Detection of Maize Dwarf Mosaic Onset in Northern Ohio

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ABSTRACT

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An ability to detect maize dwarf mosaic virus (MDMV) infections will help explain how MDM epidemics develop. Trap plant plots with and without diseased source plants, successive maize plantings, and grass weeds in tile plots were used to monitor MDM onset in northern Ohio. This area is outside the natural distribution of johnsongrass (*Sorghum halepense*), the overwintering host of MDMV-A. The average incidence of MDM in trap plants increased from 44 to 52% as the number of source plants placed at a 0.6-m distance from the trap plants increased from 25 to 100 plants. At a constant level of 100 source plants, the average incidence of MDM decreased from 52 to 33% as the distance between source plants and trap plants increased from 0.6 to 4.9 m. The decrease in MDM incidence averaged −4.8%/m of the distance from source plants and averaged +0.5% MDM for each unit increase in source plants. Successive plantings at location 2 detected MDM onset 42 and 12 days earlier in 1986 and 1987, respectively, than did the trap plant plots without source plants. MDMV was not detected in 832 weed samples collected from the field or in the six grass weed hosts grown in tile plots. Aphid populations were monitored with yellow-pan water traps. *Rhopalosiphum maidis* was significantly related to MDM onset. Aphid migration, seed transmission, and infected weed host hypotheses were evaluated as initial sources of MDMV. The weed host hypothesis best explained MDM onsets in northern Ohio.

Additional keywords: aphid vectors, corn, spatial and temporal spread.

Maize dwarf mosaic (MDM) is a widespread and important viral disease of maize (*Zea mays* L.) (4). Numerous strains of maize dwarf mosaic virus (MDMV) exist (10), but MDMV-A and -B are the most studied. Johnsongrass (*Sorghum halepense* (L.) Pers.) is considered the overwintering source of MDMV-A in several countries. Presently, there is one study relating the onset of MDM to johnsongrass (8). More than 375 annual and perennial grasses have been determined as alternate hosts of MDMV-A and/or -B under experimental conditions (15–17). Some reports, however, are contradictory. More importantly, the overwintering host of MDMV-B has not been reported.

MDM occurs predictably late in the growing season in some northeastern areas of the United States, and particularly in northern Ohio. Yet the factors responsible for this seasonal initiation of MDM remain unresolved (1,18). MDM onset appears to be limited by the availability of inoculum because the numerous aphid vector species (7) and possible weed hosts (15) have wide distributions. The relative importance of inoculum source and aphid vectors on limiting MDM onset in Ohio can be determined if time and location are controlled variables. The trap plant system previously described (5) offers this flexibility to independently study these two factors.

Trap plants have been used to assess the levels of disease intensity from location to location and from year to year (11). The efficacy of trap plants, however, depends on the susceptibility of the genotype selected to detect infection and its proximity to a virus source. Consequently, great care must be used in the selection of trap plant germ plasm. Other limitations of the trap plant system (e.g., the minimal number of plants required for early virus detection) became evident when MDM studies were undertaken in areas where the incidence of MDM was low. Thus, a need arose to reassess the trap plant system in these areas. In addition, because previous MDM studies concentrated on the seasonal development of MDM, there also was a need to determine the efficacy of trap plants for early virus detection (1,18). Our first objective in this study was to compare the trap plant system with the successive planting system and the grass weeds in tile plots for early virus detection. Our second objective included defining the relationship between quantity and distance of inoculum source to initial MDM onset and determining how onset is related to aphid populations with the use of trap plants and successive plantings. We wanted also to examine the role of grass weed hosts as sources of inoculum. Lastly, we wanted to relate our findings to the onset of MDM in northern Ohio.

MATERIALS AND METHODS

Plot locations and trap plant and aphid-trapping methodologies. Plots were located on three farms within a 10-km radius of Wooster, OH, in 1986 and 1987. Location 1A was at the Maurer farm in 1986 and at the Frye farm in 1987. Location 1B was at a second site on the Frye farm and was used for the grass host plot and the 1978–1985 trap plant plot. Locations 2 and 3 were at the Snyder and Wagner farms, respectively, in both years. Three types of experiments were done. In the first, maize hybrid WF9 × Oh51A was used as the trap plant to detect the onset of MDM. In 1987, the effect of the number of virus-infected source plants, their distance from trap plants, and the effect of aphid numbers on the time of onset and incidence of MDM in trap plants also were determined. In the second experiment, successive plantings of Seneca Chief sweet corn were used to monitor the onset of MDM. The onset of MDM in trap plants and successive plantings during 1986 and 1987 was compared to the onset in trap plants for the period 1978–1985. MDM onset also was related to aphid numbers. In the third experiment, grass hosts in tile plots were used to monitor the presence of MDMV.

