Spread of Maize Dwarf Mosaic Virus from Johnsongrass to Corn

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ABSTRACT

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Spread of maize dwarf mosaic virus (MDMV) from introduced virus-infected johnsongrass to adjacent susceptible corn in experimental plots was evaluated during 1979 and 1980. The relationship between MDM incidence in corn and distance from the source was adequately described by the model $Y = a(\exp(-bD))$, where Y is disease incidence at distance D from the source, b is the spread coefficient, and a is the scaling factor. In both years, b was significantly greater than zero, demonstrating that MDMV spread to the corn test plots. In control plots with no intentionally placed virus source, b values and disease incidence were lower than in test plots. For a single planting in 1979, the steepness of the gradient of disease incidence from the source (quantified by b) decreased with time as fewer plants remained uninfected. For two successive plantings in 1980, no significant difference in b values was observed. MDMV spread also was not related to prevalent wind direction. No spread of maize chlorotic dwarf virus (MCDV) to corn was observed by symptomatology even though 90 and 50% of the johnsongrass plants were infected in 1979 and 1980, respectively, as indicated by enzyme-linked immunosorbent assay. The leafhopper vector of MCDV, *Graminella nigrifrons*, was also present during both years. The presence of MDMV in the control plots indicates that there were virus sources other than johnsongrass or that MDMV moved more than 400 m from johnsongrass to the control plots.

Additional key words: *Sorghum halepense, Zea mays*

Johnsongrass (*Sorghum halepense* (L.) Pers.) is a perennial weed susceptible to strain A of maize dwarf mosaic virus (MDMV-A) and maize chlorotic dwarf virus (MCDV) (1,6,12). MDMV is transmitted by at least 23 aphid species (6), whereas MCDV is transmitted principally by the leafhopper *Graminella nigrifrons* (Forbes) (13). For outbreaks of MDMV-A and MCDV in areas where johnsongrass occurs, it is generally assumed that infected johnsongrass is the main virus source for initial vector acquisition and subsequent inoculation of corn (*Zea mays* L.) (1). This assumption has not been demonstrated in replicated field experiments. The source of strain B of MDMV (MDMV-B) is unknown for occurrences in either johnsongrass or nonjohnsongrass areas, although many susceptible grasses have been reported (14).

In 1979 and 1980, the spread of MDMV-A and MCDV from a small area of introduced, infected johnsongrass to plots of susceptible corn genotypes was studied at Wooster, OH, where johnsongrass is not indigenous. A nonlinear regression model was used to quantify these data and explain spread within plots.

Cooperative investigations of ARS/USDA and the Ohio Agricultural Research and Development Center (OARDC), Wooster.

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MATERIALS AND METHODS

Cultural conditions. Six 30.5 × 30.5 m (930 m^2) plots were hand-planted to 40 rows each of WF9xOh51A dent corn on 27 July 1979. Rows, planted in an east-west direction, were 76 cm apart and the planting interval within each row was ~30 cm. Plots were isolated from each other and from other known corn virus sources by at least 400 m. The southwest (SW) corner of three plots (replicates) was adjacent to a 6.1 × 6.1 m (37 m^2) area containing ~100 MDMV-MCDV-infected johnsongrass plants. These plants, in 10-cm-diameter soil plugs, had been collected near Portsmouth, OH, and were emerging from rhizomes. A bare soil area was adjacent to the SW corner of three control plots.

Disease incidence was estimated by surveying corn plants for diagnostic virus symptoms at 18, 21, 28, and 35 days after planting (9). Five north-south line markers were positioned across the corn rows at 6.1-m intervals beginning ~3 m from the western edge of each plot. Five plants to the east and west of these markers in each row were surveyed, resulting in 200 10-plant samples per plot. Disease incidence was recorded as the proportion of plants in each sample that showed disease symptoms. Straight-line distances from the johnsongrass to the center of each 10-plant sample were calculated. For regression analysis, incidence data in each plot were grouped in 1-m intervals, eg, all observations 20–21 m from johnsongrass were averaged to calculate incidence at 20.5 m.

