A Selective Toxin Produced by Phyllosticta maydis

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ABSTRACT

Culture filtrates from isolates of *Phyllosticta maydis* pathogenic to corn, inhibited root growth and shoot development of *P. maydis*-susceptible corn more than that of *P. maydis*-resistant corn. Culture filtrate from nonpathogenic *P. zeae* did not selectively inhibit growth of *P. maydis*-susceptible corn. Both *P. maydis* and *P. maydis* culture filtrate appear to be selective for corn with Texas male sterile cytoplasm.

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Additional key words: host-specific toxin, yellow leaf blight of corn.

There is a growing list of plant diseases in which host-specific toxins are known to play significant roles (10, 19). Studies of these diseases and of the toxins involved have supplied valuable tools for screening for disease resistant plants (9, 19), and have provided basic information concerning the molecular basis of disease and disease resistance (19). Specificity of the pathogen appears to be based on its ability to produce a specific chemical or group of chemicals. Susceptibility or resistance is determined by sensitivity or insensitivity of the host plants to a specific, pathogen-produced chemical (19). It is important to determine how widespread among plant diseases this type of mechanism is. Demonstration of additional host-specific toxins suggests that selective pathogen-produced metabolites may be commonly involved in plant diseases. The present study indicates that a host-selective toxin is produced by *Phyllosticta maydis*, and that it may be involved in yellow leaf blight of corn. The study was guided by techniques used to identify previously described host-specific toxins (10, 17, 18). Identification of a similar toxin was made concurrently by Comstock et al. (6). Preliminary reports have been published (5, 25).

Yellow leaf blight is a relatively new disease of corn, first observed in Ohio in 1965 (12). Corn with disease symptoms similar to those described in Ohio was reported in 1967 in Wisconsin (2), in 1968 in Pennsylvania (20) and New York (4), and at about the same time or shortly thereafter in most of the northern corn-growing States and in Canada. Although the disease is widespread, economically important losses have not occurred over wide areas. However, locally severe epiphytotics have apparently caused up to 50% loss in yield (2).

The disease now known as yellow leaf blight has also been referred to as Phyllosticta leaf spot (20). The causal organism was first thought to be *P. zeae* Stout (20, 21), which was described in 1930, but was not demonstrated to be pathogenic to corn (22). More recently the causal organism has been formally described as a new species, *P. maydis* (1). The sexual stage of this fungus is called *Mycosphaerella zeae-maydis* (13).

MATERIALS AND METHODS.—Cultures of *P. maydis* were supplied by D. M. Mukunya, Department of Plant Pathology, Cornell University. The isolates were from field-grown corn in New York which showed symptoms of yellow leaf blight. Monoconidial isolates were identified as *P. maydis* according to the description of Arny & Nelson (1), and by demonstrating pathogenicity to corn. Conidia were single celled; average size was 12.5 × 4.5 μ. One isolate produced small conidia (6.0 × 2.5 μ) which were single celled. It was nonpathogenic to corn and was tentatively identified as *P. zeae* Stout (22). Pycnidia of all isolates were produced on potato dextrose agar held at 20-22 C under artificial light.

Corn cultivars W182B, Pa83 Rf, NY821, C0109, M4, and M3, with Texas male sterile (T) cytoplasm (susceptible to *P. maydis*); and Pa33 × D410, C153 × C0109, and W182B with nonsterile (N) cytoplasm (resistant to *P. maydis*) were supplied by C. O. Grogan, Department of Plant Breeding and Biometry, Cornell University. Single crosses Pr × K61 (susceptible to *Helminthosporium carbonum* race I) and Pr 1 × K61 (resistant to *H. carbonum* race I) were produced by A. J. Ullstrup, Department of Botany and Plant Pathology, Purdue University, and were supplied by R. P. Scheffer, Department of Botany and Plant Pathology, Michigan State University. Inbreds Pa33 T (susceptible to *P. maydis*) and Pa33 N (resistant to *P. maydis*) were obtained from Pennsylvania Foundaion Seed Cooperative, Inc., Jersey Shore, Pa. Disease severity ratings (provided by D. M. Mukunya & C. W. Boothroyd) were based on both greenhouse and field inoculation tests.

