Maize Dwarf Mosaic Virus Increases Susceptibility of Sorghum and Corn to Helminthosporium maydis Race T

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ABSTRACT

Sorghum and corn plants infected with maize dwarf mosaic virus (MDMV) were more susceptible to infection by *Helminthosporium maydis* race T than those not infected by the virus. The fungus produced large, necrotic and often concentric lesions on leaves of MDMV-infected sorghum; lesions on virus-free plants remained small. Lesions caused by *H. maydis* on leaves of MDMV-infected corn, N- and T-cytoplasm, were larger and developed more rapidly than on virus-free plants. Sporulation in lesions on MDMV-infected sorghum and corn began earlier and was more abundant than on virus-free plants. Lesions similar to those resulting from infection by *H. maydis* developed on MDMV-infected sorghum leaves sprayed with *H. maydis* culture filtrate; very small necrotic lesions developed on virus-free plants. The culture filtrate caused greater water-soaking of MDMV-infected corn leaf sections than of virus-free sections.

Increased susceptibility of sorghum to *H. maydis* race T appeared to be host-dependent, since only five of sixteen hybrids tested showed a positive association between MDMV infection and *H. maydis* susceptibility.

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Viral infections have been associated with increased susceptibility of some plants to a number of fungal pathogens (1, 3, 6, 8, 14, 16, 17, 21, 23, 26). Recently, infection by maize dwarf mosaic virus (MDMV) has been shown to increase susceptibility of corn (*Zea mays* L.) to several fungal root-rot pathogens (8, 17, 26). During field testing for virus resistance in hybrids of grain sorghum (*Sorghum bicolor* Moench) in 1970, we observed that plants of cultivar McNair 652 previously inoculated with MDMV developed large, conspicuous, necrotic lesions. Healthy (nonMDMV-inoculated) plants of the same hybrid showed tiny necrotic lesions. Isolations from both types of lesions consistently yielded *Helminthosporium maydis* Nisikado & Miyake race T, which was established as the etiologic agent of the leaf spotting through fulfillment of Koch's postulates. The work reported herein was undertaken to elucidate the apparent predisposition effect of MDMV infection in sorghum and to investigate the possible occurrence of the same phenomenon in corn. A preliminary report has been published (2).

MATERIALS AND METHODS.—Sixteen grain sorghum hybrids and five each of N- and T-cytoplasm corn hybrids were used in this study. The virus used was an Alabama isolate of MDMV-A maintained in corn hybrid cultivar Hy × C103 in the greenhouse. The *H. maydis* race T used was a subculture of the original isolate from MDMV-infected McNair 652 sorghum growing on the Upper Coastal Plain Substation, Winfield, Alabama.

Susceptibility of sorghum and corn to H. maydis.—Corn plants were grown in a steam-sterilized mix of soil:peat:vermiculite (1:1:1, v/v) in plastic pots and maintained in the greenhouse; sorghum plants were grown in vermiculite in plastic pots and maintained in a controlled environment chamber. Corn seedlings were inoculated with MDMV when 7 days old (two-leaf-stage) on the first and second leaves; sorghum seedlings were inoculated when 12-14 days old (three-leaf-stage) on the second and third leaves. Inoculation was accomplished by rubbing upper surfaces of Carborundum-dusted (600-mesh) leaves with a cheesecloth pad saturated with crude sap from MDMV-infected corn seedlings. Check plants were inoculated with crude sap from healthy corn seedlings.

Sorghum and corn plants at 10-15 and 14-15 days, respectively, after inoculation with MDMV were inoculated with *H. maydis* race T by spraying (hand atomizer) with a conidial suspension in 5% sucrose (16,000 spores/ml). Plants were held in a mist chamber for 24 h and transferred to a growth chamber or greenhouse. Lesion counts were made 8 days after inoculation with *H. maydis,* and lesion areas were measured with a planimeter (Planimeter No. 702M; Lasico, Los Angeles, Calif.).