In 1980, PAG 246006 and Agway XP708 were each machine-planted in four plots on 11 June, 15 July, 8 August, and 4 September. The sweet corn hybrid Agway XP708 is susceptible to MDMV and MCDV, whereas the dent corn hybrid PAG 246006 is resistant to MDMV. Two plots were 3,906 m^2, and the other two were 976 m^2. The centers of the larger plots had a 37-m^2 area containing about 100 MDMV-MCDV-infected johnsongrass plants. The two smaller plots were used as controls. For each planting date, the two hybrids were planted in alternate rows 76 cm apart in both a north-south and east-west direction. In each plot on the first planting date, areas for two rows were left unplanted between each pair of planted rows. These originally unseeded areas were planted on the second planting date before plants from the alternate two rows of the first seeding were removed. As a result, plants of the third planting occupied the same areas as the first planting; the fourth planting occupied the same areas as the second planting. All plants were removed from a planting before plant emergence from a subsequent seeding. After emergence, plants in the perimeters of squares formed by the four intersections of the north-south and east-west planted paired rows were used as subplot sample units. Each unit contained ~10 plants of each hybrid and these plants enclosed an area of 0.58 m^2. All plants outside these units were removed, leaving a 2.3-m bare soil area between adjacent subplots.

Disease incidence was recorded as the proportion of 10 plants of each corn hybrid in each subplot that showed virus disease symptoms ~21 days after planting. As in 1979, straight-line distances from the johnsongrass (or the fallow plot center) to the center of each subplot were calculated. Incidence data

in each plot were also grouped into 1-m intervals.

Aphids were trapped in all fields during both years by using 30.5 × 30.5 × 10.2 cm yellow pan traps as described previously (7). *G. nigrifrons* leafhoppers were caught with a modified Johnson-Taylor trap (5) near the corn plots in each year. Insects were removed from the traps at periodic intervals (between daily and weekly), and daily averages were calculated.

Assays. Assays of corn for MDMV-A and MDMV-B and of johnsongrass for MDMV-A were performed by enzyme-linked immunosorbent assay (EIA) as described previously (11). Exceptions were the coating γ-globulin concentrations at 1 μg/ml and the use of γ-globulin-alkaline phosphatase conjugate dilutions of 1:800 for both MDMV-A and MDMV-B assays. The substrate (*p*-nitrophenyl phosphate) conversion reaction to form *p*-nitrophenol (yellow coloration) in the presence of alkaline phosphatase was stopped at 2 hr by addition of 0.03 ml of 3N sodium hydroxide solution per well. Each well was filled with 0.07 ml of 10% diethanolamine, pH 9.8, to permit absorbance readings at 405 nm with a Gilford Stasar II spectrophotometer equipped with a rapid sampling cuvette and coupled to a model 4009 Data Tester (Gilford Instrument Laboratories, Inc., Oberlin, OH 44074) for recording absorbance values. Mean absorbances and standard deviations were calculated for test and control samples. Test samples with differences greater than three standard deviations from the negative controls were scored positive. Negative controls in MDMV-A assays usually were MCDV-infected and uninfected corn leaf extracts, and in MDMV-B assays, MDMV-A-infected and uninfected extracts. When A_{405} and visual scorings did not agree, the visual scorings were accepted as correct, ie, a yellow color was scored positive for virus and a colorless product was scored negative.

Assays of johnsongrass for MCDV were also performed by EIA. The coating γ-globulin concentration was 1 μg/ml and the γ-globulin alkaline phosphatase conjugate dilution was 1:100 for johnsongrass samples 1–241, 1:200 for samples 242–350, 1:200 for the 1979 corn samples, and 1:800 for the 1980 corn and johnsongrass samples. Negative antigen controls were MDMV-A-infected and uninfected corn leaf extracts.

Extracts were prepared as described previously (11) except the extract was not filtered through cheesecloth or centrifuged. Antisera to both MDMV strains (7) and MCDV (2) were raised as described elsewhere.

Data analyses. The relationship between MDM disease incidence and distance from johnsongrass was described by the empirical model:

$$Y = a(exp(-bD)) \quad (1)$$

where Y is disease incidence at a distance D from the source; b is the spread coefficient; a is the scaling factor; and $exp()$ represents e (2.718) raised to a specified power, namely $-bD$ (4). Spread from the source is indicated by a gradient that is quantified by a b value significantly greater than zero. The parameters (a and b) were estimated from each data set by using ordinary least-squares regression after first transforming equation 1 to:

$$\ln(Y) = \ln(a) - bD \quad (2)$$

where ln represents the natural log transformation. Equation 2 is a straight line with slope $-b$ and intercept $\ln(a)$. The parameters were estimated for each survey time in 1979 in the plots with and without the johnsongrass virus sources. In 1980, disease symptoms were not observed in the first planting, and corn was killed by frost in the last planting. Gradients were thus measured in the two remaining plantings.