For toxin production, *P. maydis* was grown at 19 ± 1 C under continuous fluorescent light in 300-ml flasks containing 50 ml modified Fries' No. 3 basal medium (14) supplemented with 0.1% (w/v) yeast extract. After 11 days, mycelial mats were separated from the culture fluid with cheesecloth and Whatman No. 1 filter paper. The culture fluid was adjusted to pH 3.5 and stored at 3 C; solutions for assay were adjusted to approximately pH 5.5. Filtrate which had passed through a 0.22-μ Millipore filter and nonsterile filtrate gave similar results upon bioassay.

A seedling root growth bioassay was patterned after assays described for other toxins (7, 10, 14, 17, 18). Corn seedlings were produced from seeds incubated embryo-down on 100 ml water agar in 15-cm petri dishes (50 seeds/dish) for 48 hr. Seedlings with primary roots 5- to 10-mm long were placed in 10-cm petri dishes containing 10 ml White's solution (23) or culture filtrate diluted with White's solution. For this assay, all reported values are based on the average length of six primary roots after 3 days in darkness at 23 C.

A second bioassay involved utilization of excised shoots from plants grown in vermiculite in the laboratory under fluorescent light. Plants 6- to 8-cm tall (10-14 days old) were excised 2 cm above the roots and placed in 4-ml glass vials containing water or culture filtrate diluted with water. Plants were held under fluorescent light 1 to 2 days; effects on leaves were rated as 0 (no damage), 1 (necrotic flecks), 2 (extensive chlorosis and/or necrosis), or 3 (excised shoot dead).

An injection technique, used to screen corn for susceptibility to HM-T toxin (9), was used to detect toxicity of *P. maydis* culture filtrates. Aliquots (10 to 500 μl) of culture filtrate were injected into the stems of 2-week-old plants. Within 2 to 3 days, leaves were evaluated for chlorotic and necrotic streaks.

RESULTS.—Host-specific toxicity of culture filtrate from *P. maydis* was detected qualitatively with the excised shoot bioassay. *P. maydis*-susceptible excised shoots became necrotic within 24 hr after exposure to a 50-fold dilution of culture filtrate. There was little or no effect on *P. maydis*-resistant excised shoots.

The seedling root growth bioassay provided more easily quantifiable data than did the excised shoot bioassay. Diluted culture filtrate inhibited root growth of *P. maydis*-susceptible corn more than root

growth of *P. maydis*-resistant corn. The most active filtrates produced to date, caused significant inhibition of root growth of susceptible corn at a dilution of 1:400; root growth of resistant corn was inhibited at a dilution of 1:10.

The seedling root growth bioassay was used to test corn cultivars which were rated susceptible or resistant to *P. maydis* in greenhouse and field trials. In this test cultivars significantly inhibited by 1:25 dilution of culture filtrate were considered sensitive to the filtrate. All of the cultivars tested which contained T cytoplasm were susceptible to the fungus and sensitive to culture filtrate (Table 1). The three cultivars tested which contained N cytoplasm were resistant to the fungus and relatively insensitive to culture filtrate.

The specificities of *P. maydis* and *P. zeae* culture filtrates were compared. Corn susceptible to *P. maydis* was sensitive to culture filtrate from *P. maydis*, whereas corn resistant to *P. maydis* was relatively insensitive to culture filtrate (Table 2). Culture filtrate from *P. zeae* (nonpathogenic to corn) caused significant inhibition of root growth of certain cultivars at 1:50 dilution, but the pattern of inhibition was not related to susceptibility to *P. maydis* (Table 2). Hybrid Pa33 X D410 is resistant to *P. maydis*; root growth was inhibited equally by culture filtrates from *P. maydis* and *P. zeae* at 1:50 dilution. This suggests that the inhibition was not caused by the host-specific factor produced by *P. maydis*. At higher dilutions, only *P. maydis* filtrate was toxic, and its effect was specific for corn susceptible to *P. maydis*.