Trap plants were 14-day-old WF9 × Oh51A maize seedlings grown singly in 10-cm plastic pots containing autoclaved greenhouse soil. The trap plants were grown in the greenhouse and then transferred to the field for exposure to infection (7). Source plants were MDMV-infected 21-day-old Oh28 maize seedlings similarly grown in 10-cm plastic pots and then airbrush-inoculated with MDMV-A (12) at 10 days of age. At 11 days after inoculation, the source plants were placed in the field for 1 wk. Exposure of trap plants for weekly periods began on 13 May and ended on 30 September in 1986; weekly exposure periods in 1987 began on 19 May and ended on 1 September. Exposed trap plants and source plants were replaced by a new set each week. The exposed trap plants were sprayed with Malathion and returned to the greenhouse for observations; source plants were discarded. Trap plants were maintained free of insects by using Malathion sprays during a 3- to 4-wk observation period and were fertilized weekly with a 20-20-20 (N, P, and K, respectively) water-soluble fertilizer.

Aphids were collected in 30.5 cm square by 10.2 cm deep yellow-pan water traps as previously reported (7). Traps were positioned on pipe-supported metal brackets holding the trap opening 61 cm above the soil and 75 cm from the edge of the first planting of the successive plantings (1986) or at each end of and in line with the trap plant row (1987). Aphids were removed from each trap on Tuesdays and Fridays and stored in a vial of 70% alcohol for later counting and identification.

Plot designs and weed collections. The standard trap plant plot design consisted of a row of 50 trap plants spaced 0.3 m apart surrounded by a 1.5-m fallow border resulting in a 3.0- × 18-m area. This design also served as a virus-free control in 1987. The 8-yr average MDM in trap plants was based on 50 trap plants per exposure period (25 in 1978; data for 1979 and 1980 included source plants and were not used in the computation) placed in the same site at location 1. In 1986, these trap plant plots were adjacent to the fourth successively planted plots at locations 1 and 2. In 1987, the trap plant plots, the modified trap plant plots (see below), and the successive plantings at locations 1 and 3 were laid out adjacent to each other and required an area of 18 × 246 m. At location 2, the modified trap plant plots were physically separated from the trap plant plots and the successive plantings by about 800 m.

The addition of virus source to this standard trap plant configuration in 1987, hereafter referred to as the modified trap plant plot, was achieved by placement of a row of a predetermined number of infected Oh28 source plants equally spaced on each side of the row of trap plants. Treatments consisted of varying the distance between source plants and trap plants and varying the number of source plants per row. In treatments 1–3, the two rows of source plants were 0.6 m from the trap plants, but the total numbers of source plants were 25, 50, and 100 plants, respectively. In treatments 4–6, the row of source plants on each side of the row of trap plants contained 50 plants for a total of 100 source plants, but the distances between rows of source plants and trap plants were 1.2, 2.4, and 4.9 m, respectively. The subplot sizes or fallow area dimensions for each treatment layout were 4.3 × 18, 4.3 × 18, 4.3 × 18, 5.5 × 18, 7.9 × 18, and 12.8 × 18 m, for treatments 1–6, respectively. The experiment was set up as a randomized complete block with location as replication. The first set of source plants and trap plants was placed in the field on 30 June. The last set was removed on 25 August.

In the successively planted plot design, a seed drop for a 2,000-plant stand of Seneca Chief sweet corn was distributed into 20 rows, each 15 m long (locations 1 and 3), or into 12 rows, each 30.8 m long (location 2); all rows were 0.8 m apart. The first planting was placed at the north or east end of the field layout. The seeds were planted on 29 May, 11 June, 26 June, and 11 July in 1986 and on 28 May, 11 June, 25 June, 9 July, and 23 July in 1987.

Grass hosts of MDMV-A and -B (=tile plot) were planted in a plot that was laid out as a randomized complete block at location 1A in 1986. The plot was on the same site where the standard trap plant plot had been maintained during 1978–1985. Twenty plants of each of seven hosts (*Cynodon dactylon* (L.) Pers., *Sporobolus asper* (Michx.) Kunth, *S. clandestinus* (Biehler) Hitchc., *Andropogon gerardi* Vitm., *Panicum virgatum* L., *S. halepense*, and *Z. mays*) were replicated five times. Each subplot consisted of two rows of 10 clay field tiles, 30 cm long by 15 cm diameter, placed vertically into the soil to contain the test host. The tile centers were 28 cm apart within a row and between the two rows. The bare soil border surrounding the plot was 28 cm wide. A 0.8-m border of mown grass surrounded each 20-tile subplot. Seedlings of each host were started in the greenhouse and then transplanted into the tiles. The WF9 × Oh51A maize plants were set up as trap plants and changed on the same dates as the other trap plants. The other grasses remained in the field tiles until the spring of 1988.

Random collections of grass weeds and maize with and without viruslike symptoms were taken from around commercial maize fields to test for possible sources of MDMV. Collections usually were made before MDM epidemics were observed in the commercial plantings during the 1980–1987 growing seasons.

