Effect of Plant Age at Time of Inoculation with Maize Dwarf Mosaic Virus on Disease Development and Yield in Corn

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ABSTRACT

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When corn (*Zea mays*) plants of two susceptible hybrids were manually inoculated with maize dwarf mosaic virus strain A (MDMV-A) in the three-, five-, seven-, nine-, and 11-leaf stages during two growing seasons, the highest disease incidence (58%) and the greatest yield reduction (23%) were obtained in plants inoculated at the five-leaf stage. There was no significant difference in disease incidence or in yield reduction between plants inoculated in the three-leaf stage and those inoculated in the seven-leaf stage when data for the two hybrids were combined. Both sets of plants were 47-50% diseased and showed a yield reduction of 15-16%. A delay in inoculation until the 11-leaf stage resulted in a disease incidence one-half as great and a yield reduction one-third as great as those obtained for plants inoculated in the five-leaf stage. Groups of plants inoculated at different stages showed no significant difference in plant height. The average height reduction in all inoculated plants of both hybrids in the 2 yr amounted to 5.4%. Calculations of extrapolated yields for a hypothetical 100% infection rate revealed that yield losses would be surprisingly uniform (36% on the average) for plants inoculated between the three-leaf and the 11-leaf stages. Under particularly favorable conditions for infection and disease development, MDMV-A has the potential for reducing grain yield in a highly susceptible hybrid by as much as 45%.

Additional key words: maize, sugarcane mosaic virus, seedling susceptibility.

Maize dwarf mosaic virus (MDMV) has been known to occur in Mississippi since 1964 (3), but the effect of this virus on grain yield of corn (*Zea mays* L.) has been difficult to determine because of the high incidence of natural infection occurring in experimental fields. To obtain meaningful data, susceptible genotypes should be used and, at the same time, the noninoculated control plots should remain as disease-free as possible. The incidence of MDMV strain A (MDMV-A) was drastically reduced following the eradication of johnsongrass [*Sorghum halepense* (L.) Pers.], the reservoir host of MDMV-A, from an area of the University farms at Mississippi State. This offered an opportunity to grow MDMV-A susceptible corn with little interference from natural infection.

Some studies have been reported on the effect of the date of planting of corn on the natural incidence or severity of maize dwarf mosaic (MDM) (1, 2). However, the only reported study involving inoculation of corn with MDMV in the field at more than one stage of growth and relating the subsequent disease to a reduction in grain yield is that of Scheifele (4). He inoculated susceptible and resistant segregates of a three-way hybrid with MDMV-A and MDMV-B at two stages of growth: when plants had three to four leaves (early) and when they were knee high (late). The grain yields for the early MDMV-A-inoculated susceptible segregates, the late MDMV-A-inoculated susceptible segregates, and the noninoculated control plants were 38.9, 49.6, and 61.5 q/ha (62, 79, and 98 bu/acre), respectively. Compared to the control plants, the early inoculation resulted in a 36.7% reduction in grain yield and the late inoculation resulted in a 19.4% reduction in grain yield. Unfortunately, no information was given on the incidence of the disease for the two inoculation dates.

Since natural inoculation with MDMV by aphid vectors in the field is a continuous process, we wished to make the artificial inoculations at as many stages of plant growth as practically possible. We wanted to determine if a correlation could be observed between the age of the plant at inoculation and the resulting disease incidence, symptom severity, reduction in plant height, and decrease in shelled grain yield. The generally held assumption that the earlier in the life of the plant virus infection occurs, the greater the damage to the plant, was to be tested.

MATERIALS AND METHODS

Two corn genotypes were used as test plants: Mp490 × Mo12, a yellow experimental cross, and Pioneer Brand 511A, a white commercial double-cross hybrid. The latter has been grown extensively in Mississippi and other southern states. Previous tests had shown that both

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hybrids were about equally susceptible to MDMV-A. The virus used was MDMV strain A which had been isolated from johnsongrass in Yazoo County, Mississippi, in 1965. This isolate has been maintained in sweet corn cultivar Seneca Chief, which also served as the source material for the inoculum.

The juice from fresh, infected plants, which had been inoculated 3-4 wk earlier, was extracted by grinding the leaf tissue in a blender with a gradual addition of two parts of 0.05 M potassium phosphate buffer, pH 7.0, to one part of plant material (v/w) and straining the pulp through several layers of gauze. With this dilution, we obtained 2.5 ml of inoculum for each gram of leaf tissue. To prevent any deleterious effect on the virus infectivity by the heat generated from blending, we used chilled buffer solution and blended the tissue for less than 1 min at a time and placed the blender vessel in ice between the four blending runs.