TABLE 1. Effect of culture filtrate from *Phyllosticta maydis* on root growth of corn susceptible or resistant to *P. maydis*[a]

Cultivar	Disease reaction[b]	Growth of control (cm)	Inhibition[c] (%)	Toxin rating[d]
Pa33 T	S	13	78	S
W182B T	S	9	74	S
Pa83 T Rf	S	14	82	S
NY821 T	S	13	87	S
C0109 T	S	7	67	S
M4 T	S	14	67	S
M3 T	S	11	71	S
Pa33 N	R	10	20	R
C153 X C0109 N	R	11	5	R
W182B N	R	10	25	R

[a] Seeds were germinated on water agar and placed in petri dishes containing White's solution with or without culture filtrate (diluted 1:25).

[b] Rated on a 1 (no symptoms) to 5 (severe leaf blight) scale similar to that of McFeeley (12). Plants rated 2.5 or higher were considered susceptible (S); R = resistant.

[c] Percent reduction in root growth as compared with control.

[d] Inbreds which sustained inhibition of root growth which was significantly different ($P \leq 0.05$) from control were considered susceptible.

In another experiment, the abilities of culture filtrates from *P. maydis*, *P. zeae* (nonpathogenic to corn), and a *Mucor* sp. (a saprophyte) to inhibit root growth of corn seedlings were examined. *P. maydis* culture filtrate selectively inhibited root growth of corn susceptible to *P. maydis*. *P. zeae* and *Mucor* sp. filtrates inhibited root growth of both *P. maydis*-susceptible and resistant corn from 30 to 50% at dilutions as high as 1:50.

Several pathogenic isolates of *P. maydis* from different fields in New York were tested for ability to produce host-specific toxin in culture. Three other fungi isolated from corn, *P. zeae*, *Colletotrichum* sp., and *Ascochyta* sp., were included for comparison. Culture filtrates from isolates of *P. maydis* caused inhibition of root growth of *P. maydis*-susceptible corn but did not affect root growth of *P. maydis*-resistant corn (Table 3). Production of host-specific toxin in culture was not related to fungal growth. Toxin was found in filtrates ranging in pH from 3.9 to 6.6 (Table 3). Filtrates from cultures of the other three fungi did not inhibit root growth of corn at a 90-fold dilution (Table 3), but did cause some inhibition of root growth of both susceptible and resistant corn at dilutions of 1:30 or less. Growth and pH levels of culture filtrates of these three fungi were comparable to those of the *P. maydis* isolates (Table 3).

The specificity of *P. maydis* culture filtrate was compared with that of two other host-specific toxins which selectively affect certain types of corn. *Helminthosporium maydis* race T (HM-T) toxin [prepared following the procedures of Comstock and Scheffer (7)] is specific for corn containing T and certain other types of male sterile cytoplasm (7, 8, 10). *H. carbonum* race I (HC) toxin [prepared as described previously (15, 24)] is specific for corn homozygous recessive for the gene conditioning susceptibility to *H. carbonum* race I (18). HM-T toxin and *P. maydis* culture filtrate inhibited root growth of corn containing T cytoplasm, susceptible to *H. maydis* race T and *P. maydis* (Table 4). HC toxin inhibited only root growth of corn susceptible to *H. carbonum* race I.

An injection technique similar to that described for testing HM-T toxin (9) was used to determine the effect of *P. maydis* culture filtrate on greenhouse-grown corn. As with the seedling root growth bioassay, *P. maydis* culture filtrate selectively damaged corn cultivars which are susceptible to *P. maydis* (Table 5). The experiment was repeated in the laboratory with plants grown in vermiculite. *P. maydis* filtrate (10 µl/plant) caused symptoms on *P. maydis*-susceptiblc corn but had little or no effect on *P. maydis*-resistant corn. *P. zeae* filtrate (10 µl/plant) caused no damage to corn.