Data collection, assays, and analyses. The number of MDMV-infected trap plants was recorded weekly for 3–4 wk. Plants in each of the successive plantings were examined for MDM symptoms at least once beginning the third week after planting. Leaf samples from all infected trap plants from the standard trap plant plots and from the lesser of all or 10% of the infected plants in the successive plantings were bioassayed for MDMV-A and -B by rub inoculations of Oh28 maize, Monon wheat (*Triticum aestivum* L.), and Atlas sorghum (*Sorghum bicolor* L.). Grass weed hosts in the tile plot were surveyed for virus symptoms at the beginning of and two times during the season. Bioassays for MDMV-A and -B of about 10% of each grass weed host species in the tile plots were made at the ends of the 1986 and 1987 seasons and at the beginning of the 1988 season. Plant samples from collections around commercial fields were assayed for MDMV-A, -B, brome mosaic virus (BMV), and/or wheat streak mosaic virus (WSMV) by bioassays and/or enzyme-linked immunosorbent assays (ELISA), as previously modified (9).

Data from the final observation in trap plants and from the first observation of MDM symptoms in the successive plantings were used in the analyses. The data were transformed by the arcsine square root before analysis of variance (ANOVA); Duncan's new multiple range test (3) was used for mean separations. Location was used as replication in the experimental design for trap plants and successive plantings. Treatments were exposure periods or times of planting and the levels of inoculum or distances from virus inoculum in the trap plant experiment. The data are reported as nontransformed means.

Total numbers of aphids were determined on a weekly basis. When there were more than 55–60 aphids in a vial, a random subsample of 50 aphids was chosen for species identification. The species' proportion in the subsample was used to estimate the total numbers of aphids for each species in the individual collections. In 1987, when MDMV source plants were provided so that transmission of MDMV to trap plants occurred throughout the trapping season, numbers for each aphid species from the 12 traps at each location (replicate) were combined into values for each species for each 7-day period, corresponding to a trap plant exposure period. The aphid counts plus one and the infection percentages were then transformed by common logarithms and square root, respectively, before regression analysis. The RS/1 "Fit Multiple" statistical program was used for the analysis (2).

RESULTS

Standard trap plants. MDM symptoms were first detected in trap plants from the standard plot at locations 1A and 2 during the exposure period beginning on 5 August and 16 September 1986, respectively (Table 1). In 1987, none of the trap plants were infected until the week beginning on 30 June. At that time, the modified trap plant plots were started. The first of two peaks of infected plants (18 and 24% for locations 1B and 3, respectively) was observed on the week beginning 4 August. The second peak

of infected plants (64 and 88% for locations 1B and 3, respectively) was observed on 25 August. At location 2, MDM symptoms on 40 and 14% of the plants were observed only from exposure periods beginning on 25 August and 1 September, respectively.

Modified trap plants. In an ANOVA of the effect of the amount of MDMV inoculum and distance from the source, locations, exposure periods, and treatments were significant ($P \leq 0.01$). The interaction between treatments and exposure periods was not significant ($P \geq 0.05$). The highest average level of disease incidence in the modified trap plants (treatments 1–6) was 87.4%, and this occurred on 28 July (Table 2). The average level of disease incidence among the standard trap plant plots during this period averaged 4.7%.

Levels of disease incidence in the modified trap plants differed significantly between locations 1 and 3 ($P \leq 0.05$) but not between 1 and 2 or 2 and 3. A trend of greater variation among locations occurred during the earlier rather than the later part of the season when disease levels exceeded the 30–40% level.

Orthogonal polynomials were used following ANOVA to determine whether a linear or a quadratic relationship existed between amount of inoculum and disease incidence (treatments 1–3) and between distance from source and disease incidence (treatments 3–6). A highly significant linear relationship existed in both cases ($P \leq 0.01$). The quadratic relationship was not significant. The linear relationship between amount of inoculum and disease incidence was most obvious during the first six exposure periods ($P \leq 0.001$). The decrease in disease level averaged −4.8%/m of the distance from source plants, and for each unit increase in source plants, the MDM averaged +0.5%. There was no such linear relationship in the last two exposure periods ($P \geq 0.1$).

Successive plantings. Plant stands in the successive plantings at locations 1, 2, and 3 averaged 1251, 1834, and 1576, respectively, for four plantings in 1986, and averaged 1242, 1553, and 1385, respectively, for five plantings in 1987. The ANOVA for successive plantings and MDM incidence showed that plantings were significantly different at $P \leq 0.087$ and 0.01 for 1986 and 1987, respectively. The average disease incidences were 0.01, 0.0, 0.2, and 4.0% in the first, second, third, and fourth plantings, respectively in 1986. Only the fourth planting was significantly different. In 1987, the average disease incidences were 0.0, 0.0, 0.1, 0.1, and 3.9% for the first, second, third, fourth, and fifth plantings, respectively. Only the fifth planting was significantly different.