Sporulation.—Sporulation in *H. maydis* lesions was determined by the method of Hooker et al. (12). Healthy and MDMV-infected plants of McNair 652 sorghum and the five corn hybrids (N- and T-cytoplasm) were inoculated with *H. maydis* race T conidia. Eight days later, leaves were detached and cut into sections containing *H. maydis* lesions. The sections were placed on moist filter paper in petri plates and observed with 80× magnification for sporulation at 24 h intervals for 5 days.

Phytotoxicity of culture filtrate.—Filtrates were obtained from *H. maydis* cultures in Fries' liquid medium according to the method of Wheeler et al. (29) except that crude culture fluids expressed through cheesecloth were finally passed through a filter membrane of 0.2-μ pore size. The filtrate was assayed for pathotoxin (22) by the inhibition of root-elongation technique (12, 22). Seed of corn hybrid Pioneer 3030 (N- and T-cytoplasm) were placed embryo-side-down on moist filter paper in a tray. Forty-eight hours after germination, seed with primary roots 3-12 mm in length were selected for use in bioassay. Ten ml of culture filtrate were poured into a petri dish and five germinated seed were placed in each dish and allowed

Fig. 1-(A to C). Lesions on leaves of maize dwarf mosaic virus-infected McNair 652 sorghum as observed: **A)** in the field under conditions of natural infection by *Helminthosporium maydis* race T; **B, C)** in the laboratory following treatment with *H. maydis* race T conidia or culture filtrate, respectively.

TABLE 1. Number and size of lesions produced by *Helminthosporium maydis* race T on leaves of maize dwarf mosaic virus(MDMV)-infected and virus-free seedlings of five corn hybrids, N- and T-cytoplasm

Hybrid	MDMV-infected	Lesions	
		Count[a] (no./leaf)	Avg. size[b] (cm^2)
McNair × 210 V (N)	−	29.9	0.22
	+	20.0	0.50*
(T)	−	24.0	1.03
	+	26.1	1.43
McNair 508 (N)	−	21.8	0.22
	+	23.6	0.44*
(T)	−	17.1	0.95
	+	20.4	1.20*
Pioneer 3030 (N)	−	22.7	0.17
	+	33.6*	0.51*
(T)	−	13.1	0.99
	+	19.8*	1.82*
Pennington 7-C-11B (N)	−	14.5	0.26
	+	14.0	0.51*
(T)	−	16.9	0.68
	+	25.2	1.35*
Stull 400W (N)	−	22.7	0.25
	+	24.6	0.75*
(T)	−	11.1	1.03
	+	18.6*	2.50*

[a]From 4-12 plants; * = significantly different ($P = 0.05$) from lesion no. on corresponding (−) or virus-free tissue of same cytoplasm type and hybrid.

[b]From 13-42 lesions; * = significantly different ($P = 0.05$) from avg. lesion size on corresponding (−) or virus-free tissue of same cytoplasm type and hybrid.

to grow for 48 h at 25 ± 2 C in laboratory light during the day and dark during the night. In three separate assays of the filtrate involving ca. 90 seedlings per assay, elongation of primary roots of T-cytoplasm seedlings was inhibited at least 70%, compared to those in sterile water, when incubated in various dilutions of the filtrate up to 1:150 (the highest tested); root elongation of N-cytoplasm seedlings was inhibited 50% in filtrate diluted 1:10 but not at any higher dilution. This specific toxicity to T-cytoplasm indicated that the culture filtrate contained the pathotoxin ascribed to *H. maydis* race T (10, 12, 29).

Capacity of the culture filtrate to produce symptoms on sorghum and corn was determined by spraying it onto MDMV-infected and virus-free McNair 652 sorghum and Stull 400W (N- and T-cytoplasm) corn. Plants sprayed with Fries' medium and 5% sucrose served as controls.

Phytotoxic response of corn leaves to the culture filtrate also was determined by the amount of water-soaking (12, 22). Leaves from 15-day-old MDMV-infected and virus-free Stull 400W plants were detached and cut into 6.3 - 7.6 cm sections which were floated on the surface of 10 ml of filtrate, Fries' medium, or water. The amount of water-soaking was recorded after 3 days.

