Maize Dwarf Mosaic Virus Predisposes Corn to Root Rot Infection

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ABSTRACT

Corn seedlings infected with maize dwarf mosaic virus (MDMV) were more susceptible to root rot diseases incited by *Gibberella zeae* and *Helminthosporium pedicellatum* than were virus-free ones. Root rot was most severe in MDMV-diseased corn seedlings at high densities of fungus inoculum. The difference in root rot severity between MDMV- and non-MDMV-diseased corn seedlings was less in soil with high levels of inoculum.

Higher concentrations of carbohydrates and ninhydrin-positive substances were found in culture solutions around MDMV-diseased corn roots than around virus-free roots. Culture solutions from liquid culture of MDMV-infected plants supported more fungal and bacterial growth than those from virus-free plants. Weakening of the host due to MDMV infection and increasing inoculum potential due to more root leakage probably are directly associated with more severe root rot disease in MDMV-infected than virus-free corn. Phytopathology 61: 800-803.

Root rot diseases of corn cause major losses in corn production (2). Contrarily, the maize dwarf mosaic disease causes only minor yield losses. Virus infections, however, can predispose plants to infection by fungi that cause root rot diseases (6, 7, 20). *Gibberella zeae* (Schw.) Petch causes stalk rot, and both it and *Helminthosporium pedicellatum* Henry cause root rot in corn under conducive conditions. A preliminary report (13) suggests that maize dwarf mosaic virus (MDMV) increases susceptibility of corn to several fungal pathogens. We have observed MDMV-infected corn seedlings grown in unsterilized soil in clay pots that have frequently shown more browning and blackening of roots than have virus-free ones. Thus, our experiments were designed to assess the effect of MDMV infection on root rot diseases of corn caused by two fungal pathogens.

MATERIALS AND METHODS.—*Zea mays* L. 'Ohio W49' was used in all tests. Tests were conducted in a greenhouse maintained at about 24 C.

MDMV-A (4), the virus isolate used, was maintained in Ohio W49 corn. Inocula of the two root rot fungi, *G. zeae* and *H. pedicellatum*, were grown for 1 month on ground oat straw, which was previously moistened with distilled water and autoclaved.

Susceptibility test.—Corn was sown in steamed soil (1:1:2 = sand:peat:soil mixture) potted in 3-inch sterile pots, 5 seeds/pot. About 10 days after seeding, when seedlings reached the two-leaf stage, plants in 80 pots were inoculated with MDMV, and those in another 80 pots were used as virus-free controls. Seedlings were inoculated by rubbing inocula, with a pestle, on leaves predusted with 600-mesh Carborundum. Inoculum was infective crude sap extracted in 0.01 M phosphate buffer, pH 7. Control plants were dusted with Carborundum, and only the buffer was rubbed on the leaves to simulate inoculation.

About 1 week after MDMV inoculation, the third and fourth leaves showed systemic mosaic symptoms. At this stage, symptom-free plants in the MDMV-inoculated group were removed from pots. Then, the MDMV-infected and virus-free control groups were halved into subgroups for subsequent exposure to either *G. zeae* or *H. pedicellatum*. The fungal inoculum was blended into autoclaved soil at eight different inoculum soil ratios (0/1, 1/5, 1/10, 1/20, 1/40, 1/80, 1/160, and 1/320 by volume). The infested soils were put into 6-inch clay pots; then the MDMV-diseased and virus-free control corn seedlings were transplanted from 3-inch pots into separate 6-inch pots. There were five pots of MDMV-diseased corn and five pots of virus-free control corn plants at each inoculum level. Root rot severity was assessed 2 weeks after transplanting by washing soil from the roots and making observations and isolations. The experiment was repeated once.

Bioassay of root exudates.—The corn seedlings were grown in liquid culture as described previously (18). Three series of bottles were designated for virus-free control, healthy corn, or MDMV-infected corn. Immediately after MDMV inoculation, the inoculated and healthy corn seedlings were transferred to bottles. Bacteria and fungi were carried into the culturing bottle from silica sand where corn seedlings were germinated and grown until inoculation and transfer to liquid culture. No seedlings were transferred in the no-plant-control series, but a minute amount of silica sand was transferred to the control bottles to assure the transfer of the bacteria and fungi present in the sand. These bacteria and fungi were identified later.

Assay of the bacterial growth in the Hoagland's solution was made by turbidity measurements and dilution end point (DEP) assays at 4-day intervals after plants were placed in the liquid culture. Freshly made Hoagland's solution was used as a standard. DEP's were assayed on trypticase soy agar (TSA). Dilutions were made at 10^{-1} intervals in sterilized water. Inoculated

TSA plates were incubated at 24 C for 24 hr before examination of bacterial colonies. All measurements were made in triplicate, and the experiment was repeated once.