DISCUSSION.—Evidence that *P. maydis* produced a host-specific toxin is based on two observations. 1) All isolates of *P. maydis* which were tested were pathogenic and produced filtrates which were selectively toxic to *P. maydis*-susceptible corn. Several other fungi isolated from corn and a saprophytic *Mucor* sp. did not produce in culture toxic material with the same specificity as that of *P.*

TABLE 2. Effect of culture filtrate from *Phyllosticta maydis* (PM) or *P. zeae* (PZ) on root growth of corn susceptible or resistant to *P. maydis*[a]

Cultivar	Disease reaction[b]	Growth of control (cm)	% Inhibition[c] at dilution 1:50 PM	1:50 PZ	1:100 PM	1:100 PZ	1:200 PM	1:200 PZ
Pa33 T	S	13	77[d]	29[d]	46[d]	0	40[d]	0
Pa33 N	R	13	15	29[e]	3	0	0	1
W182B T	S	6	54[d]	0	51[d]	2	51[d]	0
W182B N	R	10	9	4	9	0	0	0
M3 T	S	15	64[d]	0	45[d]	15	30[d]	0
Pa33 × D410 N	R	16	28[d]	33[e]	13	0	0	0

[a] The seedling root growth bioassay was used as in Table 1.
[b] Rated as described in Table 1.
[c] Percent reduction in root growth as compared with control.
[d] Significantly different ($P \leq 0.01$) from control.
[e] Significantly different ($P \leq 0.05$) from control.

maydis culture filtrate. 2) Corn cultivars susceptible to *P. maydis* were sensitive to *P. maydis* culture filtrate. Cultivars resistant to *P. maydis* were relatively insensitive to *P. maydis* culture filtrate.

In the studies reported here, all susceptible cultivars contained T cytoplasm, whereas resistant cultivars contained N cytoplasm. Several workers (2, 3, 4, 11, 12, 21) have found that most cultivars of corn containing T cytoplasm are more susceptible to *P. maydis* than the corresponding cultivars containing N cytoplasm. In this respect yellow leaf blight is similar to southern corn leaf blight caused by *H. maydis* race T (10). Some corn cultivars with both T and N cytoplasm, however, are equally susceptible to *P. maydis* (2, 3, 4, 11). Preliminary experiments (25) have indicated that corn with N cytoplasm which is susceptible to *P. maydis* is also relatively sensitive to *P. maydis* culture filtrate. This raises the possibility that *P. maydis* is capable of producing more than one host-specific toxin. Such a phenomenon has been reported previously (16).

TABLE 3. Toxin production in culture by fungi isolated from corn[a]

Isolate	pH of filtrate[b]	Dry wt of fungus[c]	% Inhibition[d] of inbred Pa33 T	Pa33 N
Phyllosticta maydis 1	3.9	664	69	0
Phyllosticta maydis 3	4.4	616	77	0
Phyllosticta maydis 11	4.5	186	66	5
Phyllosticta maydis 24	6.6	670	77	0
Phyllosticta maydis 27	4.4	627	66	0
Phyllosticta maydis 28	4.7	314	27	0
Phyllosticta maydis 29	4.6	234	68	0
Phyllosticta zeae	4.0	587	0	0
Ascochyta sp.	4.7	284	0	0
Colletotrichum sp.	4.7	94	3	9

[a] The seedling root growth bioassay was used as in Table 1. Control roots grew 14 and 16 cm for Pa33 T (susceptible to *P. maydis*) and Pa33 N (resistant to *P. maydis*), respectively. Culture filtrates were diluted 90-fold.
[b] pH at time of harvest; pH of assay solutions was approximately 5.5.
[c] Mean wt (mg) of mycelial mats from three culture flasks.
[d] Percent reduction in root growth as compared with control.