Aphid trapping. Populations of most MDMV vector species peaked during late July to early August in both 1986 and 1987 (Tables 3 and 4). Notable exceptions were *Myzus persicae* (Sulzer) populations, which fluctuated from high to low several times during these sample periods and *Hyalopterus atriplicis* (L.), which peaked early in the sample periods. In both years, the average aphid number per collection period was lower than the corresponding 1978–1985 average (Figs. 1 and 2). The major peak in 1987 also occurred about 2 wk earlier than the 1978–1985 average. In 1987, about 94% of over 19,000 aphids collected in the 36 traps exposed for 56 days were identified as species previously recorded as MDMV vectors (6). More *Rhopalosiphum maidis* (Fitch) than *M. persicae* were present in the test areas. Both species occurred in greater numbers than the other eight vector species that had statistically similar populations of about 1–10% of the total aphids collected.

Table 5 contains estimated parameters for the stepwise regression analysis to compare 1987 populations of each aphid species at each location with the proportion of trap plants infected with MDMV. At location 1, where the fewest aphids were collected, populations of *R. maidis*, *Aphis craccivora* Koch, *Aphis gossypii* Glover, and *H. atriplicis* were significantly associated with the occurrence of MDM in trap plants. With these four species, the proportion of explained variability at this site was very high ($R^2 = 0.999$). At the other two test sites, and when data from all three sites were considered together, only *R. maidis* was significantly associated with infection in trap plants, suggesting that it was the most important vector in 1987.

Aphid populations and MDM onset in trap plants and successive plantings. The associations between aphid numbers and the detection of MDM onset in trap plants and successive plants for 1986 and 1987 and for the 1978–1985 period are shown in

TABLE 1. Percentage of maize dwarf mosaic virus infection in WF9 × Oh51A trap plants with standard trap plant design at Frye (1), Snyder (2), and Wagner (3) locations in Wooster, OH, in 1986, 1987, and 1978–1985[a]

		Exposure date[b]																		
Year	Location[c]	19 May	26 May	2 June	9 June	16 June	23 June	30 June	7 July	14 July	21 July	28 July	4 Aug	11 Aug	18 Aug	25 Aug	1 Sept	8 Sept	15 Sept	22 Sept	29 Sept
1986	1A	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	10
1987	1A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	...	...	...	...
	1B	0	0	0	0	0	0	2	0	2	2	6	18	8	14	64	70	...	...	...	...
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	40	14	...	...	...	...
	3	0	0	0	0	0	0	0	4	2	2	8	24	20	20	88	71	...	...	...	...
1978–85[d]	1A	0	0	0	0	0	1	<1	<1	<1	1	1	8	5	<1	8	20	11	6	6	0

[a] Based on 50 trap plants per location except for 25 plants in 1978 and 100 plants in 1985, 1986 (1A = tile plot), and 1987 (1A = tile plot).
[b] Actual beginning date for 1987; all other years, beginning date occurred during the 7-day period.
[c] Field site (1A) at the Frye location was the same for all years except it was in a nearby field in 1983. Field site 1B at the Frye location was 250 ft north of field site 1A and adjacent to the modified trap plants. Standard trap plant plots at locations 1B and 3 were adjacent to modified trap plant plots.
[d] Data for 1979 and 1980 included source plants and are not presented here.

TABLE 2. Average percentage of infection in WF9 × Oh51A plants of the modified trap plant plots at different exposure periods at the Frye, Snyder, and Wagner locations in Wooster, OH, in 1987[a]

	Average percentage of infection by exposure period								Average	
Treatment[b]	30 June	7 July	14 July	21 July	28 July	4 Aug	11 Aug	18 Aug	6 wk	8 wk
1	11.7	20.6	38.3	43.7	85.4	62.7	46.6	38.8	43.7	43.5
2	15.1	22.2	53.8	50.0	91.5	72.6	55.3	39.5	50.9	50.0
3	17.8	28.7	61.7	54.8	96.9	74.7	43.1	39.2	55.8	52.1
4	10.6	20.6	54.7	46.8	92.6	77.3	51.6	43.0	50.5	49.7
5	13.2	19.0	37.5	42.9	85.4	67.2	48.5	34.9	44.2	43.6
6	4.3	7.9	31.7	39.1	72.6	52.4	27.6	30.7	34.7	33.3
Average	12.1 e	19.9 de	46.3 bc	46.2 bc	87.4 a	67.8 b	45.5 bc	37.7 cd	46.6	45.4

[a] Numbers marked with different letters differ significantly at $P \leq 0.05$ with Duncan's new multiple range test.
[b] Treatments 1, 2, and 3 had a constant distance of 0.6 m between source plants and trap plants, but the numbers of source plants varied and were 25, 50, and 100 plants, respectively, for each treatment. Treatments 4, 5, and 6 each had 100 source plants, but the distances between source plants and trap plants for each treatment varied and were 1.2, 2.4, and 4.9 m, respectively.

