Distribution of Maize Dwarf Mosaic and Aphid Vectors in Ohio

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ABSTRACT

A survey of commercial corn fields and corn seedling trap plants revealed that maize dwarf mosaic (MDM) is most prevalent in southern Ohio from mid-July through mid-September. During this period, at sites where virus-infected source plants are present, increases in aphid populations result in corresponding increases in MDMV transmission. Most MDM in Ohio results from strain A. MDM rarely occurs in the northwestern Corn Belt area of Ohio.

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Maize dwarf mosaic (MDM) first appeared in Ohio in 1963 (21). By 1964, MDM was identified in 79 of Ohio's 88 counties and yield loss in corn (*Zea mays* L.) was estimated at 5 million bushels (bu) (5, 7). MDM was most prevalent in the southern one-third of the state in bottom lands near infestations of Johnsongrass [*Sorghum halepense* (L.) Pers.] (7, 24).

When a mechanically transmissible virus was isolated from the diseased corn in Ohio in 1963 (20), it was named the maize dwarf mosaic virus (MDMV) (22) and was further characterized by Williams and Alexander (21) and Bancroft et al. (2). Based on symptoms and other evidence, it now appears that MDMV, one other virus (4, 14), and possibly other insect-vectored pathogens (6, 15) are involved in the disease complex in southern Ohio.

The original descriptions of MDM in southern Ohio referred to a mosaic or mottle that appeared at the base of young leaves followed by leaf reddening and severe stunting (7, 21). These symptoms accurately describe a disease complex that currently exists in that area. However, some of these symptoms (leaf reddening and severe stunting) may be attributed more appropriately to diseases of corn caused by pathogens other than, or in addition to, MDMV. Symptoms characteristic of infection by MDMV alone appear ca. 5 days after inoculation and include a light-green/dark-green mosaic or mottle at the base of the youngest leaves (21). Later, chlorotic streaks may appear that extend from the base of a developing leaf and narrow towards the tip. The type of mosaic or mottle pattern may vary, depending on the strain or isolate of MDMV (8, 9, 25). In maturing plants, the mosaic symptoms tend to fade, and diseased plants may be only slightly less green than healthy plants. Plant height is commonly reduced ca. 10-15% due to proportionately shortened internodes (J. K. Knoke, *unpublished*).

All strains of MDMV are easily transmitted to corn by rubbing developing leaves with freshly expressed juice from diseased tissue. MDMV has been transmitted by many species of aphids (Aphididae) in greenhouse tests (2, 18). The virus is stylet-borne by its aphid vectors (10). Aphids can acquire the virus from infected tissue within 10 s, retain their infectivity for ca. 30 min, and inoculate test plants within 15 s (12, 19). Many species of aphids have been found in the vicinity of fields with diseased plants, and many of them may be significant vectors of MDMV (3, 16).

The present study was undertaken to determine the seasonal and geographical distribution of MDM and aphids in Ohio, and to determine the strains of MDMV responsible for the disease. Except where otherwise indicated, we will use MDM to refer to the disease induced by one or more strains of MDMV and not to the disease complex mentioned previously.

MATERIALS AND METHODS.—In 1968, a survey of virus diseases was made during July and August that included commercial corn fields in 42 counties extending along the eastern and western borders of Ohio, from north to south in the center of the state, and then westward along the Ohio River. One or two fields in each county were surveyed, and two 100-plant samples per field were selected from; (i) the 10th and 30th row from a field edge, or (ii) each of two rows, 20 rows apart, beginning no less than 9.14 m (30 ft) from a field edge. At each site, the total number of plants that showed symptoms in 100 consecutive plants was recorded. If viral symptoms were observed, a leaf sample was taken and later bioassayed to determine the identity of the virus.

In 1970, 16 test sites were established in the southern and western areas of the state to determine seasonal incidence and distribution of corn viruses and populations of associated vectors. Trap plants (for measuring the incidence of virus) and a yellow pan trap were exposed at each site.

The trap plants were individual corn seedlings (a MDMV susceptible corn hybrid, Wf9 × Oh51A) planted in 0.102-m (4-inch) diam plastic pots in the greenhouse at Wooster and transported to the test sites 14 days later. They were left in the field 7 days and returned to a greenhouse where they were observed two to three times for mosaic symptoms during 3-4 wk. Each 30.5-m (100-ft) row of 50 trap plants was centrally located in a 3.05- × 33.55m (10 × 110-ft) fallow area cleared of vegetation each week. Plastic cups partially buried in the soil served as receptacles for the pots and also provided adequate water for the plants during the week.