RESULTS.—*Susceptibility of sorghum and corn to H. maydis*.—Tiny (<2-mm diam) necrotic lesions developed on all sorghum plants whether MDMV-infected or not. Large (2- to 15-mm diam) necrotic, and often concentric, lesions typical of *H. maydis* race T in the field developed only on MDMV-infected plants (Fig. 1-A,B) of hybrids AKS-614, Dorman BR-100, Excel Bird-Go, Funks BR-79, and McNair 652; large lesions failed to develop on Asgrow Bravis R, Bird-A-Boo, DeKalb BR-64, DeKalb E-57, Funks BR-630, Funks G-766W, Ga. 615, McNair 546, Oro, Penn Grain BR, and Shoo-Bird.

Lesions developed on all corn plants inoculated with *H. maydis* race T conidia; however, lesions on MDMV-infected plants were significantly larger than those on virus-free plants (Table 1). Increased lesion size in association with MDMV infection occurred on plants of both cytoplasm types in hybrids McNair 508, Pioneer 3030, Pennington 7-C-11B, and Stull 400W, but only on N-cytoplasm plants of McNair × 210 V; magnitude of the increase ranged from 1.2-3.0× among the hybrids. Lesions on virus-free, N-cytoplasm plants were of the 'resistant' type typically associated with *H. maydis* race T infection; the larger, more progressive lesions produced on MDMV-infected, N-cytoplasm plants tended to resemble the susceptible reaction of T-cytoplasm plants to race T infection (Fig. 2). Significant increases in lesion numbers occurred on MDMV-infected Pioneer 3030 (N- and T-cytoplasm) and Stull 400W (T-cytoplasm only) plants; otherwise, no effect of MDMV infection on lesion incidence was noted.

Sorghum and corn plants inoculated only with MDMV developed the mosaic symptom typically associated with infection by this virus.

Sporulation.—Sporulation in *H. maydis* lesions on

MDMV-infected McNair 652 sorghum and Stull 400W corn began sooner, and/or was more abundant, than on virus-free plants (Table 2); data for the four other corn hybrids were similar to those for Stull 400W.

Phytotoxicity of culture filtrate.—The filtrate from *H. maydis* race T culture produced necrotic-concentric lesions typical of *H. maydis* infection only on MDMV-infected sorghum plants (Fig. 1-C); virus-free plants developed tiny necrotic lesions. In corn, the culture filtrate produced chlorotic-necrotic lesions (similar to those produced by *H. maydis* race T) on MDMV-infected and virus-free T-cytoplasm and also on MDMV-infected N-cytoplasm, but not on virus-free N-cytoplasm plants. The culture filtrate caused a greater amount of water-soaking of leaf sections of MDMV-infected plants of N- and T-cytoplasm corn than of virus-free plants (Table 3). Fries' medium at 0 and 1:10 dilutions caused an equal amount of water-soaking in leaf sections from MDMV-infected and virus-free corn, but none at higher dilutions.

DISCUSSION.—The predisposition effect of MDMV infection appeared similar to that reported by Lamey and Everette (14) for hoja blanca virus (HBV). *Cochliobolus miyabeanus,* a facultative parasite, produced larger lesions on HBV-infected rice leaves than on HBV-free ones; lesion development by *Piricularia oryzae,* a facultative saprophyte, was not affected by HBV infection. In our studies, lesions were produced on all corn and sorghum leaves inoculated with *H. maydis* race T; however, invasion and sporulation by the fungus were more extensive in MDMV-infected leaves. As Tu and Ford (26) pointed out in discussing the predisposition relationship of MDMV infection and root rot in corn, the vigor of MDMV-infected plants might be sufficiently reduced, rendering them more susceptible to other pathogens. A number of physiological and cytological changes occur in MDMV-infected tissues that could create a more suitable substrate for a subsequently invading pathogen such as *H. maydis*. Accumulation of amino acids, amides, carbohydrates, and macro- and micro-elements has been associated with some MDMV infections (7, 15, 26) as have disruption of cells (13, 18) and increased digestibility of infected tissues (4). Thus, increased susceptibility of MDMV-infected tissues could be due to increased levels and accessibility of nutrients which can be used by *H. maydis* for colonization of the tissue and sporulation. Such an effect would be similar to that resulting from an action ascribed to a pathotoxin produced by *H. maydis* race T (9, 11).