The inoculum was taken to the field in an ice chest and only a portion was used for inoculation at a time. Five g of 22-μm (600-mesh) silicon carbide was mixed with 350 ml of inoculum, and the abrasive was kept in susepension by periodically shaking the plastic bottle containing the inoculum. Inoculation was made with an artist's air brush operated at a constant air pressure of 90 psi (6.3 kg/cm^2), and the nozzle was kept less than 2 cm from the leaf surface. The tractor, from which the inoculation was made by two persons (each inoculating one row of plants), was driven at the slowest speed possible without stalling (approximately 1.6 km/hr). We sprayed the inoculum to as much leaf surface as time permitted, aiming the nozzle first at the leaf whorl.

The experiment was conducted in the same field in 1975 and 1976. We used a randomized complete block design with four replications. A plot consisted of two adjacent rows, each 5.08 m (16.67 ft) long, with rows spaced 1.02 m (40 inches) apart. Thirty seeds were planted per row, and plants were thinned to 20 per row. Thus, each plot comprised 40 plants. This corresponded to 38,800 plants/hectare (15,700 plants/acre).

Five inoculations were made, each on progressively older plants, and plants in one plot of each replication were left noninoculated to serve as checks. The first inoculation was made after all seedlings had fully emerged, at that time when most seedlings had reached the three-leaf stage. Other plots were inoculated at two-leaf intervals until it was no longer possible to move the tractor through the plots without seriously damaging the plants. Thus, plants were inoculated in the three-, five-, seven-, nine-, and 11-leaf stages.

Six wk after the last inoculation, disease incidence and severity were determined. Each plant was rated for disease severity on a scale of 1 to 4, with 1 representing symptomless plants, and 2, 3, and 4 denoting mild, intermediate, and severe mosaic symptoms, respectively. The amount of mosaic symptom covering the total foliage of each plant served as the basis for determining the symptom severity. The mean disease severity index for each inoculation date was calculated by adding the ratings assigned to individual diseased plants and dividing the sum by the number of diseased plants.

To determine the plant height, all plants in each plot were measured after the ears were fully developed. The amount of lodging in each plot was recorded at the time of harvest. All ears in each plot were harvested, weighed, and the moisture content of the grain was determined. The plot ear weights were converted to kilograms or quintals of shelled grain per hectare at 15.5% moisture.

In calculating the extrapolated grain yield, the assumption was made that all inoculated plants at each of the five leaf stages became infected. The extrapolated grain yield for each treatment (stage of plant growth at inoculation) was calculated as follows (NI = noninoculated):

(i) (total percent of infected plants in treatment)−(percent of infected plants from natural infection in NI check) = (percent of plants infected from inoculation in treatment);

(ii) 100%−(percent of plants infected from inoculation in treatment) = (percent of plants not infected due to inoculation in treatment);

(iii) (yield of NI check) × [(percent of plants not infected due to inoculation in treatment) ÷ 100] = (yield from healthy plants in treatment);

(iv) (total yield in treatment) − (yield from healthy plants in treatment) = (yield from infected plants in treatment); and

(v) [(yield from infected plants in treatment) ÷ (percent of plants infected from inoculation in treatment)] × 100 = (extrapolated yield from treatment).

RESULTS

The two corn hybrids used in this study reacted differently to inoculation with MDMV-A at the five plant ages tested. Hybrid Mp490 × Mo12 responded with the greatest percent of infected plants when inoculation took place in the five- or seven-leaf stage, whereas Pioneer 511A developed the greatest percent of infection when plants were inoculated in the three- or five-leaf stage. When inoculation was delayed beyond the seven-leaf stage, infection of Mp490 × Mo12 decreased sharply, while the percentage of Pioneer 511A plants infected remained relatively high through the 11-leaf stage (Table 1). These different levels of susceptibility of the two hybrids with plant age were reflected also in their respective disease severity indices. Whereas in Mp490 × Mo12 the disease severity indices decreased gradually with advance in plant age at inoculation time, from 3.7 to 2.4, they remained fairly constant over plant ages in the case of Pioneer 511A, varying between 3.6 and 3.9 (Table 1). During the 2 yr of this study, natural infection by MDMV in noninoculated check plots ranged from 2.5 to 9.3%, with a mean disease incidence of 3.5% for Mp490 × Mo12 and 6.3% for Pioneer 511A (Table 1).

Although both hybrids are considered to be highly susceptible to MDMV-A, there was no appreciable dwarfing of the diseased plants. The average height reduction from all inoculation dates for Mp490 × Mo12 and Pioneer 511A was 3.5% and 7.1%, respectively. The greatest height reduction obtained in this study was 8.4%, which occurred in plants of Pioneer 511A inoculated in the seven-leaf stage.