Assay for fungal growth was a DEP made on the same samples used in DEP assays of bacteria. The assays were made in petri dishes with 2% potato-dextrose agar acidified with a few drops of 5% lactic acid. The plates were incubated at 23 C for 2 days before colony counts were made. All measurements were triplicated, and the experiment was repeated once.

Determination of carbohydrates and amino acids in culture solution.—Liquid culture solutions were sampled in quadruplicate at 4-day intervals for carbohydrates and amino acid determinations. Sampling was begun 4 days after plants were transferred to liquid culture.

Carbohydrates were determined semiquantitatively with Dreywood's anthrone reagent (12). Appropriate amounts of reagents were added to 5 ml of the sample solution; after reacting, the absorbancy was measured at 620 nm.

Total ninhydrin-positive materials were measured by the method of Moore & Stein (11). The samples were diluted 20 times with distilled water, and appropriate amounts of reagents were added to 1 ml of the diluted sample. The resultant solutions were measured at 570 nm in a spectrophotometer.

Analysis of free amino acids in corn.—Leaves and roots of MDMV-infected and healthy corn grown in liquid culture were harvested for amino acid analysis 4 weeks after inoculation. Samples for analysis for free amino acids were prepared as before (8). Analyses were made in a Technicon automatic amino acid analyzer as described previously (19).

RESULTS.—MDMV infection increased susceptibility of corn seedlings to *G. zeae* and *H. pedicellatum* (Table 1). In soil with high inoculum densities (⅕ to 1/20), virus-free and MDMV-diseased corn were equally diseased (Fig. 1). But at low inoculum levels (1/40 to 1/320), seedlings infected with MDMV showed more root rot than did virus-free ones. Differences in root rot severity between MDMV-disease and virus-free corn decreased with increasing inoculum densities (Table 1, Fig. 1).

The growth of corn seedlings was retarded in the initial stages of root rot. Curling and rolling of leaves followed when *G. zeae*-induced root rot spread to become sheath and stalk rot. Eventually, the seedlings died, and a white mycelial mass often encircled the basal part of the corn seedling near the culture surface.

TABLE 2. Concentration of bacteria and fungi in nutrient culture solutions, sampled at 4-day intervals, after transferring healthy and maize dwarf mosaic virus (MDMV)-infected corn to the solutions

Sample interval	Dilution end point		
	No-plant	Healthy	MDMV-infected corn
days		*bacteria*	
4	10^{-2}	10^{-3}	10^{-4}
8	10^{-3}	10^{-3}	10^{-5}
12	10^{-3}	10^{-4}	10^{-5}
16	10^{-4}	10^{-5}	10^{-6}
20	10^{-4}	10^{-5}	10^{-7}
24	10^{-5}	10^{-6}	10^{-7}
28	10^{-5}	10^{-6}	10^{-8}
		fungi	
4	10^{-2} (2)[a]	10^{-2} (3)	10^{-2} (3)
8	10^{-2} (4)	10^{-2} (4)	10^{-2} (4)
12	10^{-3} (2)	10^{-3} (4)	10^{-3} (6)
16	10^{-3} (6)	10^{-3} (7)	10^{-3} (10)
20	10^{-3} (12)	10^{-4} (3)	10^{-4} (4)
24	10^{-4} (9)	10^{-5} (2)	10^{-5} (9)
28	10^{-4} (12)	10^{-5} (3)	10^{-5} (13)

[a] Numbers in parentheses refer to number of colonies at dilution end point.

Culture solutions, collected at various intervals after growing MDMV-diseased corn, supported more bacterial growth than did those of comparable virus-free ones (Table 2, Fig. 2). Controls, where no corn seedling was present, had the lowest bacterial and fungal populations (Table 2). Virus-free plants secreted utilizable nutrients from roots into the solution, but the amount secreted was smaller than that from MDMV-diseased plants.

The bacteria in the solution were identified as *Bacillus* spp. and *Xanthomonas* spp. (3, 15, 16), and the proportion of these two genera in the population was ca. 85% and 15%, respectively. The fungi in the solution were identified as *Fusarium moniliforme* var. *subglutinans* Wr. & Reinking, *Aspergillus* sp., *Penicillium* sp., *Cladosporium herbarum* S. F. Gray, and others (*Monilia* sp., *Helminthosporium* sp., and *Colletotrichum* sp.); their respective chances of occurrence were 91, 4, 3, 1, and 1%.

