Epiphytotics of Maize Dwarf Mosaic and Maize Chlorotic Dwarf Diseases in Ohio

Raymond Louie, J. K. Knoke, and D. T. Gordon

Research Plant Pathologist, Department of Plant Pathology; Research Entomologist, Department of Entomology, North Central Region, ARS, USDA; and Associate Professor, Department of Plant Pathology, respectively; Ohio Agricultural Research and Development Center, Wooster 44691.

The authors express appreciation to R. J. Anderson, S. Keller, J. Yamazaki, and N. Yamazaki for their technical assistance.

Published with approval of the Director of the Ohio Agricultural Research and Development Center as Journal Article No. 143-73. Based on cooperative investigations of the Agricultural Research Service, U.S. Department of Agriculture, and the Ohio Agricultural Research and Development Center, Wooster, Ohio 44691.

Accepted for publication 11 June 1974.

ABSTRACT

Maize dwarf mosaic (MDM) and maize chlorotic dwarf (MCD) epiphytotics were studied in southern Ohio, where these diseases occur naturally. Successively-planted field plots and 14-day-old corn plants (trap plants), exposed to field conditions for weekly intervals throughout the season, were used to monitor the development of the epiphytotics. Diagnostic symptoms of MDM included mosaic, fleck, and ring patterns; a veinbanding symptom was diagnostic for MCD. Delayed development of MDM and MCD epiphytotics was associated with early field plantings. Both MDM and MCD epiphytotics began in early June. The incidence of MDM subsequently increased and remained high throughout the season. MCD incidence increased to a peak in mid-July, then sharply declined. The potential occurrence of each disease was measured more accurately with trap plants than with successive field plantings.

Phytopathology 64:1455-1459

Additional key words: *Zea mays* L.

Maize dwarf mosaic virus (MDMV) and maize chlorotic dwarf virus (MCDV) have been identified in diseased corn (*Zea mays* L.) from southern Ohio (1, 9). Symptoms of mosaics, chlorotic flecks and rings, and various mottle patterns on leaves (4) and a slight stunting were observed on plants infected with various strains of MDMV (R. Louie and J. K. Knoke, *unpublished*). In preliminary studies, MCDV was found in corn plants with stunt-like symptoms from southern Ohio and five southern states (1). In greenhouse tests, MCDV induced symptoms (5) similar to those reported for the Ohio corn stunt agent (CSA-OH) (7). Field symptoms of corn plants naturally infected with MCDV have been only partially described (2).

This paper reports on symptoms diagnostic of MCDV infections in field corn and two methods to estimate the disease potential [sensu Tammen (8)] of MDM and MCD.

MATERIALS AND METHODS.—Epiphytotics of MDM and MCD were studied in field plots near Portsmouth, Ohio. Five corn hybrids (Funk 23413, Pioneer Brand 309B, Pioneer Brand 3188, Pioneer Brand 3179, and WF9×Oh51A) were planted at ca. 2-wk intervals, from 25 May to 19 July. The 21 June planting was lost because of flooding of the plot area. Each plot [5.1 m × 20.5 m (16.6 ft × 100 ft)] was a 5 × 5 latin-square design. Twenty-five seeds of each hybrid were planted in a 6.1 m (20-ft) row for each replicate.

The incidences of MDM and MCD in field plots were determined by observations of disease symptoms, beginning 2 wk after planting and then weekly until crop maturity or 18 September. Incidence of MDM was determined by the percentage of plants with characteristic chlorotic patterns. Throughout the season, random leaf samples were bioassayed for MDMV to test the accuracy of the visual observations. The methodology for the bioassay has been previously described (3).