TABLE 1. Effect of maize dwarf mosaic virus strain A on two corn hybrids inoculated at five stages of growth in the field for 2 yr

Number of leaves at inoculation	Mp490 × Mo12						Pioneer 511A					
	Disease incidence (%)	Symptom severity index	Reduction in height (%)	Stalk lodging (%)	Grain yield (kg/ha)	Reduction in yield (%)	Disease incidence (%)	Symptom severity index	Reduction in height (%)	Stalk lodging (%)	Grain yield (kg/ha)	Reduction in yield (%)
3	41.9[a]	3.7[b]	4.1	14	5,656	19	51.1	3.6	7.5	20	5,675	12
5	63.0	3.1	5.6	17	5,041	28	51.1	3.7	7.5	18	5,349	17
7	61.0	2.9	4.3	18	5,757	18	38.4	3.9	8.4	19	5,650	13
9	29.1	2.7	2.4	15	6,102	13	43.8	3.8	6.2	13	5,945	8
11	16.8	2.4	1.3	20	6,315	10	38.0	3.8	5.7	18	6,121	5
Non-inoculated	3.5	3.0	0.0	6	6,993	0	6.3	2.9	0.0	14	6,460	0

[a]Average percent of infected plants of four replications in each of 2 yr, based on a maximum of 320 plants inoculated at each of the five growth stages plus average percent of infected plants from natural infection in noninoculated plots.

[b]Symptom severity index is based on a rating scale of 1 (symptomless) to 4 (most severe mosaic symptom) and was calculated from the ratings of infected plants only.

TABLE 2. Pooled data on disease incidence, symptom severity, plant height, lodging, and grain yield in Mp490 × Mo12 and Pioneer 511A corn hybrids inoculated with maize dwarf mosaic virus strain A at five stages of growth for 2 yr

Number of leaves at inoculation	Plants infected/ plants inoculated	Symptom severity index[a]	Reduction in height (%)	Stalk lodging (%)	Observed			Extrapolated		
					Diseased plants (%)	Grain yield (kg/ha)	Decrease in yield (%)	Diseased plants (%)	Grain yield (kg/ha)[b]	Decrease in yield (%)
3	291/625	3.7 v[c]	5.7 w	16.6 v	46.6 w	5,669 wx	15.7	100.0	4,200	37.5
5	355/623	3.3 v	6.6 w	15.7 vw	57.7 v	5,198 x	22.8	100.0	3,836	43.0
7	309/625	3.4 vw	6.6 w	18.4 v	49.7 vw	5,706 wx	15.2	100.0	4,451	33.8
9	231/633	3.2 w	4.4 w	12.9 vw	36.6 x	6,026 w	10.4	100.0	4,526	32.7
11	171/623	3.1 wx	3.5 w	18.0 v	27.5 y	6,221 vw	7.5	100.0	4,501	33.1
Noninoculated	31/639	2.8 x	0.0 v	9.7 w	4.9 z	6,723 v	0.0			

[a]Symptom severity index is based on a rating scale of 1 (symptomless) to 4 (most severe mosaic symptom) and was calculated from the ratings of infected plants only.

[b]In calculating the extrapolated grain yield it was assumed that all inoculated plants became diseased.

[c]Means within a column not followed by the same letter differ significantly, $P = .05$.

The two hybrids also behaved differently with regard to stalk lodging. Inoculation with MDMV-A did not contribute markedly to lodging in Pioneer 511A, but in Mp490 × Mo12 three times as many inoculated as uninoculated plants lodged. However, there was no significant difference in the amount of lodging among the groups of plants of both hybrids inoculated at different stages of growth (Table 1 and 2).

All MDMV-A-inoculated sets of plants of both hybrids, regardless of the age at which they were inoculated, produced lower yields of shelled grain than the noninoculated check plants. The only groups of plants that did not differ significantly in yield ($P = 0.05$) from the checks were those inoculated in the 11-leaf stage, but even they consistently yielded lower than the noninoculated plants (Table 1 and 2). The greatest reduction in grain yield occurred when plants of either hybrid were inoculated in the five-leaf stage. There was no difference in yield in either hybrid, between plants inoculated in the three-leaf stage and those inoculated in the seven-leaf stage, although these two sets of plants differed substantially in disease incidence. Hybrid Mp490 × Mo12 had about 45% more infected plants in the group inoculated in the seven-leaf stage than in that inoculated in the three-leaf stage. On the other hand, Pioneer 511A had about 33% more infected plants in plots inoculated in the three-leaf stage than in those inoculated in the seven-leaf stage. In both hybrids the decrease in yield became smaller as the inoculation was postponed beyond the seven-leaf stage. A delay in inoculation until the 11-leaf stage still resulted in a 5-10% yield reduction, depending on the hybrid.