The yellow pan traps 0.305- × 0.305- × 0.102-m (12- × 12- × 4-inch) galvanized metal painted inside and out with "Canary Yellow," Pratt and Lambert Effecto Enamel® (Pratt and Lambert Inc., New York, N.Y.) and patterned after Moericke (11), were each located in the center of a row of trap plants. The trap was three-fourths filled with water and positioned on a metal bracket so the upper edge of the trap was 0.305 m (1 ft) above the soil. Each week (each day at Portsmouth), the aphids were removed from the traps with a small brush, or the water and insects were poured into a large funnel with a small square of fine mesh nylon screen attached to its base. Then the insects (or the insects and screen) were placed in vials of 70% alcohol and returned to the laboratory for sorting and counting.

For bioassays, seedlings (three- to five-leaf stage) of 'Oh28' corn, 'Monon' wheat [*Triticum aestivum* (L.)] and 'Atlas' sorghum [*Sorghum bicolor* (L.) Moench] were inoculated with inoculum prepared from 1 g of suspect corn leaf tissue ground with a pestle in a mortar containing 3 ml of 0.01 M phosphate buffer adjusted to pH 6.5. The test plants were inoculated by dipping either a forefinger or a two-layer cheesecloth pad into a suspension of inoculum containing 1% 600-mesh silicon carbide and rubbing the leaves twice.

Bioassay test plants and trap plants were held in separate greenhouses and observed 2-3 times for mosaic symptoms during a 3-4 wk period. The greenhouse temp ranged between 27-32 C during the day and 24-27 C at night. All plants were kept free of insects by fumigation and were fertilized regularly.

Mosaic symptoms on infected trap plants or bioassay test plants were normally observed 7-10 days after inoculation. The presence or absence of symptoms in a corn-wheat-sorghum bioassay series permitted us to identify the viruses present. Systemic mosaic symptoms on corn and sorghum identified MDMV-A. Mosaic symptoms on corn alone and local lesions on sorghum identified MDMV-B (13). Systemic symptoms on corn and wheat identified wheat streak mosaic virus (WSMV) (23). However, serological methods were necessary to confirm double infections by MDMV-A and MDMV-B.

For the serological assays, juice from infected tissue was diluted with a physiologically buffered saline (PBS) solution (0.85% NaCl plus 0.01 M sodium phosphate adjusted to pH 7.0) and centrifuged for 15 min at 10,000 *g*. The supernatant fraction served as the antigen source. Antigens were diluted to 1:4 in two-fold steps with PBS solution. MDMV-A and MDMV-B antisera were prepared according to a modification of the procedure of Shepherd (17); WSMV antiserum was prepared as described by Williams et al. (23). MDMV-A and MDMV-B antisera were cross-absorbed with MDMV-B and MDMV-A, respectively. The heterologous antigens were prepared by extracting virus-infected plants with PBS (1:2). Equal volumes of antisera and extracts were mixed and incubated at room temp for 2 h. The precipitates were removed by centrifugation of the mixtures at 10,000 rpm for 10 min. The supernatant fractions were used in second cross-absorptions of the antisera with the heterologous antigens. The supernatant fractions from the second cross-absorptions were diluted with PBS to give a dilution of 1:8. This dilution, used in all tests, was optimum for detection of virus in extracts. Diluted cross-absorbed antisera did not react with their respective heterologous antigens. Antigen controls included extracts from healthy, MDMV-A-, MDMV-B-, and WSMV-infected corn leaves. The microprecipitin test, modified from Ball (1), was used for assays. Test droplets in plates were incubated at room temp for 1-4 h and then overnight at 4-6 C before reactions were recorded.

