal stunting disease of corn in southern and adjacent corn belt states of the USA.

Maize chlorotic dwarf probably has been in this region since the early 1960's when the corn-stuntlike disease first became important. Early accounts of disease symptoms generally match symptom descriptions recently reported. Also, authors familiar with the disease since the early to mid-1960's have not indicated a substantial change in symptomatology in the past decade (35, 45, 50).

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