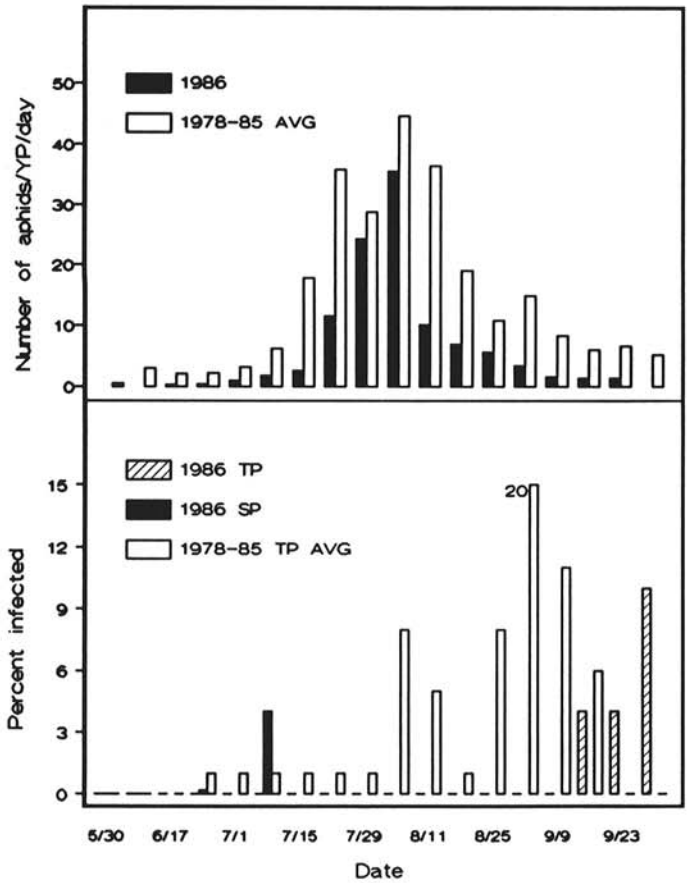

Fig. 1. Associations between aphid numbers and the onset of MDM in trap plants (TP) and successive plantings (SP) for 1986 and the period 1978–1985.

Figures 1 and 2. The highest weekly averages were 35.6 and 22.4 aphids per trap per day for the weeks beginning 8 August 1986 and 28 July 1987, respectively. These were a little more than three-fourths and one-half as high as the 8-yr average. MDM onset in successive plantings and trap plants occurred before aphid population peaks. The onset of MDM in successive plantings occurred earlier than the onset in trap plants (26 June planting observed on 5 August vs 16 September for trap plants in 1986, and 23 July planting observed on 13 August vs 25 August for trap plants in 1987 for location 2). MDM in trap plants in 1986 and 1987 followed the 1978–1985 trend when high levels occurred after aphid population peaks.

MDMV assays. Bioassays of infected trap plants in 1987 indicated 24 of 24 and 0 of 24 plants infected with MDMV-A and -B, respectively. ELISA of infected maize plant samples from successive plantings indicated 19 of 61 and 42 of 61 plants infected with MDMV-A and -B, respectively, in 1986, and 43 of 62 and 19 of 62 for MDMV-A and MDMV-B, respectively, in 1987. There were no doubly infected plants. In 1987, more trap plants and plants in successive plantings were infected with MDMV-A than with MDMV-B, apparently reflecting the proximity of the MDMV-A source plants. Grass weeds in the tile plot never developed viral symptoms nor did they test positive by ELISA (0 of 178) or by bioassays (0 of 50, in 1988) throughout the 3-yr exposure period. In the 1980–1987 period, 832 weed samples from areas adjacent to fields of commercial sweet corn in northern Ohio were tested for virus infection to determine the frequency occurrence of various viruses affecting maize. None of the weed samples of 118 *Agropyron repens* (L.) P. Beauv., 127 *Dactylis glomerata* L., 89 *Festuca arundinacea* Schreb., 130 *Lolium perenne* L., 113 *Phleum* spp., and of 233 other minor species of weed samples that were collected were found infected with either MDMV-A or -B. However, two of two *Digitaria ischaemum* (Schreb.) Schreb. ex Muhl., two of two *Echinochloa crusgalli* (L.) P. Beauv., three of three *Panicum dichotomiflorum* Michx., one of four *Setaria viridis* (L.) P. Beauv., and 13 of 14 *Setaria*

TABLE 3. Populations of aphid vectors of maize dwarf mosaic virus collected in yellow-pan water traps at the Frye, Snyder, and Maurer locations in Wooster, OH, in 1986

