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

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Journal Series Paper 6-810044 of the Alabama Agricultural Experiment Station.
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^{++}, and ninhydrin-positive substances than washings from virus-free seedlings; and, leachates from MDMV-infected leaves contained greater amounts of phenol sulfuric acid-, 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 (5,10). 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 (8). Tu and Ford (15) 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 (3) 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 leafspot (2).

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 (1). 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. 'H60XC103,' which were grown from seed in vermiculite in 10-cm-diameter plastic pots in a Conviron 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 12,110 lux was furnished by a mixture of fluorescent and incandescent bulbs. Plants were watered daily and fertilized periodically with Hyponex (Hydroponic 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 gauze 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 Martinson (14). 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 10^3 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 ×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 then 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

0031-949X/82/11150003/$03.00/0

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 (Psycrotherm, 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 SRC-16 B2, Industrial Instruments, Cedar 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, Norfolk, 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 × 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 quantified 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 using 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^2 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 1. Prepenetration activities of conidia of *Helminthosporium maydis* race O on leaf sections from 15-day-old maize dwarf mosaic virus (MDMV)-infected and virus-free corn seedlings

	Leaf sections[a]	
Activity	MDMV-infected (%)	MDMV-free (%)
Conidia forming one germ tube	31.8	48.4
Conidia forming two germ tubes	68.2	51.6
Germinating conidia producing one appressorium	22.3	16.7
Germinating conidia producing two appressoria	20.8	8.2
Germinating conidia producing three appressoria	7.0	3.4
Germinating conidia producing four appressoria	2.3	0.7

[a]Six hours after inoculation with 300–400 conidia in two experiments.

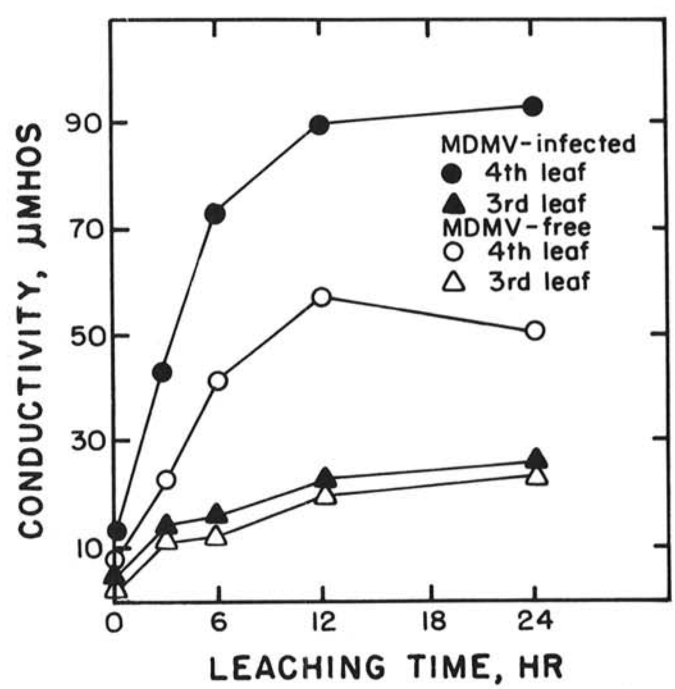

Fig. 1. Conductivity of washings from maize dwarf mosaic virus (MDMV)-infected and virus-free corn seedlings in the three- and four-leaf stage. Each data point is an average of eight determinations in two experiments; differences between values for four-leaf stage MDMV-infected and virus-free seedlings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

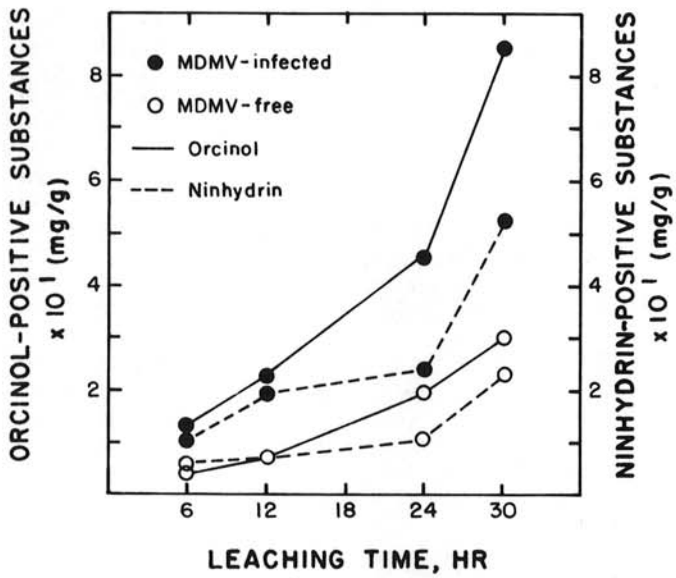

Fig. 2. Concentrations of orcinol- and ninhydrin-positive substances in leachates from leaf pieces from maize dwarf mosaic virus (MDMV)-infected and virus-free corn seedlings in the four-leaf stage. Each data point is an average of 9–12 determinations in three experiments; differences between values for MDMV-infected and virus-free leaf pieces were significant ($P = 0.05$; F-test) at each time interval.

concentration of α- or β-D-glucose was the same in both types of leachates (averages, three experiments).