In the DEP assay for fungi, no difference in DEP

TABLE 1. Root rot severity (percentage of roots showing blackening and browning) of virus-free and maize dwarf mosaic virus (MDMV)-infected corn plants exposed to various concentrations of *Gibberella zeae* and *Helminthosporium pedicellatum* inoculum

Corn	Fungus	Concentration of fungal inoculum in soil[a]							
		1/5	1/10	1/20	1/40	1/80	1/160	1/320	Check[b]
		%	%	%	%	%	%	%	%
MDMV-infected	*G. zeae*	70	70	65	60	55	50	50	10
	H. pedicellatum	65	60	50	50	45	40	40	5
Virus-free	*G. zeae*	60	60	50	40	30	30	20	0
	H. pedicellatum	55	40	30	30	30	20	20	0

[a] Volume basis.
[b] No fungal inoculum.

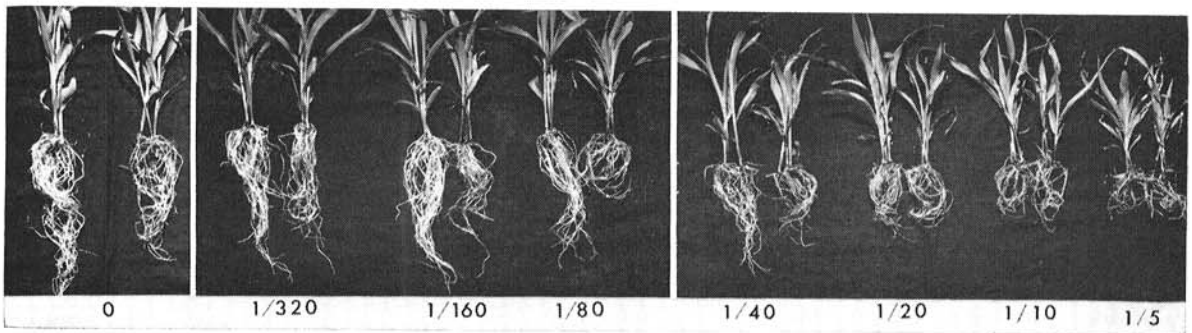

Fig. 1. Severity of root rot at different inoculum levels (0 through 1/5) of *Gibberella zeae* in virus-free (at left) and maize dwarf mosaic virus-infected corn (at right of each pair).

occurred between the solutions in which healthy and diseased plants were grown. More fungal colonies, however, were noted at the DEP from the solutions where MDMV-diseased plants were grown than in those from virus-free plants (Table 2, Fig. 2).

The culture solutions contained higher concentrations of ninhydrin-positive substances and carbohydrates where MDMV-infected corn was grown than in solutions where virus-free healthy corn was grown (Table 3).

Leaves and roots of MDMV-infected corn generally contained larger amounts of free amino acids than did those of virus-free corn. The infected leaves and roots contained, respectively, 28 and 12% more free amino acids than did their virus-free counterparts (Table 4). The increase in free amino acids in roots of MDMV-infected corn was 16% less than that of MDMV-infected leaves.

Discussion.—MDMV-infected corn seedlings were more severely diseased by root rot fungi than were virus-free ones. This confirms the earlier observation (13) that MDMV-diseased corn was more susceptible to root rots. That MDMV-infected corn is more root-rot susceptible is possibly linked to two factors: (i) an increase in host susceptibility in roots of the virus infected corn; and (ii) an increase of the inoculum potential sensu Garrett (9) of the rhizosphere of the MDMV-infected roots. Beute & Lockwood (1) found similarly that roots of virus-infected peas exuded two-three times more amino acids and 25-50% more carbohydrates than did roots of healthy peas which favored pathogenesis by *Aphanomyces euteiches*.

MDMV-infected corn may have reduced the host vigor considerably, permitting its roots to be more susceptible to fungal invasion. MDMV-infected corn is known to have less photosynthetic ability and more respiratory activity (17), and it also contains more free amino acids per unit of tissues than does virus-free corn (Table 4). Roots of MDMV-infected corn had ca. 12% more free amino acids than did roots of virus-free healthy corn (Table 4), even though the leakage of ninhydrin-positive substances from the roots was considerably higher than that from virus-free ones (Table 3). The greater increase of free amino acids in leaves than in roots of MDMV-infected corn is proba-