Aphid	Mean per trap per day for 7-day period[a]															Average	Percentage of 15-wk total
	17 June	24 June	1 July	8 July	15 July	22 July	29 July	5 Aug	12 Aug	19 Aug	26 Aug	2 Sept	9 Sept	16 Sept	23 Sept		
Rhopalosiphum maidis	0.03	0.00	0.02	0.59	0.63	6.80	14.60	24.88	4.99	0.77	0.49	1.48	0.67	0.86	0.68	3.83	53.38
Myzus persicae	0.00	0.00	0.03	0.08	0.16	0.98	2.85	1.15	1.29	3.23	3.55	1.16	0.45	0.08	0.19	1.01	14.11
Aphis gossypii	0.00	0.02	0.03	0.02	0.21	0.71	1.53	2.80	0.69	0.16	0.07	0.03	0.00	0.00	0.00	0.42	5.82
Therioaphis maculata	0.00	0.03	0.01	0.08	0.08	0.42	1.43	1.12	0.11	0.24	0.15	0.04	0.01	0.00	0.01	0.25	3.46
Hyalopterus atriplicis	0.04	0.13	0.49	0.16	0.27	0.30	0.20	0.45	0.13	0.21	0.07	0.08	0.08	0.03	0.02	0.18	2.47
Aphis craccivora	0.02	0.03	0.09	0.12	0.18	0.19	0.16	0.80	0.57	0.51	0.26	0.03	0.04	0.04	0.07	0.21	2.89
Hyadaphis erysimi	0.05	0.10	0.08	0.11	0.31	0.09	0.24	0.34	0.20	0.07	0.03	0.02	0.04	0.01	0.00	0.11	1.57
Three species[b]	0.09	0.05	0.11	0.33	0.71	1.80	2.16	1.78	0.90	0.99	0.58	0.24	0.08	0.09	0.04	0.66	9.24
Unidentified aphids	0.07	0.05	0.12	0.30	0.07	0.25	1.05	2.27	1.22	0.74	0.37	0.30	0.21	0.25	0.37	0.51	7.09
Total aphids	0.30	0.41	0.98	1.79	2.62	11.54	24.22	35.59	10.10	6.92	5.57	3.38	1.58	1.36	1.38	7.18	...

[a] Dates are the actual beginning dates in 1986.
[b] Total for *Macrosiphum euphorbiae*, *Aphis maidiradicis*, and *Dactynotus ambrosiae*.

TABLE 4. Populations of aphid vectors of maize dwarf mosaic virus collected in yellow-pan water traps near Wooster, OH, in 1987

Aphid	Mean per trap per day for 7-day period[a]								Average	Percentage of 8-wk total
	30 June	7 July	14 July	21 July	28 July	4 Aug	11 Aug	18 Aug		
Rhopalosiphum maidis	0.01	0.14	0.84	7.38	13.44	5.84	1.59	0.47	3.71	39.4
Myzus persicae	0.18	0.50	3.50	1.78	2.81	4.78	1.43	2.42	2.18	23.1
Aphis gossypii	0.07	0.21	1.67	2.46	1.54	0.90	0.29	0.23	0.92	9.8
Therioaphis maculata	0.02	0.16	0.83	1.46	1.19	0.54	0.12	0.10	0.55	5.9
Hyalopterus atriplicis	0.56	1.13	0.45	0.51	0.24	0.26	0.06	0.11	0.42	4.4
Aphis craccivora	0.03	0.17	0.65	0.60	0.70	0.24	0.34	0.11	0.36	3.8
Hyadaphis erysimi	0.01	0.03	0.19	0.56	0.88	0.60	0.12	0.08	0.31	3.3
Three species[b]	0.01	0.16	0.86	1.08	0.57	0.34	0.15	0.21	0.42	4.5
Unidentified aphids	0.03	0.17	0.50	1.04	1.04	0.84	0.47	0.37	0.56	5.9
Total aphids	0.92	2.68	9.49	16.87	22.42	14.34	4.56	4.08	9.42	...

[a] Dates are the actual beginning dates in 1987.
[b] Total for *Macrosiphum euphorbiae*, *Aphis maidiradicis*, and *Dactynotus ambrosiae*, each species having less than 2% of the 8-wk total.

P. Beauv. spp. were infected with MDMV-B, but the infected plants were found in late August and early September. BMV and WSMV were detected in 32 of 42 and 18 of 60 samples tested, respectively. During the same period, 266 maize leaf samples with virus symptoms were randomly collected from commercial sweet corn fields. MDMV-A, -B, or both MDMV-A and -B were identified from 20, 184, and 35 maize leaf samples, respectively. BMV and both BMV and MDMV-B were detected from two different maize leaf samples. Maize subtle mosaic virus was detected from 25 maize leaf samples.