RESULTS AND DISCUSSION.—The survey of commercial corn fields in 1968 detected MDM in 13 southern counties, WSM in two northern counties, and no virus disease in 27 counties (Fig. 1). Of the 7,400 plants observed in counties where MDM was detected, an average of 18% were infected with MDMV-A. However, the incidence of MDM varied considerably among fields within counties. For example, on July 30, the percentage of diseased plants in fields ranged from 7% to 100% in Washington County and from zero to 96% in Morgan County. Overall, more diseased plants were detected during July than August, which suggests a possible masking of symptoms as the plants matured. Also, the prevalence of MDM in fields decreased with increased distance northward from the Ohio River. MDMV-B was not detected in any leaf samples tested in 1968. Thus, in the 1968 survey, different varieties and ages of corn and marked variations in incidence of disease in fields within a single county were encountered. These results, therefore, provided only an indication of the incidence of MDM in a given area. Apparently, the northwestern Corn Belt area of the state was relatively free of MDM in 1968.

Results of the more extensive investigation carried out with trap plants in 1970 (Fig. 2) are in Table 1. In the southern areas of the state, infection varied considerably from a low of 0.6% in Brown County to a high of ca. 45% in Scioto and Clinton Counties. A high incidence of MDM (avg 28%) occurred in bottomland sites where

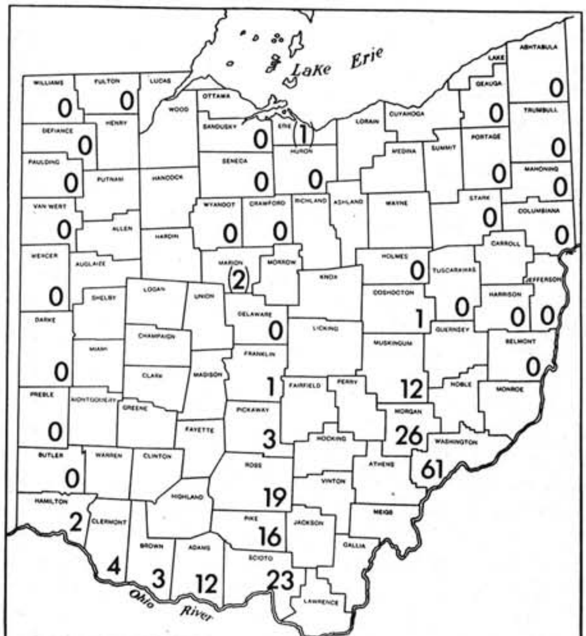

Fig. 1. Counties surveyed and percentages of plants infected with MDMV or (in parentheses) WSMV in commercial corn plantings. 1968.

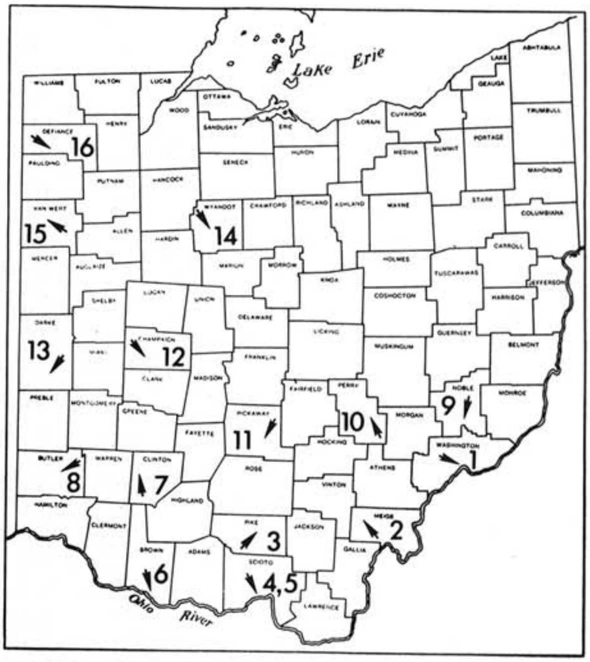

Fig. 2. Locations of trap plant exposure sites and aphid traps. 1970. Number = site number, arrow tip = site location.

Johnsongrass was present; in upland sites lacking Johnsongrass, incidence was low (avg 2.2%). Percentages of infected trap plants at sites in central and northwest Ohio were generally less than 1% for the season except at the southernmost site in Pickaway County. There Johnsongrass was present, and 4% of the plants were infected.