To learn what potential MDMV-A has in reducing grain yield in highly susceptible corn hybrids, we calculated the average hypothetical yields for the two hybrids for a situation in which all inoculated plants would have become infected. Our calculations were based on the actual yield reductions obtained for specific percentages of infected plants inoculated at the five growth stages. These calculations revealed that in a highly susceptible hybrid the yield reduction due to infection by MDMV-A would be fairly uniform regardless of when inoculation took place between the three- and 11-leaf stages, and would amount to an average yield reduction of about 36% (Table 2). The maximum extrapolated yield reduction for the two hybrids at 100% infection was 43.0% for plants inoculated in the five-leaf stage. By comparison, the greatest yield reduction we observed was with Mp490 × Mo12 when it was inoculated in the five-leaf stage. Here, the yield was 28% below that of the noninoculated plants (Table 1).

DISCUSSION

Corn plants inoculated with MDMV-A at the earliest stage of growth (three leaves) were not the most severely affected by this disease. Neither the amount of infection nor the reduction in plant height nor the yield reduction was greater in plants inoculated at the three-leaf stage than in those inoculated at the five-leaf stage. Another surprising result was the lack of difference in yield depression between plants inoculated in the seven-leaf stage and those inoculated in the three-leaf stage, although distinct differences existed between incidences of disease in these two groups of plants. No correlation between the severity of leaf symptoms (amount and intensity of mosaic) and the degree of height reduction was found. Although the greatest plant height reduction occurred in plants inoculated in the five-leaf stage (Mp490 × Mo12) or the seven-leaf stage (Pioneer 511A), these height reductions did not differ significantly (Duncan's multiple range test) from those obtained for plants inoculated earlier or later. The magnitude of reductions in plant height we obtained in this and other studies (5) does not indicate that infection with MDMV-A causes dwarfing in corn. Hence, the designation of the disease as maize dwarf mosaic appears inappropriate, at least for the hybrids tested in these studies.

In susceptible corn, MDMV-A can reduce yields substantially. Scott and Nelson (5) obtained an average reduction in shelled grain yield of 28% for hybrids having one resistant and one susceptible parent. In single-cross hybrids in which both parental lines were susceptible to MDMV-A, the average yield reduction for six such crosses was 40%. In the present study, the average yield reductions were generally smaller. However, an indication of the potential of this virus for reducing grain yields was gained with the cross Mp490 × Mo12, which is made up of two susceptible inbreds. When plants of this hybrid were inoculated with MDMV-A in the five-leaf stage in 1975, 84% of the inoculated plants became infected, and the resulting yield reduction was 46.5%. This decrease in yield was greater than the 43% extrapolated yield reduction (with an assumed 100% infection) which, however, represented an average for the 2 yr.

When the data for each hybrid are examined separately, it becomes apparent that no simple or direct relationship exists between the percent of MDM-diseased plants and grain yield. We obtained the same yield reduction for groups of plants differing markedly in their disease incidences, as was the case with Mp490 × Mo12 inoculated in the three-leaf and the seven-leaf stages. Both groups of plants showed yield reductions of 18-19%, yet they differed in disease incidence by approximately 45%.

Corn genotypes equally susceptible to MDMV-A in the early seedling stage may respond differently to infection with this virus at later stages of growth. This was true in the case of the two hybrids chosen for this study. In an earlier test, which served as the basis for selecting these hybrids, Mp490 × Mo12 and Pioneer 511A had 92.5 and 90.0% diseased plants, respectively, when inoculated with MDMV-A in the early seedling stage. Results from the present study showed that Mp490 × Mo12 became progressively more resistant as the inoculation was delayed beyond the seven-leaf stage, whereas Pioneer 511A remained susceptible to infection by MDMV-A to a fairly high degree through the 11-leaf stage. However, in both hybrids the percentage of yield reduction decreased with postponement in inoculation from the five-leaf through the 11-leaf stage. The two hybrids also differed in the extent and intensity of their leaf symptoms. The symptom severity indices paralleled the disease incidences in that they decreased in Mp490 × Mo12 and remained essentially unchanged in Pioneer 511A with advance in the age of plants at the time of inoculation.

Protection of the two hybrids from infection by MDMV-A until most plants had at least 11 leaves would

have resulted in a yield reduction which was only one-third as great as that expected in plants of these hybrids inoculated in the five-leaf stage. Therefore, if a MDMV-A-susceptible hybrid produces exceptionally high yields, it might be economically profitable to grow such a hybrid and protect it from infection with MDMV-A by aphids in the early stages of growth. This could be done in some areas by planting as early as possible for the seedlings to escape the main flights of aphids. The small yield reduction resulting from late infection might be more than compensated by the high yielding capacity of that particular hybrid.

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