DISCUSSION

Trap plants, successive plantings, and grass weed hosts were used to determine MDM onset. Grass weeds are implicated as a natural source of MDMV (15) and the use of perennial grass weeds as test plants should most closely approximate the overwintering of MDMV in nature. MDMV, however, was not detected in the grass weeds in tile plots during the 3-yr exposure period. The reason for the lack of infection is unknown, but the physiological state of the plants in late season may have increased their resistance to MDMV infection at a time when aphid activity was greatest. In addition, the small plot size most likely reduced the probability of infection. The lack of infections in grass weeds in tile plots suggested that this method, using these grass species, will not be useful for studying MDM onset in maize.

Trap plant and successive planting methods were useful for monitoring MDM onsets. Both have several attractive attributes; they also have major limitations. The trap plant/source plant method allows for precise control of the exposure period. When exposure periods are short, confounding effects of secondary spread are eliminated. The method was suitable for detecting and measuring disease in areas where the incidence of MDM is high (11). This method, however, is labor-intensive and requires much greenhouse space. Use of 50 plant units often may be the minimum for virus detection. Successive plantings have other limitations. They require suitable weather for seedbed preparation, seed germination, and plant growth. Seeding plots according to a schedule is always problematic. Furthermore, successive planting designs are usually confounded by secondary spread. Successive plantings were useful at the Wooster location for the detection of MDM onset because it is outside the natural range of johnsongrass, and low MDM incidences are known to occur only late in a growing season (R. Louie and J. K. Knoke, *unpublished*). In this study, the successive plantings detected MDM onset 42 and 12 days earlier in 1986 and 1987, respectively, than did the standard trap plant plots. The larger plot size of the successive plantings most likely increased its effectiveness.

Some interpretation problems occurred in the ANOVA where treatments were replicated by location because of the large land area requirement. For example, infected plants in the successive plantings averaged 0.3, 0.1, and 0.1% on 5–6 August for the third planting and averaged 1.2, 14.8, and 1.0% on 29 August for the fourth planting, for locations 1, 2, and 3, respectively, in 1986. In 1987, infected plants averaged 0.5, 9.0, and 0.0% on 31 July for the fourth planting and averaged 3.6, 2.4, and 5.9% on 13 August for the fifth planting, for locations 1, 2, and 3, respectively. Location was not significant based on ANOVA, whereas differences in a particular planting at different locations appeared to be substantial. The differences may not be significant because there were so few instances of high levels of infections when comparing locations over all time periods. These few instances of high levels of infections could be biologically significant. It means that MDM epidemics are related to factors that are site-specific. The raw data, in fact, suggested an interaction of virus source with vectors at the different locations. The effect of location could be demonstrated by repeating the experiment over a period of years and then analyzing the interaction of the location by planting time with the year as replication.

Aphid populations in 1986 and 1987 differed from the 1967–1977 and 1978–1985 averages for northern Ohio. Compared to the 1967–1977 average population, both 1986 and 1987

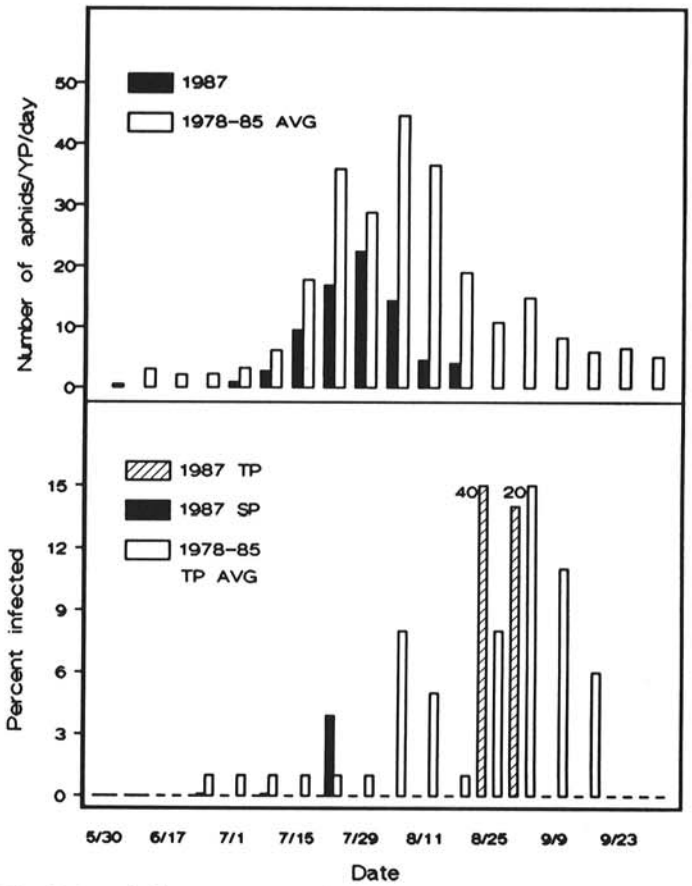

Fig. 2. Associations between aphid numbers and the onset of MDM in trap plants (TP) and successive plantings (SP) for 1987 and the period 1978–1985.