In 1979, analysis of variance (ANOVA) was used to determine the effect of virus source and time since planting on spread of MDMV (represented by b) and on disease incidence. The experiment consisted of a randomized factorial with replicate, virus source, and time since planting as factors. In 1980, ANOVA was used to determine the effect of planting time, direction, and virus source on spread (b) and disease incidence. The 1980 experimental design was a randomized factorial with replicate, planting time, direction, and virus source as factors. Furthermore, the logit transformation (15) was made on the MDM incidence data in 1979 and regressed against time, using first-difference regression (10). Separate regressions were performed for the test and control plots. The first-difference regression parameter is an estimate of the apparent infection rate sensu Vanderplank (10).

RESULTS

1979. Results of assays of johnsongrass plants from the three test plots were: Plot 1—85 of 96 assayed plants infected with MDMV-A (89%) and 92 of 96 with MCDV (96%); Plot 2—94 with MDMV-A (94%) and 93 with MCDV (93%) per 100 assayed plants, respectively; Plot 3—75 with MDMV-A (77%) and 78 with MCDV (80%) per 97 assayed plants, respectively. Thus, for the three johnsongrass source plots, MDMV-A incidence was 87% and MCDV incidence was 90%.

Corn plants with mosaic symptoms (32 days after planting) in the control plots were assayed for MDMV-A and MDMV-B by EIA. Assay results for plants from the three plots were: Plot 1—eight infected with MDMV-A and nine with MDMV-B per 10 assayed plants, Plot 2—four with MDMV-A and 10 with MDMV-B per 10 assayed plants, and Plot 3—six with MDMV-A and 10 with MDMV-B per 10 plants. Thus, among the corn plants with mosaic symptoms in the three control plots, 60% were infected with MDMV-A, 97% with MDMV-B, and 57% doubly infected with both strains.

MDM incidence in relation to distance from johnsongrass virus source for one test plot at 18 days after planting (first assessment date) is presented in Figure 1. This pattern was typical for all plots adjacent to a virus source in 1979 and also was typical for the second and third plantings of 1980. The predicted levels of disease incidence as far as 40 m from the johnsongrass virus source for the first three assessment dates in 1979 (based on regression analysis) are plotted in Figure 2. The predicted values represent the average levels of MDM incidence as a function of distance from the johnsongrass. All plants were infected by 35 days after planting in the plots adjacent to johnsongrass, and therefore no gradients existed.

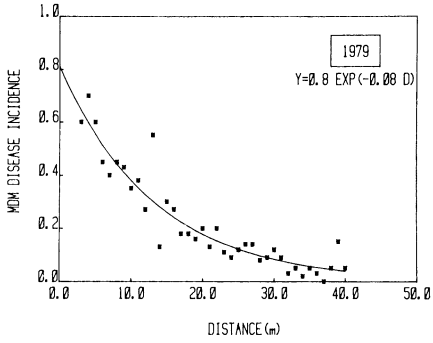

Fig. 1. Maize dwarf mosaic (MDM) incidence in relation to distance from virus-infected johnsongrass 18 days after planting in a single plot. Predicted incidence based on the estimated parameters of the nonlinear regression equation is represented by the solid line. Coefficient of determination equaled 0.88.

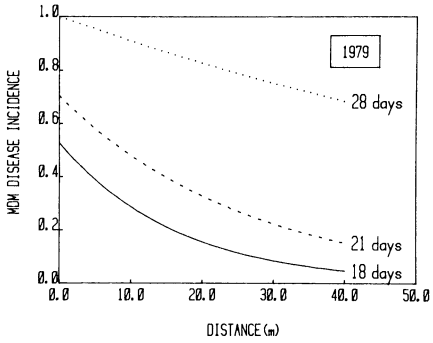

Fig. 2. Average predicted maize dwarf mosaic (MDM) incidence in relation to distance from a small area of virus-infected johnsongrass at three assessment times after planting in 1979. Predicted values were obtained by estimating the parameters of equation 1 at each of the three assessment times for each replicate, then averaging the estimated parameters for all three replicates.

ANOVA indicated a significant effect of time and treatment (johnsongrass vs. control) on the spread coefficient b. The gradients flattened over time in test plots (Table 1, Fig. 2), and by 35 days no gradient existed. Some control plots exhibited a negative gradient ($b \leq 0$), ie, increasing disease incidence with distance from the fallow ground. This indicated sources of MDMV other than the introduced johnsongrass. ANOVA indicated significant effects of time, treatment, and the treatment × time interaction on MDM incidence. Incidence increased over time both in the controls and test plots (Table 2). The significant interaction indicated that the rate of disease increase differed between the controls and test plots. This was substantiated by differences in the apparent infection rates (r). The test plots (adjacent to johnsongrass) had an r value equal to 0.49/day; control plots had an r value equal to 0.27/day. These two values were significantly different from each other ($P = 0.05$) according to a t test.