To determine diagnostic symptoms for MCD, field corn-leaf samples with various symptoms were assayed for MCDV by rate-zonal centrifugation. Leaf samples were collected weekly over a 9-wk period and stored frozen until assayed. Thawed leaf samples were ground in 0.1 M potassium phosphate, pH 7.0 (1 g tissue: 1 ml buffer) and the extracts clarified by chloroform treatment (one-half volume). Clarified extracts were centrifuged at 40,000 rpm for 60 min in the Beckman Type 50 Ti rotor (Beckman Instruments, Inc., Palo Alto, California). Pellets were suspended in extraction buffer (ca. one-tenth volume) and layered onto sucrose density-gradient columns for assay. Gradients were prepared by successively layering solutions of 400, 300, 200, and 100 mg sucrose per ml 0.1 M phosphate, pH 7.0, in cellulose nitrate tubes. The volumes used were: 2.0, 3.6, 3.6, and 1.8 ml, respectively, for the Beckman SW-41 Ti rotor; 1.0, 1.3, 1.3, and 0.65 ml for the SW-50.1 rotor; and 0.9, 1.0, 1.0, and 0.5 ml for the SW-56 Ti rotor. Gradient columns were allowed to diffuse for at least 18 h at 3-4 C and then warmed to room temp before use. Gradient columns were centrifuged at 20 C for c. 60 min at 40,000 rpm, c. 40 min at 45,000 rpm, or c. 30 min at 50,000 rpm for the SW-41 Ti, SW-50.1, or SW-56 Ti rotor, respectively. Centrifuged gradient columns were scanned photometrically at 254 nm by means of an ISCO Model 640 Density-Gradient Fractionator and UA-2 Ultraviolet Analyzer

(Instrumentation Specialities Co., Lincoln, Nebraska).

MCDV was considered present if centrifuged gradient columns contained a peak at the same position as a tobacco mosaic virus (TMV) or a known MCDV preparation. The reference TMV was layered on a gradient column along with the concentrated extract or on one gradient column of a set (six) centrifuged at the same time. The known MCDV was layered on one gradient column of a set. MDMV, the only other virus detected in this study, did not sediment to the same depth in similar comparisons. Further details of the assay are to be published elsewhere.

Fifty plants each of Aristogold Bantam, P309B, P3179, and WF9×Oh51A were exposed for 7-day periods (trap plants) in the field to estimate the potential disease incidence under natural field conditions. The estimate was based on the percentage infection of MDMV or MCDV in the surviving trap plants during each week, and it was called the disease intensity of MDM or MCD. The methodology for trap plants has been previously described (3). Five plants per replication of each corn hybrid were placed in four rows as a randomized complete block containing 10 replications. The trap plants were separated by 0.6 m (2 ft) within rows and 1.5 m (5ft) between rows. The site [7.6 m × 33.5 m (25 ft × 110 ft)] was maintained free of other vegetation. After 1 wk of exposure, trap plants were sprayed with malathion and returned to Wooster, where the two viruses do not occur naturally. The hybrids Aristogold and WF9×Oh51A were placed in greenhouses for observation of MDM and MCD symptoms for a 3- to 5-wk period. The hybrids P309B and P3179 were transplanted into a field at Wooster and observed for diagnostic symptoms.

RESULTS.—*Diagnostic symptoms of MCD and MDM in naturally-infected corn.*—Studies of incidence

TABLE 1. Assays of corn (*Zea mays* L.) leaf tissues for maize chlorotic dwarf virus by rate-zonal centrifugation

Leaf symptoms	No. of Assays Positive	Negative
Veinbanding only[a]	14	0
Veinbanding first, followed by other symptoms[b]	17	0
Other symptoms only[c]	5	0
Other symptoms first, followed by veinbanding[b]	23	1
Veinbanding and other symptoms occurring concurrently[a]	166	10
Symptomless[a]	1	18
Symptomless first, followed by veinbanding or other symptoms[b]	8	0

[a]The leaf symptom observed at time of harvest for assay.
[b]Symptoms observed one week after leaf was harvested for rate-zonal assay.
[c]Other symptoms included leaf reddening, mottling, stunting, marginal leaf chlorosis, chlorosis at base of leaf whorl, and leaf tearing.

