Relation of Maize Dwarf Mosaic Virus Infection to Increased Susceptibility of Corn to Helminthosporium maydis Race O

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Accepted for publication 17 May 1982.

ABSTRACT

Stevens, C., and Gudauskas, R. T. 1982. Relation of maize dwarf mosaic virus infection to increased susceptibility of corn to Helminthosporium maydis race O. Phytopathology 72:1500-1502.

Helminthosporium maydis race O produced greater numbers of lesions and larger lesions on corn seedlings infected with maize dwarf mosaic virus (MDMV) than on virus-free seedlings. Germination rates of H. maydis conidia were similar on leaves of MDMV-infected and virus-free seedlings; however, conidia on MDMV-infected leaves produced more germ tubes and more than twice the number of appressoria as conidia on virus-free leaves. Washings from MDMV-infected seedlings showed greater conductivity and higher concentrations of K\(^+\), Cu\(^{2+}\), and ninhydrin-positive substances than washings from virus-free seedlings; and, leachates from MDMV-infected leaves contained greater amounts of phenol sulfuric acids, orcinol-, and ninhydrin-positive substances and total amino acids than leachates from virus-free leaves. Increased susceptibility of MDMV-infected corn to H. maydis appeared to be related to altered permeability of virus-infected tissues and consequent increased exudations that enhanced germ tube formation and colonization by the fungus.

Some viral infections have been shown to increase the susceptibility of plants to diseases caused by other pathogens; however, few explanations for the altered susceptibility have been proposed. Corn plants infected with maize dwarf mosaic virus (MDMV) are more susceptible to several fungal root rots and at least one foliar disease than are virus-free plants. Tu and Ford (1973) found that leakage of carbohydrates and ninhydrin-positive substances was higher from roots of MDMV-infected corn, and they suggested that the increased exudation might increase the inoculum potential of root-infecting fungi in the rhizosphere and thereby the likelihood of their establishment in the roots. A similar mechanism had been proposed earlier by Beute and Lockwood (1973) for the increased severity of root rots in virus-infected peas. Increased exudation of virus-infected tissues with consequent enhancement of inoculum potential has also been proposed as a primary mechanism in the increased susceptibility of virus-infected gladiolus to Curvularia leaf spot.

A previous study in this laboratory showed that Helminthosporium maydis Nishikado & Miyake race T produced more and larger lesions on leaves of MDMV-infected corn and sorghum than on leaves of virus-free plants. The work reported here was undertaken to determine possible mechanisms for this increased susceptibility of MDMV-infected corn by using H. maydis race O as the fungal pathogen.

MATERIALS AND METHODS

Plants. All studies were conducted with corn seedlings, Zea mays L. ‘H60XCl03,’ which were grown from seed in vermiculite in 10-cm-diameter plastic pots in a controlled-environment growth chamber (Controlled Environment LTD., Winnipeg, Manitoba, Canada R3H 0W9) programmed for 12-hr day at 29 C and 12-hr night at 21 C. Light intensity of approximately 12,100 lux was furnished by a mixture of fluorescent and incandescent bulbs. Plants were watered daily and fertilized periodically with Hyponex (Hydronic Chemical Co., Copley, OH 44321).

Virus and fungus. The virus used in this study was MDMV strain A (MDMV-A) originally isolated from naturally infected corn in Alabama and maintained in the greenhouse by mechanical transfer to corn seedlings. The fungus, H. maydis (Cochliobolus heterostrophus Drechs.) race O, ATCC 24273 (American Type Culture Collection, Rockville, MD 20852), was maintained on potato-dextrose agar (PDA) in petri plates at 28 C under fluorescent light in an incubator and transferred at intervals of 1 or 2 wk.

Viral inoculum was prepared from corn seedlings infected with MDMV for 2–3 wk by grinding infected leaves in a mortar with a few drops of water. Inoculations were made by the gauge pad method onto Carborundum-dusted leaves of 7-day-old corn seedlings (two-leaf stage). Conidial suspensions of H. maydis were prepared from 10- to 14-day-old cultures on PDA.

Conidium germination and appressorium formation. The percentage of H. maydis conidia that germinated and produced appressoria on MDMV-free and MDMV-infected leaves was determined by the procedures of Trainor and Martison (1974). Leaf sections from 15-day-old corn seedlings (four-leaf stage) were placed on moist filter paper in petri dishes and inoculated by placing one drop of aqueous suspension containing \(10^7\) conidia per milliliter on each section. Leaf sections were incubated in the light at 28 C and periodically some leaf sections were removed, treated with a small amount of 1% acid fuchsin in lactophenol, and observed at \(\times 100\).