Ninhydrin-positive substances and amino acids of leachates and washings. Leachates from MDMV-infected leaves contained about twice as much ninhydrin-positive substances as leachates from MDMV-free leaves (Fig. 2). Analyses of 24-hr washings showed no significant differences in ninhydrin-positive substances in washings from seedlings in the three-leaf stage; however, washings from MDMV-infected seedlings in the four-leaf stage contained twice as much ninhydrin-positive substances as washings from fourth leaf stage, virus-free seedlings (*unpublished*).

Total amino acid content of leachates from MDMV-infected leaves was 1,326 μg/g dry wt leaf tissue as compared to 417 μg/g for leachates from MDMV-free leaves (averages, three experiments). The amino acids detected were alanine, γ-aminobutyric acid, aspartic acid, cysteine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine, and valine. Each occurred in higher (twofold to sixfold) concentration in leachates from MDMV-infected leaves; largest increases in concentration were noted with methionine, valine, glutamic acid, γ-aminobutyric acid, and proline. Low concentrations of phenylalanine were found in leachates from MDMV-infected leaves, but none was detected in leachates from virus-free leaves.

DISCUSSION

These results confirm an earlier report that MDMV-infected corn is more susceptible to *H. maydis* than virus-free corn, and they suggest a possible basis for this increased susceptibility. The permeability of corn leaves infected with MDMV was altered, as evidenced by the increased leakage of electrolytes and other substances in these tissues. The increased levels of ninhydrin-positive substances and cations leaked onto surfaces of MDMV-infected leaves may have contributed to the increase in germ tube and appressorium formation by *H. maydis* observed on these leaves. Other reports have indicated that chemicals released from within plants may influence appressorium formation (6). An increase in germ tube and appressorium formation could increase the probability of successful penetration by *H. maydis.*

The number and size of lesions caused by *H. maydis* also could have been affected by the increased exudation of amino acids and carbohydrates into intercellular spaces of MDMV-infected leaves. Such leakage might have altered the nutritional status of the tissues 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

1. Beniwal, S. P. S., and Gudauskas, R. T. 1974. Maize dwarf mosaic virus increases susceptibility of sorghum and corn to *Helminthosporium maydis* Race T. Phytopathology 64:1197-1201.
2. Beute, M. K. 1973. Increased leaf exudation enhances Curvularia leaf spot severity in virus-infected gladiolus. Phytopathology 63:1204-1205.
3. Beute, M. K., and Lockwood, J. L. 1968. Mechanism of increased root rot in virus-infected peas. Phytopathology 58:1643-1651.
4. Bradfute, O. E., and Robertson, D. C. 1981. Electron microscopy of viruses and virus-infected cells of maize. Pages 25–32 in: Virus and viruslike diseases of maize in the United States. D. T. Gordon, J. K. Knoke, and G. E. Scott, eds. Southern Coop. Series Bull. 247. June 1981. OARDC, Wooster, OH. 218 pp.
5. Dickinson, C. H. 1979. External synergisms among organisms inducing disease. Pages 97–111 in: Plant Disease, An Advanced Treatise. Vol. IV. How Pathogens Induce Disease. J. G. Horsfall and E. B. Cowling, eds. Academic Press, Inc., New York. 466 pp.
6. Emmett, R. W., and Parbery, D. G. 1975. Appressoria. Annu. Rev. Phytopathol. 13:147-167.
7. Ford, R. E., and Tu, J. C. 1969. Free amino acid contents in corn infected with maize dwarf mosaic virus and sugarcane mosaic virus. Phytopathology 59:179-182.
8. Gudauskas, R. T., and Ford, R. E. 1981. Physiology of disease. Pages 85–87 in: Virus and viruslike diseases of maize in the United States. D. T. Gordon, J. K. Knoke, and G. E. Scott, eds. Southern Coop. Series Bull. 247. June 1981. OARDC, Wooster, OH. 218 pp.
9. Keleti, G., and Lederer, W. H. 1974. Handbook of Micromethods for the Biological Sciences. Von Nostrand-Reinhold Company, New York. 166 pp.
10. Powell, N. T. 1979. Internal synergisms among organisms inducing disease. Pages 113–133 in: Plant Disease, An Advanced Treatise. Vol. IV. How Pathogens Induce Disease. J. G. Horsfall and E. B. Cowling, eds. Academic Press, Inc., New York. 466 pp.
11. Robinson, P. W., and Hodges, C. F. 1977. Effect of nitrogen fertilization on free amino acid and soluble sugar content of *Poa pratensis* and on infection and disease severity by *Drechslera sorokiniana.* Phytopathology 67:1239-1244.
12. Shishiyama, J., Egawa, H., Mayama, S., and Akai, S. 1969. Role of amino acids in the development of *Helminthosporium* blight disease of rice plant and some enzyme activities relating to amino acid metabolism in the host-parasite interaction. Mem. Coll. Agric., Kyoto Univ., Jpn. 95:7-34.
13. Sweeley, C. C., Bentley, R., Makita, M., and Wells, W. W. 1963. Gas-liquid chromatography of trimethylsilyl derivatives of sugars and related substances. J. Am. Chem. Soc. 85:2497-2507.
14. Trainor, M. J., and Martinson, C. A. 1978. Nutrition during spore production and the inoculum potential of *Helminthosporium maydis* Race T. Phytopathology 68:1049-1053.
15. Tu, J. C., and Ford, R. E. 1971. Maize dwarf mosaic virus predisposes corn to root rot infection. Phytopathology 61:800-803.
16. Tukey, H. B., Jr., Tukey, H. B., and Wittwer, S. H. 1958. Loss of nutrients by foliar leaching as determined by radioisotopes. Proc. Am. Soc. Hortic. Sci. 71:496-506.