The seasonal occurrence of MDM in the three areas of Ohio in 1970 is represented in Fig. 3. MDM was initially detected the first week in May in southern Ohio, but the incidence remained generally low throughout May and June. The highest incidence of MDM occurred from mid-July through mid-September. At two lowland test sites in Scioto and Clinton counties, respectively, infection of the trap plants was generally above 90% from July 20 through Sept. 14. At two highland test sites in Meigs and Brown counties, respectively, trap plants usually remained healthy; less than 1% were infected during July, and only 2.5% were infected during August.

Estimates of aphid populations in southern Ohio ranged from a low of 2.6 aphids/trap per day for the season in Brown County to a high of 23.6 at the Scioto County-south site (Table 1). Fewer aphids were trapped at the highland test sites than at the lowland sites. Aphids were more numerous in southern Ohio (avg 8.7/trap per day) than in central (avg 6.6) or northwestern Ohio (avg 5.6). Across central Ohio, populations of aphids were five times higher in the western area of the state than in the eastern area.

Average populations of aphids in the southern, central, and northwestern areas of the state (Fig. 4) suggest regional differences in the occurrence of these potential MDMV vectors. In the south, populations increased rapidly early in the season and had three distinct peaks

TABLE 1. Description of trap plant exposure sites, percentage of trap plants infected with MDMV, and aphid populations in Ohio (1970)

Site no.	County	Johnson-grass present in area	Trap plants: Total no. exposed	Trap plants: infected (%)	Aphids trapped[a]: Total for season	Aphids trapped[a]: Avg/trap /day	Ratio of % infected plants to aphids/trap/day
	South						
1	Washington	+	1,264	27.1	1,485	7.9	3.43
2	Meigs	−	1,044	1.7	407	2.8	0.61
3	Pike	+	1,250	9.0	1,698	9.1	0.99
4	Scioto-North[b]	+	1,276	13.9	2,018	11.1	1.25
5	Scioto-South[b]	+	1,231	44.9	4,273	23.6	1.90
6	Brown	−	1,038	0.6	342	2.6	0.23
7	Clinton	+	1,194	45.3	1,075	5.7	7.95
8	Butler	−	1,299	4.2	1,330	7.1	0.59
	Central						
9	Noble	−	914	0.1	310	2.0	0.05
10	Perry	−	1,042	0.0	560	3.6	
11	Pickaway	+	1,209	4.1	1,129	6.5	0.63
12	Champaign	−	946	0.3	1,507	10.8	0.03
13	Darke		983	0.0	1,499	10.3	
	Northwest						
14	Wyandot		1,225	0.5	1,030	5.5	0.10
15	Van Wert		1,220	0.2	1,027	5.7	0.04
16	Defiance		1,203	0.2	924	5.5	0.04

[a]Yellow pan trap.
[b]Sites on same farm ca. 402.5 m (0.25 mile) apart.

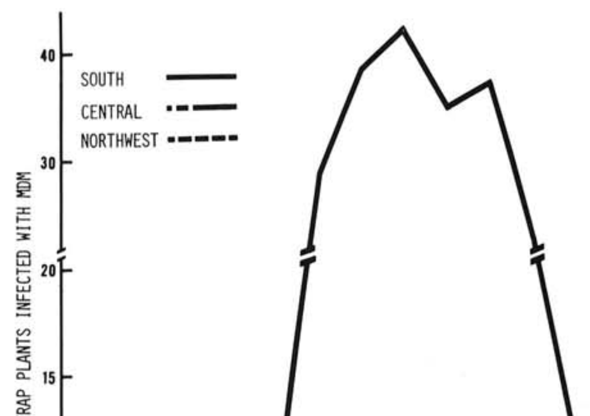

Fig. 3. Incidence of MDM in Ohio as measured by trap plants. 1970. South = average for sites 1-8, central = average for sites 9-13, northwest = average for sites 14-16.

(June 8, Aug. 17, and Oct. 12). The highest population during one week occurred in the northwest area of the state. There the number of aphids increased slowly in the spring to one major peak (52/trap per day) on Aug. 3. Seasonal fluctuations in population across central Ohio appeared to be more like those in southern Ohio than those in northwestern Ohio, though the early season peak across central Ohio was almost absent. Populations reached the seasonal high in all three state areas in early August.