Lesion production. Comparative susceptibility of MDMV-infected and virus-free corn plants to H. maydis was determined by spraying (with a hand atomizer) 15-day-old seedlings with a conidial suspension in 5% sucrose (600 conidia per milliliter; 20 ml on five plants). Inoculated plants were maintained under high humidity in a mist chamber for 24 hr. Numbers of H. maydis lesions per square centimeter of leaf tissue were determined at 2 days after inoculation and length of lesions at 3 days after inoculation.

Collection and analyses of leaf leachates and washings. To provide leachates, the third (immediately above inoculated leaves) and fourth leaves were removed from a group of 15-day-old corn seedlings, half of which were infected (8 days) with MDMV and half of which were virus-free. Laminae were cut into 1 cm\(^2\) pieces, which were suspended in sterile distilled water in flasks at the rate of 3 g/50 ml. Washings were collected from intact corn seedlings with primary leaves and secondary roots removed. Plants in the three- (11-day-old; no visible symptoms on MDMV-inoculated plants) and four-leaf stages (15-day-old; mosaic symptom visible on MDMV-inoculated plants) were inverted and leaves were immersed in flasks of distilled water at six plants per
100 ml of water following a modification of the procedure of Tukey et al. (16). Streptomycin sulfate was added to the bathing solution at 50 ppm in all experiments. All flasks containing leaf pieces or seedlings were placed in a rotary incubator shaker (Pycrotherm, New Brunswick Scientific Co., Inc., New Brunswick, NJ 08903) set at 28 C and ~160 rpm. Replicate flasks were removed periodically, and 20- and 10-ml samples for leaf leachates and washings, respectively, were stored in vials at 0 C.

Conductivity measurements of leaf washings were made with a conductivity bridge (model SRB-16 B3, Industrial Instruments, Cedars Grove, NJ 07009) and a cell with a constant K = 0.1 (Yellow Springs Instrument Co., Yellow Springs, OH 45387). Washings were analyzed for K', Ca++, Mg++, Cu++, and Zn with atomic absorption spectrophotometers (Perkin-Elmer, Norwalk, CT 06856, and Instrumentation Laboratory Inc., Wilmington, MA 01887).

Phenol sulfuric acid-, orcinol-, and ninhydrin-positive substances in samples of leaf leachates and washings were determined by standard procedures (9). For dry weight determinations, leachates from leaf pieces were dried under a stream of air and weighed. For identification of sugars and free amino acids, 100-ml samples of leachates were concentrated to 10 ml at reduced pressure with a rotary flash evaporator at a temperature no greater than 55 C (Buchler Instruments, Fort Lee, NJ 07024). The concentrated leachates were centrifuged with a clinical centrifuge and the supernatants were fractionated by slow passage through a 25 X 1-cm cation exchange column (Bio-Rad AG50WX8 hydrogen form, Bio-Rad Laboratories, Richmond, CA 94804). For sugar analyses, the column was washed with 50 ml of distilled water and eluates were evaporated to dryness at 55 C. Residues were taken up in 10 ml of anhydrous pyridine, which was then evaporated completely at room temperature under nitrogen. Sugars were separated and quantitated by gas-liquid chromatography of trimethylsilyl derivatives according to the method of Sweeley et al. (13) by using a Hewlett-Packard 5710A gas chromatograph with an ionization detector. The column consisted of a 4-foot, 2% OV-101 100/120 WHP chromosorb, and was programmed at a rate of 4 C/min from 150-210 C. Identification of sugars was based on retention times of trimethylsilyl derivatives of authentic sugar standards (Monosaccharide Kit, Applied Science Laboratories, Inc., State College, PA 16801). For amino acid analyses, amino acids were eluted from the cation column with 50 ml of 3N ammonium hydroxide and evaporated, and the residues were taken up in citrate buffer and analyzed with a Beckman model 121-M amino acid analyzer by the methods of Ford and Tu (7).

Data were subjected to analysis of variance and tested for significance by the F-test.

RESULTS

Conidium germination and appressorium formation. There were no differences in rates of germination of H. maydis conidia on MDMV-infected and virus-free leaf sections 3, 6, and 12 hr after inoculation (unpublished); however, conidia on MDMV-infected sections produced more germ tubes and over twice the number of appressoria as conidia on virus-free leaf sections (Table 1). At 6 hr after inoculation, the average lengths of germ tubes and appressoria from 50 conidia on MDMV-infected leaf sections were 110 and 9 μm, respectively; each value was significantly different (P = 0.05) from the corresponding average length of 200 μm for germ tubes and 6 μm for appressoria from conidia on virus-free leaves.