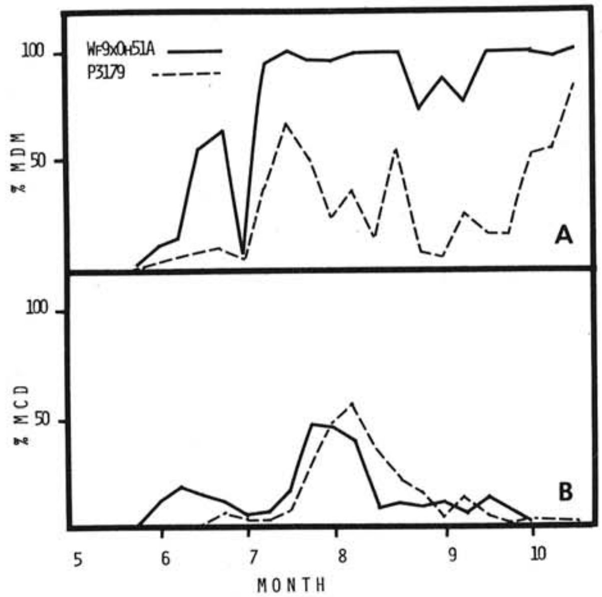

Fig. 1. Percentage infection of trap plants with (A) maize dwarf mosaic virus (MDM) or (B) maize chlorotic dwarf virus (MCD) from Portsmouth, Ohio. Percentage maize dwarf mosaic and maize chlorotic dwarf was based on mosaic and veinbanding symptoms, respectively. Plants of hybrid P3179 from 9 May - 22 August and 29 August - 17 October were transplanted to field plots or placed in greenhouses, respectively, for observation of symptoms.

TABLE 2. Relationship of planting date and incidence of maize dwarf mosaic (MDM) in five corn hybrids at Portsmouth, Ohio, 1972

Weeks after planting	Planting date	% MDM in corn hybrids[a] 1	2	3	4	5	Observation date
2	25 May	0	0	0	0	0	14 June[b]
	9 July	74	32	11	53	0	26 July
4	25 May	0	0	0	0	0	21 June
	9 July	98	66	57	90	10	8 August
8	25 May	83	24	2	34	0	18 July
	9 July	93	82	80	100	8	12 Sept[c]

[a]Corn hybrids: 1, WF9×Oh51A; 2, P309B; 3, P3179; 4, P3188; 5, F23413.
[b]Percentage MDM 3 wk after planting.
[c]Percentage MDM 9 wk after planting.

and intensity of MCD required recognition of diagnostic symptoms. Veinbanding and corn stunt-like symptoms were associated with MCDV infection (Table 1). Corn stunt-like symptoms included marginal chlorosis and splitting of the leaves, blotchy yellow or red leaves, and shortened upper internodes. Macroscopically, veinbanding appeared as narrow bands of green next to the secondary veins with fine chlorotic stripings over the smaller veins. This symptom was more pronounced on the abaxial side of the leaf and may be found on the lower, upper, or on all leaves of a plant. Leaves with veinbanding symptoms were dull and rough on the adaxial side, in contrast to the shiny and smooth adaxial surfaces of healthy leaves. Diseased leaves were not as easily torn from the leaf sheath as were healthy leaves. Rate-zonal assays confirmed visual observations of MCD in 95.4% of the determinations. Corn hybrid F23413 showed beinbanding without stunt-like symptoms to a higher degree than the other four lines. None of the 1,186 trap plants transplanted into the field at Wooster, including 198 plants that developed veinbanding symptoms, showed corn stunt-like symptoms. Similarly, corn stunt symptoms were not observed on trap plants placed in the greenhouse for observation.

Bioassay of 895 random leaf samples with mosaic, fleck, or ring patterns for MDMV positively confirmed visual ratings.

Estimates of MDM and MCD disease potentials.—1) Disease intensity of MDM and MCD.—Estimates of MDM and MCD disease potentials during a season were made by observing the percentage infection in trap plants and comparing the disease incidence in plants of each successively planted plot. Infected trap plants indicated the presence of MDMV inoculum and vectors at the Portsmouth site as early as the week ending 16 May. Two of four corn hybrids were selected to illustrate disease intensity in Fig. 1-A. After mid-June, the disease intensity of MDM remained above a 50% level in corn hybrid WF9×Oh51A, except during a flooding period at the end of June. During the weeks ending on 11 July to 22 August, the disease intensity reached the 90-100% level. It then dropped to a 60-90% level for 3 wk and resumed at the 90-100% level again during the weeks ending on 12 September to 17 October. The other, more resistant, corn hybrid P3179 (significant, $P = 0.05$) reflected similar trends, but to a lesser degree.

Maize chlorotic dwarf was observed as early as the week ending on 6 June (Fig. 1-B). From 20 June to 11 July, the disease intensity of MCD was severely reduced by rain and a flood. The disease intensity of MCD was highest during the 2 wk ending on 1 and 8 August; it dropped to zero by the week ending on 17 October.