Fig. 2-(A to D). Lesions produced by *Helminthosporium maydis* race T on leaves of Stull 400W corn. **A)** Virus-free, N-cytoplasm; **B)** maize dwarf mosaic virus-infected, N-cytoplasm; **C)** virus-free, T-cytoplasm; **D)** maize dwarf mosaic virus-infected, T-cytoplasm.

Foliar necrosis of various patterns in sorghum has been reported as a symptom of infection by MDMV or other strains of sugarcane mosaic virus (5, 20, 25). Some of these reactions (5, 20) are similar to those we described, and some of the symptoms we observed on McNair 652 plants in the field (Fig. 1-A) possibly were of this type, i.e., due to MDMV infection alone. However, the predisposition effect was demonstrated under conditions in which a conspicuous necrotic spotting occurred on

TABLE 2. Sporulation of *Helminthosporium maydis* race T on individual lesions on leaves of maize dwarf mosaic virus-infected and virus-free sorghum and corn (N- and T-cytoplasm) plants

Plant	Virus-infected	Sporulation ratings[a] after incubation for				
		24 h	48 h	72 h	96 h	120 h
McNair 652 sorghum	−	0.0	0.0	0.0	0.0	0.1
	+	0.0	0.3	0.7	1.4	1.5
Stull 400W corn (N)	−	0.0	0.4	0.8	1.2	1.7
	+	0.2	1.1	1.4	2.0	2.5
(T)	−	0.8	1.3	2.0	2.6	3.1
	+	1.3	2.0	2.7	3.6	3.9

[a]Average rating of 13-39 lesions in two experiments where 0 = no sporulation; 1 = 1 to approximately 200 spores; 2 = 201-500; 3 = 501-1,000; 4 = 1,001-2,000; 5 = over 2,000. Beginning at 24 h for corn and 48 h for sorghum, figures for sporulation on virus-infected seedlings were significantly different ($P = 0.05$) from those for virus-free.

TABLE 3. Phytotoxic response of leaf sections of maize dwarf mosaic virus-infected and virus-free Stull 400W, N- and T-cytoplasm, corn seedlings floated for 3 days on culture filtrate from *Helminthosporium maydis* race T, Fries' medium, or water

Treatment	Dilution	Phytotoxicity[a] N-cytoplasm Virus-free	N-cytoplasm MDMV-infected	T-cytoplasm Virus-free	T-cytoplasm MDMV-infected
Culture filtrate	0	0.0	1.0*	3.3	4.7*
	1:10	0.0	0.7*	1.8	2.8*
	1:50	0.0	0.3	1.0	1.7
	1:150	0.0	0.3*	0.3	1.2*
Fries' medium	0	0.3	0.3	0.7	0.7
	1:10[b]	0.3	0.3	0.8	0.7
Water	---	0	0	0	0

[a]Average amount of water-soaking on six leaf sections in two experiments where: 0 = none; 1 = 1-20% leaf area involved; 2 = 21-40%; 3 = 41-60%; 4 = 61-80%; 5 = 81-100%. * = rating significantly different ($P = 0.05$) from that for corresponding virus-free tissue of same cytoplasm type.

[b]No water-soaking occurred at any higher dilution.

MDMV-infected sorghum following inoculation with *H. maydis* race T conidia or culture filtrate containing pathotoxin (Fig. 1-B,C) that did not occur in the absence of either pathogen. Fulfillment of Koch's postulates, and studies on lesion size and sporulation, substantiate increased susceptibility of MDMV-infected sorghum to *H. maydis*.

Susceptibility of sorghum to *H. maydis* in the field (24) and greenhouse (19) has been reported. Recent studies (27, 28) involving known races of the fungus have indicated it to be a nonpathogen or only weakly pathogenic on sorghum. Presumably, all of these investigations involved virus-free plants. In our study, increased susceptibility of sorghum to *H. maydis* appeared to be host-dependent, since only 5 of 16 hybrids tested showed a positive relationship between MDMV infection and susceptibility to *H. maydis*.

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