An indication of the occurrence of viruliferous aphids at the various test sites is in Table 1. For each aphid trapped at the test site in Clinton County, 7.95% of the trap plants were infected. In the adjacent county (Brown), only 0.23% of the plants were infected for each aphid trapped. Thus, 35 times more viruliferous aphids were present at the Clinton County site than at the Brown County site. This higher rate of transmission at the Clinton County site was probably related to the presence of infected Johnsongrass in the immediate area of the test site, which would permit a high percentage of those aphids present to acquire and transmit the virus the short distance. At the southern Ohio test sites where Johnsongrass was found in proximity (sites 1, 3, 4, 5, & 7), an average 3.1% infection occurred in the trap plants for each aphid compared with only a 0.48% infection (sites 2, 6, & 8) where Johnsongrass was absent. Similarly, infection per aphid was ca. 15 and 35 times greater in southern than in central and northwestern Ohio, respectively. This larger percentage of infected aphids, coupled with a 57% higher population in southern than in northwestern Ohio, suggests that MDMV-susceptible

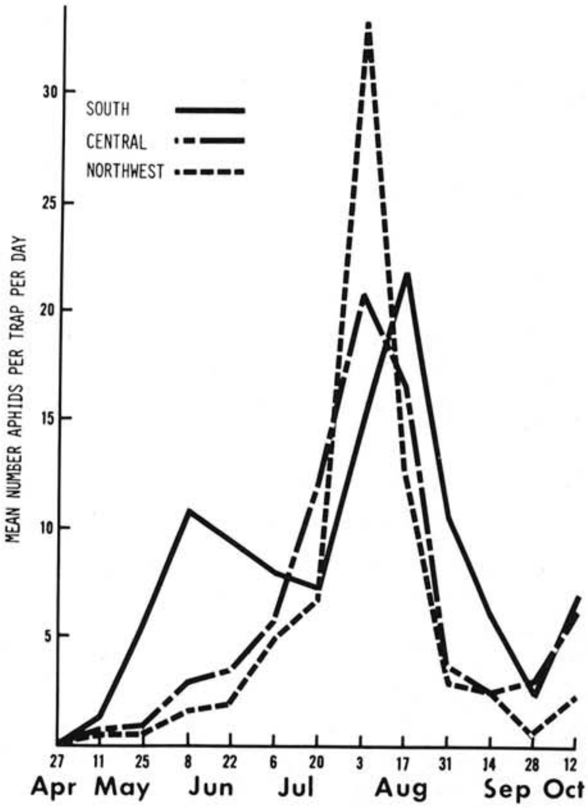

Fig. 4. Occurrence of aphids in Ohio as measured by yellow pan traps. 1970. South = average for sites 1-8, central = average for sites 9-13, northwest = average for sites 14-16.

corn varieties planted in southern Ohio would be 55 times more likely to become infected with MDMV than such corn planted in northwestern Ohio.

The ratio of MDMV-infected plants to number of aphids also changed throughout the season at individual test sites (Fig. 5). Generally, the ratio remained low during May and June indicating that few aphids were

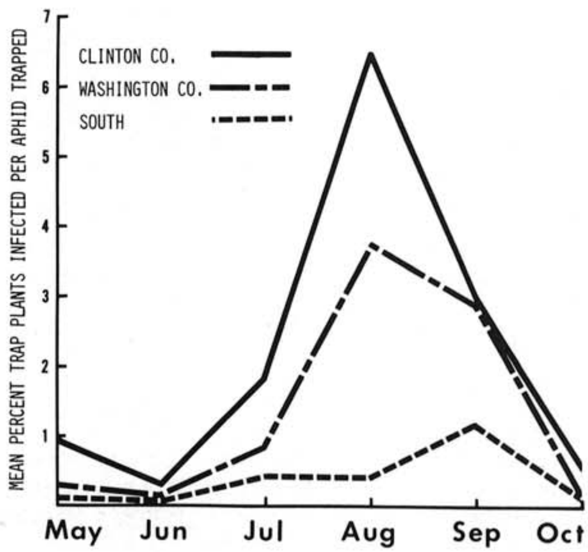

Fig. 5. Ratio of MDM diseased trap plants to aphid populations in Ohio. 1970. South = average for sites 1-8, Clinton Co. = site 7, Washington Co. = site 1.