2) Incidence of MDM and MCD in field plots.—MDM potential at the Portsmouth site also was estimated from the disease incidence in five corn hybrids in five successive plantings. The incidences of MDM in the 25 May and 9 July plantings are found in Table 2. Plantings later than 9 July showed similar trends, but the rate of disease increase was faster. Two to 3 wk after planting, MDM symptoms were observed in corn planted 7 June, 9 July, 19 July, and 1 August. Early planting reduced the incidence of MDM in the most resistant hybrid (F23413), but not in the most susceptible hybrid (WF9×Oh51A). At 8 wk after the corn was planted on 25 May and 9 July, 0 and 8%, respectively, of the plants of F23413 were diseased. In contrast, disease incidence was high in WF9×Oh51A regardless of the time of planting (83 and 93% of the plants in the 25 May and 9 July plantings, respectively). The highest incidence of MDM 8 wk after planting generally occurred in corn planted on 9 July.

The incidences of MCD in two of five plantings are found in Table 3. In plants of the 25 May planting, maize chlorotic dwarf symptoms were first observed on 11 July. The incidence of MCD was highest at 6 to 8 wk in three of the five plantings. Plants of the 1 August planting grew poorly, and visual diagnosis of MCD was difficult. These factors contributed to a lower incidence of MCD observed in the 1 August planting. A similar decrease in disease incidence was noted in hybrids WF9×Oh51A and P3188, planted on 9 July and 19 July and observed on 20 September. This apparent decrease was caused by leaf-reddening and chlorosis, which masked the diagnostic veinbanding symptom. Also, southern corn leaf blight was severe on P3188. Based on obsevations in late August, none of the hybrids was resistant to MCDV. Hybrids planted on 19 July had the greatest incidence of MCD.

Leaf-reddening which has been associated with corn virus diseases, was found in 39.8, 38.1, 17.4, 13.8, and 11.4% of all plants in corn hybrids WF9×Oh51A, P309B, P3179, F23413, and P3188, respectively, by late August or mid-September. Leaf reddening occurred on 31.7, 6.2, 35.6, 27.9, and 1.4% of all plants in the plantings on 25 May, 7 June, 9 July, 19 July, and 1 August, respectively.

DISCUSSION.—The recent discovery of a new virus, MCDV, in corn in southern Ohio (2) has helped to clarify

TABLE 3. Relationship of planting date and incidence of maize chlorotic dwarf (MCD) in five corn hybrids at Portsmouth, Ohio, 1972

Weeks after planting	Planting date	% MCD in corn hybrids[a]					Observation date
		1	2	3	4	5	
3	25 May	0	0	0	0	0	14 June
	9 July	1	0	0	2	2	2 August
4	25 May	0	0	0	0	0	21 June
	9 July	1	2	2	10	0	8 August
8	25 May	4	8	11	10	9	18 July
	9 July	70	91	94	57	82	12 Sept[b]

[a]Corn hybrids: 1, WF9×Oh51A; 2, P309B; 3, P3179; 4, P3188; 5, F23413.
[b]Percentage MCD 9 weeks after planting.

the etiology of the stunting symptoms and indicated that a disease complex is involved. Whether additional disease agents, e.g. CSA-OH (7), contribute to the stunting symptoms to any significant extent remains to be ascertained. From present evidence (1, 6, 7), we believe it likely that CSA-OH and MCDV are synonyms for the same pathogen.

The association of veinbanding and corn stunt-like symptoms in 95% of the field samples with MCDV strongly implicates the virus in the production of stunt-like symptoms in corn in southern Ohio. However, the occurrence of only veinbanding in the absence of corn stunt-like symptoms in trap plants returned to Wooster suggests that the frequent association of these symptoms in infected field plants depended on a factor (or factors) not present in Wooster. In southern Ohio, MCD symptoms were found alone or in combination with MDM symptoms in infected corn by late June and early July. It was late July before we determined a consistent association of veinbanding symptoms with MCDV infection. Most of the leaf samples assayed were collected during August and September, when leaf-reddening became common and prominent. Corn stunt-like symptoms apparently are influenced also by the genetic background of the corn line, as indicated by the relatively low incidence of corn stunt-like symptoms in corn hybrid F23413. Other pathogens may be involved in the production of these symptoms.