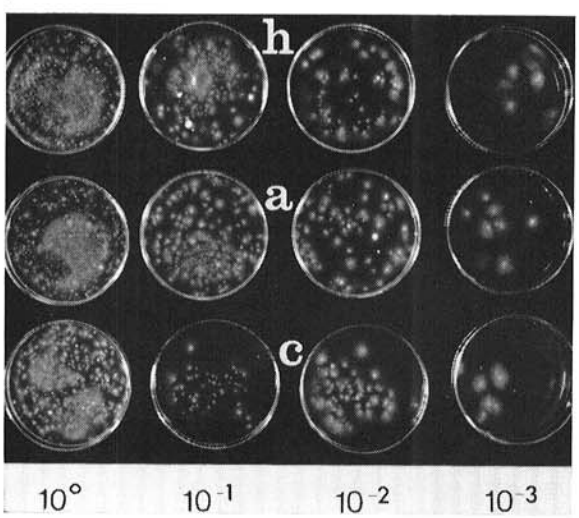

Fig. 2. Dilution end point assay for fungal population in culture solutions of no-plant control (c), healthy corn (h), and maize dwarf mosaic virus strain A-infected corn (a). Samples were taken 16 days after corn was transferred to the liquid cultures.

Table 3. Concentration of ninhydrin positive substances and carbohydrates in Hoagland's solution at various intervals after corn was grown in nutrient solution

Sampling interval	No-plant	Healthy	Maize dwarf mosaic virus-infected
days	*ninhydrin-positive substances*[a]		
4	0.25[b]	0.43	0.53
8	0.30	0.53	0.88
12	0.35	0.60	0.90
16	0.35	0.72	0.90
	carbohydrates		
4	0.04[c]	0.12	0.20
8	0.04	0.18	0.40
12	0.04	0.25	0.50
16	0.05	0.30	0.56

[a] For measuring the ninhydrin-positive substances, the solutions were diluted 20 times before adding reagents.

[b] The absorbancy was measured at 570 nm; average of four measurements.

[c] The absorbancy was measured at 620 nm; average of four measurements.

TABLE 4. Concentration of free amino acids (μmoles/g of tissue) in leaves and roots from healthy and maize dwarf mosaic virus-A (MDMV-A)-infected corn plants, after they were grown in balanced nutrient solution

Amino acid	μmoles/g			
	Healthy leaves	Infected leaves	Healthy roots	Infected roots
Aspartic acid	0.168[a]	0.353	0.273	0.393
Threonine	0.091	0.106	0.088	0.097
Serine	0.433	0.566	0.221	0.241
Glutamic acid	0.778	1.067	0.631	0.628
Citrulline	0.013	0.028	0.008	0.014
Proline	0.046	0.068	0.059	0.060
Glycine	0.447	0.329	0.117	0.195
Alanine	0.725	1.025	0.558	0.593
Valine	0.054	0.067	0.099	0.112
Cystine	ND[b]	0.007	ND	0.009
Methionine	0.011	0.018	0.027	0.005
Isoleucine	0.027	0.043	0.082	0.086
Leucine	0.036	0.044	0.110	0.127
Tyrosine	0.034	0.043	0.049	0.042
Phenylalanine	0.019	0.028	0.042	0.050
Ornithine	0.024	0.019	0.016	0.024
Lysine	0.069	0.065	0.122	0.127
Histidine	0.017	0.015	0.036	0.034
Asparagine	0.094	0.208	0.064	0.069
Total	3.086	4.099	2.602	2.906

[a] An average of two runs.
[b] ND = not detectable.

bly also due to some leakage of free amino acids from roots into the liquid growth medium. The membrane permeability of root cells may be altered by the virus infection to allow increased leakage of substances.

Many of the free amino acids that occurred in relatively high concentrations in the plant tissues (e.g., aspartic acid, alanine, glutamic acid) are good nitrogen sources for the growth of some root rot fungi in vitro (10). These amino acids usually increased substantially in leaves as well as in roots of MDMV-infected corn (Table 4). Thus, the increase in susceptibility to root rot fungi by MDMV-infected corn may have been expected because of the combination of reduced host vigor and increased concentrations of free amino acids in vivo.

Based on our bioassay and biochemical analyses of culture solutions, the leakage of ninhydrin-positive substances and carbohydrates was higher in roots of MDMV-infected corn than in roots of virus-free corn (Table 3), and the exudates were capable of supporting more bacterial and fungal growth (Table 2). Schroth & Cook (14) showed that the susceptibility of three bean varieties to preemergence damping-off caused by *Rhizoctonia solani* was positively correlated with the quantities of ninhydrin-positive, silver nitrate-positive, and ultraviolet fluorescing substances. These substances also were found to enhance the fungal growth and bacterial activity (5). Therefore, we assume that the increased root exudation from the root system of MDMV-infected corn enhanced the fungal growth and provided a higher inoculum potential of the root rot fungi in the root rhizosphere. This increased the chance for successful establishment of the root rot fungi in plant roots.

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