ANOVA indicated a significant effect only of time on the number of trapped aphids. For the weekly periods ending 14, 21, 28, and 35 days after planting, the number of aphids per trap per day averaged 74, 11, 11, and 12, respectively.

Plants with MCDV symptoms were not observed in any of the plots. An average of 333 *G. nigrifrons* leafhoppers per day were trapped during the first 28 days after planting.

1980. Johnsongrass plants from the two test plots were assayed for MDMV-A and MCDV early in the season. Forty-seven and 40 plants of the 53 assayed were infected with MDMV-A and MCDV, respectively, for Plot 1; 40 and 12 of 50 assayed plants were infected with MDMV-A and MCDV, respectively, for Plot 2. For these samples, overall percentages of infection were 84% for MDMV-A and 50% for MCDV. These johnsongrass plants were transplanted from the johnsongrass plots established in 1979.

Predicted MDM incidences in XP708 in relation to distance from johnsongrass for the second and third plantings of 1980 are plotted in Figure 3A. The predicted values for the four directions away from johnsongrass are presented in Figure 3B. Only the presence of johnsongrass significantly affected the spread coefficient or disease incidence (Table 3). All other main effects and all interactions were nonsignificant. The control plot exhibited no gradients (b was not significantly different from zero) and had about half the MDM incidence of the plots adjacent to johnsongrass (Table 3).

ANOVA indicated a significant effect only of planting time on the number of trapped aphids. During the second and third plantings, the number of aphids per trap per day averaged 15 and five, respectively.

MCDV symptoms were not observed on plants of either hybrid. An average of 144 and 196 *G. nigrifrons* leafhoppers per day were trapped during the second and third planting, respectively.

DISCUSSION

Researchers discussing the survival of MDMV-A and MCDV between growing seasons have identified johnsongrass as the principal reservoir (1,6,16). Although it has been amply demonstrated that johnsongrass is readily infected by both viruses in experimental inoculations (6,12) and these viruses have been identified in field-grown johnsongrass (6,13), there are no reports of the incidence of the two viruses in a large sample of field-collected plants. Our EIA results show a high incidence of both viruses (87% of plants infected with MDMV-A and 90% with MCDV in 1979) in a collection of 350 plants and suggest that this incidence should provide an ample source for vectors to transmit the two viruses to the developing corn crop.

The presence of MDM disease gradients ($b > 0$) away from virus-infected johnsongrass indicated that johnsongrass served as a source of MDMV-A. The significantly higher level of MDM incidence in the test plots compared with the controls also suggests that johnsongrass was the source of MDMV at least for the initial infections. The occurrence of MDMV-A in the control plots suggests that the virus may have spread beyond the immediate vicinity of the johnsongrass source located at a minimum of 400 m from the control plots. It is also possible that other sources of the virus existed and

Table 1. Average values of the spread coefficient[x] in corn plots adjacent to johnsongrass or bare soil (controls) in 1979

Time[y]	Johnsongrass	Control	Mean[z]
18	0.061	−0.006	0.028 a
21	0.038	−0.015	0.012 a
28	0.010	−0.032	−0.011 b
35	0.000	−0.032	−0.016 b
Mean[z]	0.027 A	−0.021 B	

[x] Spread coefficient (b) is a measure of disease gradient from a source and was estimated from equation 2.
[y] Days after planting.
[z] Means followed by the same letter (capital or lower case) are not significantly different according to Duncan's modified least significant difference test ($P \simeq 0.05$).

Table 2. Average values of maize dwarf mosaic incidence[x] in corn plots adjacent to johnsongrass or bare soil (controls) in 1979

Time[y]	Johnsongrass[z]	Control[z]
18	0.160 c	0.003 b
21	0.318 c	0.011 b
28	0.782 b	0.059 b
35	1.000 a	0.252 a

[x] Incidence equals the proportion of plants infected by MDMV.
[y] Days after planting.
[z] Means followed by the same letter within a single column are not significantly different according to Duncan's modified least significant difference test ($P \simeq 0.05$).