Singular characteristics in the progress of MDM and MCD epiphytotics were revealed by using different corn hybrids in successive biweekly field plantings, and as trap plants. A first contrast is the time difference required for host response to infection in field plants as compared to trap plants. As a result, it is difficult to determine accurately factors such as, which vector species are most efficient in the field, what environmental effects are most influential, or what causes plants to be most susceptible. This variation is avoided in trap plants because they are removed from the field after a 7-day exposure. The time period for host response to infection is then independent of what happens in the field. For example, as judged by trap plant responses, MDM and MCD both began appearing at a significant level in early June. However, because of a variation in time of host responses in field plants, MDM symptoms were first found in mid-June and MCD symptoms in mid-July in the 7 June planting. A second contrast is the phenomenon of disease escape from MDM in resistant corn hybrids. For example, few plants of corn hybrid P3179 in the 25 May planting, but 80% of the plants from the 9 July planting were MDMV-infected at 8 wk after planting. Because disease incidence is associated with time of planting, plants of P3179 from the 9 July planting will all be judged susceptible, regardless of their obvious relative resistance to MDMV when rated under less severe conditions. All corn hybrids were similarly susceptible to MCDV, regardless of planting date. However, because these hybrids were chosen specifically for studying MDM, other hybrids could possibly be selected to demonstrate disease escape from MCD. Finally, the incidence of disease in trap plants revealed fluctuating levels of activity of viruliferous vectors during the season for both viruses. This trend was less evident in plants of the successively-planted field plots.

The different patterns of epiphytotic development for the two diseases probably reflect a different vector situation. The progress of the MDM epiphytotic may be explained by assuming the occurrence of many species of aphid vectors over an extended period of time, or continuous presence of several efficient vector species during this period of time. In contrast, the progress of the MCD epiphytotic indicated a single, or few, leafhopper vector species, with a clearly marked increase in population size, followed by a marked decrease. These interpretations are compatible with the known number of vector species of the two viruses (5).

Trap plants, but not successively-planted plots, are sufficiently sensitive for measuring the threshold levels of inocula, vectors, and suscepts. A disease potential exists when threshold levels of these major factors are reached. The degree of this potential we call "disease intensity." Disease intensity is the product of all interactions among the above factors, plus, for a specific time interval, the environment that results in disease in trap plants. Successively-planted plots permitted only measurement of disease incidence. This is too limiting for understanding the features of epiphytotic development. For example, predictions of the relative activities of vectors, not possible with disease-incidence measurements alone, are possible with disease-intensity measurements. The variability in the time interval from infection to symptom expression, and the continuing decrease in plants available to express disease symptoms, are responsible for the limitations of the disease-incidence measurements.

The exposure time of 1 wk for trap plants represented the shortest exposure period that was logistically feasible. Longer periods decreased the sensitivity of the method (R. Louie and J. K. Knoke, *unpublished*). When disease intensity was at a high level, moderately resistant hybrids gave better measurements than susceptible hybrids. Thus, selection of trap plants should include susceptible and moderately susceptible hybrids to ensure the sensitivity of the method throughout the epiphytotic development.

LITERATURE CITED

1. BRADFUTE, O. E., R. E. GINGERY, D. T. GORDON, and L. R. NAULT. 1972. Tissue ultrastructure, sedimentation and leafhopper transmission of a virus associated with a maize dwarfing disease. J. Cell. Biol. 55:25a (Abstr.).
2. BRADFUTE, O. E., R. LOUIE, and J. K. KNOKE. 1972. Isometric virus-like particles in maize with stunt symptoms. Phytopathology 62:748 (Abstr.).
3. KNOKE, J. K., R. LOUIE, R. J. ANDERSON, and D. T. GORDON. 1974. Distribution of maize dwarf mosaic and aphid vectors in Ohio. Phytopathology 64:639-645.
4. LOUIE, R., and J. K. KNOKE. 1970. Evidence for strains of maize dwarf mosaic virus in southern Ohio. Phytopathology 60:1301 (Abstr.).
5. NAULT, L. R., W. E. STYER, J. K. KNOKE, and H. N. PITRE. 1973. Semipersistent transmission of leafhopper-borne maize chlorotic dwarf virus. J. Econ. Entomol. 66:1271-1273.
6. ROSENKRANZ, E. 1969. Two types of corn stunt in the USA. Phytopathology 59:1047 (Abstr.).
7. ROSENKRANZ, E. 1969. A new leafhopper-transmissible corn stunt disease agent in Ohio. Phytopathology 59:1344-1346.

8. TAMMEN, J. 1965. Discussion on paper, The dynamics of inoculum, by R. R. Baker. Page 318 *in* K. F. Baker and W. C. Snyder, eds. Ecology of soil-borne plant pathogens. University of California Press, Berkeley, Los Angeles.
9. WILLIAMS, L. E., and L. J. ALEXANDER. 1965. Maize dwarf mosaic, a new corn disease. Phytopathology 55:802-804.