Lesion production. H. maydis produced significantly (P = 0.05) more (2.8 cm) vs 1.7) and larger (3.9 mm in length vs 2.3 mm) lesions on MDMV-infected seedlings than on virus-free ones (averages from 12-18 plants in three experiments).

Conductivity and cations of washings. Washings from MDMV-infected seedlings in the four-leaf stage showed 56-100% greater conductivity (Fig. 1) and significantly (P = 0.05) more K' (27 ppm vs 16) and Cu++ (0.2 ppm vs 0.01) than washings from virus-free, four-leaf-stage seedlings.

Dry weights and carbohydrates of leachates and washings. Average dry weight of leachates after 24 hr of incubation of MDMV-infected leaf pieces was 351 mg/g dry wt of leaf tissue (four determinations, one experiment), which was significantly higher (P = 0.05) than the 154 mg/g average weight for leachates from virus-free leaves. Average amount of phenol sulfuric acid-positive substances in leachates from MDMV-infected leaves after 24 hr was 37 mg/g dry wt of leaf tissue (16 replications, four experiments), which also was significantly higher (P = 0.05) than the 21.5 mg/g in leachates from virus-free leaves. Leachates from MDMV-infected leaves contained two to three times more orcinol-positive substances than leachates from virus-free leaves (Fig. 2). There were no significant differences in carbohydrate levels in washings from MDMV-infected and virus-free seedlings in the three- or four-leaf stages (unpublished).

α-D-Arabinose, α-D-ribose, α-D-fructose, α-D-galactose, α-D-glucose, β-D-glucose, two unidentified pentoses, and four unidentified hexoses were detected in leachates by gas chromatography. Concentrations of the identified pentoses and α-D-galactose were twofold to fivefold higher in leachates from MDMV-infected leaves than in those from virus-free leaves;

<table>
<thead>
<tr>
<th>Leaf sections</th>
<th>MDMV-infected (%)</th>
<th>MDMV-free (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conidia forming one germ tube</td>
<td>31.8</td>
<td>48.4</td>
</tr>
<tr>
<td>Conidia forming two germ tubes</td>
<td>68.2</td>
<td>51.6</td>
</tr>
<tr>
<td>Germinating conidia producing one appressorium</td>
<td>22.3</td>
<td>16.7</td>
</tr>
<tr>
<td>Germinating conidia producing two appressoria</td>
<td>20.8</td>
<td>8.2</td>
</tr>
<tr>
<td>Germinating conidia producing three appressoria</td>
<td>7.0</td>
<td>3.4</td>
</tr>
<tr>
<td>Germinating conidia producing four appressoria</td>
<td>2.3</td>
<td>0.7</td>
</tr>
</tbody>
</table>

*Six hours after inoculation with 300-400 conidia in two experiments.

Fig. 1. Conductivity of washings from maize dwarf mosaic virus (MDMV)-infected and virus-free corn seedlings in the three- and four-leaf stage. Each data point is an average of eight determinations in two experiments; differences between values for four-leaf stage MDMV-infected and virus-free seedlings were significant (P = 0.05; F-test) at each time interval.

Vol. 72, No. 11, 1982 1501
leading to greater growth and colonization by the fungus and consequently more extensive lesion development. Loss of metabolites by the leaf cells also could have affected host resistance mechanisms.

Total amino acid content and concentrations of individual amino acids were higher in leachates from MDMV-infected leaves than in those from virus-free leaves. Previous work has shown that free amino acid content of leaves and roots also is generally higher in MDMV-infected corn (7,8). Amino acids showing the greatest increases in our study included methionine, valine, glutamic acid, γ-aminobutyric acid, and proline. Aspartic and glutamic acids have been shown to increase the size of lesions on rice leaves infected with Cochliobolus miyabeanus (12), and increases in free glutamic acid and proline contents of Poa pratensis leaves have been positively correlated with lesion ratings and disease severity associated with infection by Drechslera sorokiniana (11).

These results agree with previous findings that increased susceptibility of virus-infected tissues to fungal disease may be related, at least in part, to increased leakage in the tissues (2,3,15). Alterations in tissue structure and in several physiological processes have been associated with MDMV infection in corn (4,8), which could affect permeability and/or other properties of tissues and lead to increased susceptibility to H. maydis.

LITERATURE CITED