TABLE 5. Estimated parameters[a] and regression statistics[b] for the stepwise regression analysis of the proportion of trap plants infected with maize dwarf mosaic virus on the populations of individual aphid species at Frye (1), Snyder (2), and Wagner (3) locations in Wooster, OH, 1987

Location	*Y*-intercept	Estimated parameters				S	*F*	df	R^2
		Rm	Ac	Ag	Ha				
1	30.67	31.1 (0.77)	−40.3 (1.43)	7.1 (0.83)	−4.7 (0.92)	0.70	1,092.2	4,3	0.999
2	13.14	15.0 (2.93)				7.18	26.2**	1,6	0.814
3	21.36	13.4				8.36	18.4**	1,6	0.754
All	13.37	15.1 (1.83)				8.19	67.9**	1,22	0.755

[a] Coefficients for species in the regression model after stepwise elimination, with standard deviations in parentheses; Rm = *Rhopalosiphum maidis*, Ac = *Aphis craccivora*, Ag = *Aphis gossypii*, Ha = *Hyalopterus atriplicis*.
[b] S = standard deviation about the regression surface; *F* = *F*-statistic; ** = significant at $P = 0.01$; R^2 = coefficient of determination.

populations peaked in early August and at a level much below the 10-yr average of 300 aphids per trap per day in late August (6). Although the numbers of aphids per yellow pan per week were lower in these 2 yr than in previous years, the occurrence of high levels of MDM in late season remained constant. *M. persicae*, the second most numerous species, was not statistically associated with the occurrence of MDMV transmission in 1987. This variance was expected because it previously was implicated as a probable vector in Ohio in only 4 of 6 yr and was more important in the southern than in the northern areas of the state (13).

The trap plant and assay data in these studies lend support to the hypothesis that MDM onset in northern Ohio results from the interactions of aphid vectors with site-specific infected weed host(s). At locations 1 and 3, where the source plants were no more than 75 m away, infections in the standard trap plants began simultaneously with the exposure of virus source plants. At location 2, the source plants were over 800 m away and infections in the standard trap plants occurred only in late season, reflecting the natural inoculum source at that site. We also confirmed Abt's (1) finding that MDMV-B was most often isolated from assays of infected maize and weeds from commercial fields in northern Ohio. This occurrence of MDMV-B in the most northern maize fields in Ohio argues against migration of viruliferous vectors from southern states or southern Ohio where MDMV-A predominates (7). MDMV-B also was the predominant strain isolated from the successive plantings at the Wooster location in 1986. However, in 1987, MDMV-A predominated when MDMV-A was used as the virus source in the modified plots, and this source was probably more significant to the spread of MDMV in our plots than the natural inoculum source.

Continued transmission of MDMV from source plants to trap plants in the modified trap plant plots showed that aphid species transmitting MDMV were present throughout the season. The effect of late season viruliferous aphids at the different locations also was apparent in the modified trap plant plots. The increase in disease incidence with increased amounts of virus inoculum and the decrease in disease incidence with increased distance from the virus source were most obvious in the first six exposure periods. This association was not obvious in exposure periods seven and eight, and reflected the increased activity of viruliferous aphids that tempered the effects of inoculum source and the distance from source (6).

The spatial distributions of infected plants in the successive plantings' plots were few, widely distributed, and isolated. Subsequent occurrences of infected plants clustered around the earlier ones. This pattern of spread is best explained by infected seed or by the initiation of infection within the plot from a few viruliferous aphids (14).

The occurrences of MDMV-A mostly in southern and MDMV-B mostly in northern Ohio do not support the aphid flight hypothesis of Zeyen et al (19). The absence of virus infections, in either early and mid-season commercial plantings (1; R. Louie and J. K. Knoke, *unpublished*) or in our early successive plantings when vector populations were low, also do not support a seed transmission hypothesis for MDM onset. Our observations in this study suggested that virus inoculum and not aphid vectors was most likely the limiting factor for disease onset in northern Ohio. The occurrence of MDM later in the season and the difficulty in finding alternate host(s) of MDMV-A or -B reaffirm the suggestion of an uncommon or rarely infected summer weed host (15). The weed host is possibly a symptomless carrier and/or one with low virus titer.

The weed host hypothesis was favored by Abt (1) and Studenroth (18). Based on the late season infections in trap plants and successive plantings, the random spatial patterns of disease plants in successive plantings, and assay data of weeds and infected maize, we similarly concluded that MDM onsets in northern Ohio were most likely associated with an uncommon or rarely infected weed host(s) that is not yet discovered. Furthermore, we concluded that the onsets of MDM were site-specific.

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