TABLE 2. Strains of MDMV in Ohio as determined by bioassays and serological assays of trap plants in 1970

Exposure sites (county)	Trap plants (no.)		Plant assay (%)			
				Infected with MDMV		
	Infected[a]	Assayed	Healthy	A	B	AB
South						
Washington	343	86	1	74	1	23
Meigs	18	15	13	67	0	20
Pike	112	62	8	86	0	6
Scioto[b]	730	150	9	72	2	17
Brown	6	5	0	80	20	0
Clinton	541	99	5	88	3	4
Butler	54	31	0	90	0	10
Central						
Noble	1	0				
Perry	0	0				
Pickaway	50	23	0	96	0	4
Champaign	3	0				
Darke	0	0				
Northwest						
Wyandot	6	5	0	80	20	0
Van Wert	2	2	50	0	50	0
Defiance	2	0				
Total plants		478	28	380	10	60

[a]Determined visually on basis of typical symptoms.
[b]Trap plants from both north and south sites.

viruliferous during this period. Then from June through August, a 20-fold increase in the number of viruliferous aphids occurred at the Washington and Clinton county sites. This increase may have been the result of an increase in the number of diseased plants near the test sites, an increase in the suitability of these same plants as virus sources for aphids, and/or the presence of more efficient vector species. Conversely, the subsequent drop in the rate of transmission after August (or after September in the majority of southern test sites) may have resulted either from the advanced maturity of nearby corn and Johnsongrass, which made them less suitable for aphid probing and as source plants (19), or from a decline in the numbers of efficient vectors.

However, in southern Ohio, from mid-July to mid-September, an increase or decrease in aphid populations from one week to the next generally resulted in an increase or decrease in the incidence of infected trap plants. Therefore, with a relatively uniform quantity and quality of source plants, the amount of transmission was directly related to the number of aphids present.

Determinations of the MDMV strains infecting trap plants from 10 sites were made with bioassays and/or serological techniques. Approximately 25% of the plants with mosaic symptoms were assayed (Table 2). MDMV-A, the most prevalent strain, occurred alone in 79.5% of the plants; MDMV-B occurred alone in only 2.1% of the plants; and mixed infections of the two strains were detected in 12.6% of the assayed trap plants. Although MDMV-A was prevalent in the southern half of the state, it was also detected in four plants in the northwest area, some distance from the normal range of natural infestations of Johnsongrass. MDMV-B, formerly observed more often in northern Ohio, was detected in ca. 15% of the plants assayed from all test sites in southern Ohio; thus, this strain is distributed in Ohio like MDMV-A. WSMV was not detected in the plants assayed in 1970.

The time and magnitude of virus transmission and identification of the virus are both essential to establish standard planting dates for corn lines and to accurately interpret the disease ratings in studies of resistance. The use of trap plants permitted us to determine the magnitude of virus spread at various sites throughout the season. Assayed infected trap plants demonstrated that the incidence of the strains in Ohio was ca. 85% MDMV-A and 15% MDMV-B.

In Ohio, MDM occurs mainly in the southern counties, and the epiphytotics require both infected source plants and sufficient aphid populations. At test sites in the more northern counties (sites 12-16), populations of aphids were adequate to spread the virus but the absence or low incidence of source plants restricted transmission. In southern counties where source plants were abundant, the transmission of MDMV to trap plants was more directly related to populations of aphids.