Table 3. Average values of the spread coefficient[x] and maize dwarf mosaic incidence[y] in corn plots adjacent to johnsongrass or bare soil (control) 21 days after planting in 1980

	Johnsongrass	Control
Spread coefficient[z]	0.094 a	0.001 b
Disease incidence[z]	0.169 A	0.092 B

[x] Spread coefficient (b) is a measure of disease gradient from a source and was estimated from equation 2.
[y] Incidence equals the proportion of plants infected by MDMV.
[z] Means followed by the same letter (capital or lower case) in a single row are not significantly different according to Duncan's modified least significant difference test ($P \simeq 0.05$).

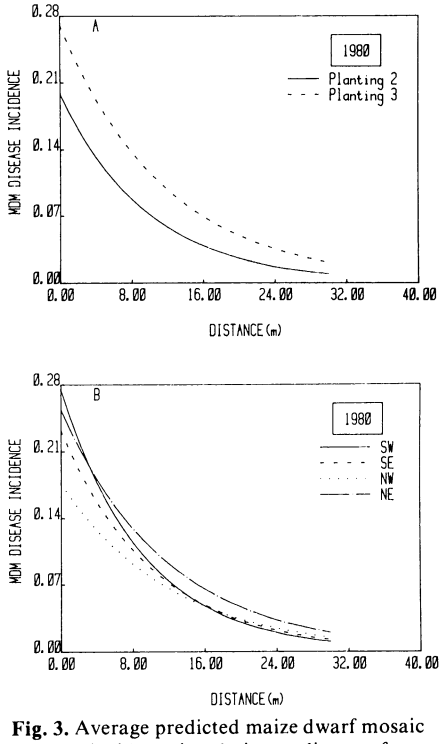

Fig. 3. Average predicted maize dwarf mosaic (MDM) incidence in relation to distance from a small area of virus-infected johnsongrass at **(A)** two planting dates and **(B)** four directions from the virus source in 1980. Predicted values were obtained by estimating the parameters of equation 1 for each planting time, direction, and replicate, then averaging the estimated parameters for both replicates.

contributed to MDMV-A occurrence in these nonjohnsongrass plots. This conjecture is supported by the even greater incidence of MDMV-B in samples from the control plots; johnsongrass does not serve as a source for this strain. Unfortunately, we have no information on the likely MDMV-B source, although on other occasions we have reviewed possible sources (1).

Several models have been proposed for describing disease-distance relationships (3,8) and no single model appears to be appropriate for all diseases. The exponential model of equation 1 gave the best fit for the majority of the data sets based on coefficients of determination (R^2) and the random pattern of the residuals (L. V. Madden, *unpublished*). For example, the exponential model (equation 1) fit the data in Figure 1 with $R^2 = 0.88$. Gregory's "log-log" model, $Y = aD^{-b}$, fit the same data with $R^2 = 0.72$. All regressions of ln (Y) on D (ie, equation 2) in the test plots except for 35 days after planting in 1979 were significant at $P < 0.05$. Gregory (3) stated that in a single planting, infection gradients flatten over time as fewer plants remain uninfected and secondary foci develop. We confirmed this postulate in 1979 (Fig. 2, Tables 1 and 2). In the successive (independent) plantings of 1980, where flattening of gradients need not occur, we observed no difference either in gradients or MDM disease incidence (Fig. 3, Table 3).

Surprisingly, we did not find an influence of direction from johnsongrass on spread MDMV (Fig. 3B) even though the prevalent wind direction was from the SW. This could be attributed to averaging wind directions for extended periods of time (~3 wk). Air actually moved in all directions to some extent during those periods. Brief gusts of wind may be sufficient to carry aphids in any direction.

The failure to observe MCDV-infected corn plants by symptomatology in 1979, even though EIA detected the virus in johnsongrass, was attributed to prominent MDM mosaic symptoms masking the characteristic veinclearing symptoms of MCDV. EIA also detected MCDV in 19 of 30 MCD-symptomless corn plants collected in test plots and 24 of 30 MCD-symptomless plants from control plots (*unpublished*). To alleviate the problem of potential symptom masking in 1980, a corn line that was resistant to MDMV but susceptible to MCDV was planted with an MDMV-susceptible line. Nevertheless, no MCDV-infected plants were observed in the field even though 50% of the johnsongrass plants were infected, according to EIA results. It is possible that there were errors with EIA for detecting MCDV in corn, although there have never been indications that the technique was performed incorrectly. The vector of MCDV was also present during both years. We are thus unable to explain the lack of spread of MCDV.

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