TABLE 4. Comparative specificities of three host-specific toxins which affect corn[a]

Cultivar	Susceptible to:	Growth of control (cm)	% Inhibition[b] by toxin HM-T toxin[c]	HC toxin[c]	PM toxin[d]
Pa33 T	*H. maydis* race T *P. maydis*	15	42[e]	0	61[e]
Pa33 N	None of the test fungi	15	15	5	0
M3 T	*H. maydis* race T *P. maydis*	22	40[e]	10	53[e]
Pr × K61	*H. carbonum* race I	17	0	59[e]	0
Prl × K61	None of the test fungi	15	4	0	0

[a] The seedling root growth bioassay was used as in Table 1.
[b] Percent reduction in root growth as compared with control.
[c] Partially purified preparations were used at 0.67 μg/ml.
[d] Culture filtrate was diluted 100-fold.
[e] Significantly different ($P \leq 0.001$) from control.

TABLE 5. Effect of injection with *Phyllosticta maydis* culture filtrate on greenhouse-grown corn[a]

Inbred	Disease reaction[b]	Rating[c]		
		Undiluted	1:10 dilution	Water control
Pa33 T	S	58	18	0
Pa33 N	R	3	0	0
W182B T	S	65	17	0
W182B N	R	13	5	0
C0109 T	S	79	15	0

[a] Aliquots (0.5 ml) of toxin solution were injected by hypodermic syringe into corn stems.

[b] Rated as described in Table 1.

[c] The rating was the mean value from five replicate plants and was calculated from:

$$\frac{\%\text{ leaves affected per plant}}{\text{severity of symptoms}}$$

Severity of symptoms = 1 (more than 50% of tissue chlorotic or necrotic), 2 (chlorotic streaking but no necrosis), or 3 (slight chlorosis at the point of injection).

The problems involved with the use of unpurified culture filtrates were demonstrated in experiments with nonpathogenic *P. zeae* and saprophytic *Mucor* sp. Both fungi produced metabolites in culture which inhibited root growth of corn at dilutions as high as 1:50. The pattern of inhibition among corn cultivars did not follow the host range of *P. maydis*. Therefore, the "toxins" produced by these fungi are different from the host-specific factor produced by *P. maydis*. Since neither *P. zeae* nor *Mucor* are known to be pathogens, the "toxins" they produce cannot be construed as playing a role in disease development. It is possible that certain isolates of *P. maydis* also are capable of producing "toxins" similar to those produced by the nonpathogens. Such materials could interfere with the bioassay for host-specific toxin activity, as reported for *H. maydis* race T (7).

Not all corn cultivars were equally sensitive to the "toxins" produced by nonpathogenic fungi (Table 2). Thus, toxic fungal culture filtrates may discriminate among genotypes of higher plants, but this does not necessarily indicate a role for such "toxins" in disease.

There is a previous report of a toxin produced by *Phyllosticta* sp. pathogenic to corn (11). Undiluted culture filtrate caused some degree of inhibition of root growth of all corn lines tested. Sensitivity to undiluted culture filtrate was not correlated with susceptibility to *Phyllosticta* sp. Diluted culture filtrate did not inhibit root growth of corn (11). It appears that the selectively toxic factor reported in the present study is different from the toxic activity described by Koons (11).

The host-specific toxin found in culture filtrates of *P. maydis* is different from the host-specific toxin produced by *H. carbonum* race I. However, it may be similar to HM-T toxin produced by *H. maydis* race T because both toxins are selective for corn with T cytoplasm and because both toxins cause damage to mitochondria from corn with T but not N cytoplasm (6).

There are indications that *P. maydis* toxin can be used to identify plants susceptible to *P. maydis* in greenhouse (Table 5) and field tests (V. E. Gracen & O. C. Yoder *unpublished*), using an injection technique (9). This technique permits the selection of breeding material which is resistant to *P. maydis*.

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