LITERATURE CITED

1. BALL, E. M. 1961. Serological tests for the identification of plant viruses. American Phytopathological Society, St. Paul, Minnesota. 16 p.
2. BANCROFT, J. B., A. J. ULLSTRUP, M. MESSIEHA, C. E. BRACKER, and T. E. SNAZELLE. 1966. Some biological and physical properties of a midwestern isolate of maize dwarf mosaic virus. Phytopathology 56:474-478.
3. BLAIR, B. D. 1970. Aphids collected from a Scioto County, Ohio, corn field and areas bordering the field. J. Econ. Entomol. 63:1099-1101.
4. BRADFUTE, O. E., R. LOUIE, and J. K. KNOKE. 1972. Isometric viruslike particles in maize with stunt symptoms. Phytopathology 62:748 (Abstr.).
5. ELLETT, C. W., B. F. JANSON, and L. E. WILLIAMS. 1965. Maize dwarf mosaic in Ohio in 1964. Plant Dis. Rep. 49:615-616.
6. GRANADOS, R. R., K. MARAMOROSCH, T. EVERETT, and T. P. PIRONE. 1966. Leafhopper transmission of a corn stunt virus from Louisiana. Phytopathology 56:584 (Abstr.).
7. JANSON, B. F., L. E. WILLIAMS, W. R. FINDLEY, E. J. DOLLINGER, and C. W. ELLETT. 1965. Maize dwarf mosaic: New corn virus disease in Ohio. Ohio Agric. Exp. Stn. Res. Circ. 137. 16 p.
8. LOUIE, R., and J. K. KNOKE. 1970. Evidence for strains of maize dwarf mosaic virus in southern Ohio. Phytopathology 60:1301 (Abstr.).
9. MACKENZIE, D. R., C. C. WERNHAM, and R. E. FORD. 1966. Differences in maize dwarf mosaic virus isolates of the northeastern United States. Plant Dis. Rep. 50:814-818.
10. MESSIEHA, M. 1967. Aphid transmission of maize dwarf mosaic virus. Phytopathology 57:956-959.
11. MOERICKE, V. 1950. Uber das Farbsehen der Pfirsichblattlaus (Myzodes persicae Sulz). Z. Tierpsychol. 7:265-274.
12. NAULT, L. R., H. J. HARLAN, and W. R. FINDLEY. 1971. Comparative susceptibility of corn to aphid and mechanical inoculation of maize dwarf mosaic virus. J. Econ. Entomol. 64:21-23.
13. PAULSEN, A. Q., and W. H. SILL, JR. 1970. Absence of cross-protection between maize dwarf mosaic virus strains A and B in grain sorghums. Plant Dis. Rep. 54:627-629.
14. PIRONE, T. P., O. E. BRADFUTE, P. H. FREYTAG, M. C. Y. LUNG, and C. G. PONELEIT. 1972. Virus-like particles associated with a leafhopper-transmitted disease of corn in Kentucky. Plant Dis. Rep. 56:652-656.
15. ROSENKRANZ, E. 1969. A new leafhopper-transmissible corn stunt disease agent in Ohio. Phytopathology 59:1344-1346.
16. SHAUNAK, K. K., and H. N. PITRE. 1971. Seasonal alate aphid collections in yellow pan traps in northeastern Mississippi: possible relationship to maize dwarf mosaic disease. J. Econ. Entomol. 64:1105-1109.
17. SHEPHERD, R. J. 1965. Properties of a mosaic virus of corn and Johnson grass and its relation to the sugarcane mosaic virus. Phytopathology 55:1250-1256.
18. STONER, W. N., L. E. WILLIAMS, and L. J. ALEXANDER. 1964. Transmission by the corn leaf aphid, Rhopalosiphum maidis (Fitch) of a virus infecting corn in Ohio. Ohio Agric. Exp. Stn. Res. Circ. 136. 4 p.
19. TU, J. C., and R. E. FORD. 1971. Factors affecting aphid transmission of maize dwarf mosaic virus to corn. Phytopathology 61:1516-1521.
20. WILLIAMS, L. E., and L. J. ALEXANDER. 1964. An unidentified virus isolated from corn in southern Ohio. Phytopathology 54:912 (Abstr.).
21. WILLIAMS, L. E., and L. J. ALEXANDER. 1965. Maize dwarf mosaic, a new corn disease. Phytopathology 55:802-804.
22. WILLIAMS, L. E., L. J. ALEXANDER, W. R. FINDLEY, E. J. DOLLINGER, R. W. RINGS, and R. E. TREECE. 1964. Maize dwarf mosaic. Ohio Rep. 49:88-91.
23. WILLIAMS, L. E., D. T. GORDON, L. R. NAULT, L. J.

ALEXANDER, O. E. BRADFUTE, and W. R. FINDLEY. 1967. A virus of corn and small grains in Ohio, and its relation to wheat streak mosaic virus. Plant Dis. Rep. 51:207-211.

24. WILLIAMS, L. E., O. W. SPILKER, and C. W. ELLETT. 1968. Distribution and severity of corn viruses in Ohio in 1965. Pages 55-57. *in* W. N. Stoner, ed. Corn (maize) viruses in the continental United States and Canada. USDA, ARS Bulletin 33-118. 95 p.

25. ZUMMO, N., and D. T. GORDON. 1971. Comparative study of five mosaic virus isolates infecting corn, Johnson grass, and sorghum in the United States. Phytopathology 61:389-394.