Host Preference Correlated with Chlorate Resistance in *Macrophomina phaseolina*

C. A. S. PEARSON, J. F. LESLIE, and F. W. SCHWENK, Department of Plant Pathology, Throckmorton Hall, Kansas State University, Manhattan 66506

ABSTRACT

Pearson, C. A. S., Leslie, J. F., and Schwenk, F. W. 1987. Host preference correlated with chlorate resistance in *Macrophomina phaseolina*. Plant Disease 71:828-831.

Isolates of *Macrophomina phaseolina* from maize and soybean field soils and plant tissues were collected from 13 states in the central and eastern United States. The chlorate phenotype of each isolate was determined after subculture on a minimal medium containing 120 mM potassium chlorate. In general, maize isolates were chlorate-resistant, whereas soybean isolates were chlorate sensitive. Isolates from soil populations of the fungus could be partitioned into three phenotypic classes (dense, feathery, and restricted). Isolates with a dense phenotype (chlorate-resistant) preferentially colonized maize tissues, whereas isolates with feathery and restricted phenotypes (chlorate-sensitive) preferentially colonized soybean tissues. The effects of a 2-yr crop rotation on soil and tissue populations of *M. phaseolina* also were examined in a field in southeastern Kansas. Only isolates with the feathery and restricted phenotypes were detected in this field. The *M. phaseolina* soil population was significantly lower following a maize-after-maize cropping sequence (37.5 propagules per 5 g of soil) than it was following a soybean-after-soybean cropping sequence (68.3 propagules per 5 g of soil). Quantitatively, higher levels of fungus were obtained from soybean tissue (25.6 propagules per 100 mg) than from maize tissue (2.4 propagules per 100 mg). Host preference appears to be exhibited in both the *M. phaseolina*:maize and the *M. phaseolina*:soybean pathosystems.

Additional key words: charcoal rot, crop rotation, *Glycine max, Zea mays*

Macrophomina phaseolina (Tassi) Goid. is the causal agent of charcoal rot. Many economically important hosts, including cereals, legumes, vegetables, fruits, and fiber crops, are attacked by this anamorphic fungus (9). Although only one species is currently recognized (29), variability in morphology and pathogenicity has been observed among isolates obtained from different hosts (26). Also, different isolates from a single host plant may vary in these characters (8). Such variability has made it difficult to group isolates, although several attempts have been made (14,26). We have used growth in a minimal medium containing chlorate to subdivide this species into three physiologically distinct classes (22,23).

Chlorate, an analog of nitrate, has been used to study nitrogen assimilation in bacteria, algae, fungi, and higher plants (1,3,6,28). The only known route for nitrate assimilation is by reduction to nitrite via the enzyme nitrate reductase; nitrite is then reduced to ammonia (10,11,16). Nitrate reductase also can reduce chlorate to chlorite (6). The resulting accumulation of chlorite is presumably poisonous to cells (28). Generally, fungal strains that have functional nitrate reductase are chlorate-sensitive, whereas those that are unable to catabolize nitrate are chlorate-resistant (11,16).

Isolates of some fungal species such as *Fusarium moniliforme* (15,25), *F. oxysporum* (3–5,24), *F. solani* (3), *Aspergillus flavus* (19), and *Verticillium albo-atrum* (13) behave in a peculiar fashion when grown on an agar medium that contains potassium chlorate. When these fungi are placed on chlorate medium, their growth is initially inhibited. Spontaneous sectors often form that grow poorly when placed on a minimal medium containing nitrate as the sole nitrogen source; i.e., the strains can no longer use nitrate as a nitrogen source. Two genetically distinct mutant sectors derived from the same parental strain can complement one another, resulting in good growth when a heterokaryon is formed on a minimal medium containing nitrate as the sole nitrogen source (24,25).

Unlike these fungi, *M. phaseolina* does not produce chlorate-resistant sectors when placed on a minimal medium containing potassium chlorate (22). Instead, differences in chlorate sensitivity are observed among isolates. Isolates from maize are chlorate-resistant and grow in a dense pattern on chlorate minimal medium, whereas isolates from soybean are sensitive to chlorate and grow in either a feathery or a restricted pattern. Nitrogen source utilization studies indicate that maize isolates utilize nitrate less efficiently than soybean isolates (23). This difference correlates well with the differences observed in maize and soybean xylem sap nitrogen composition (17,20).

Although *M. phaseolina* is considered plurivorous, preliminary evidence from a limited number of isolates from Kansas suggested that isolates with one of the chlorate phenotypes preferentially colonize maize, whereas isolates with the other chlorate phenotypes preferentially colonize soybean (22). The objectives of the current study were to: 1) characterize soil and tissue populations of *M. phaseolina* from several areas of the central and eastern United States with respect to their chlorate phenotypes and 2) determine if host preference existed in populations of *M. phaseolina* in vivo. Because host preference could result in selection against certain members of the population, the effects of crop rotation on the relative frequency of strains with different chlorate phenotypes in soil and host tissue also were examined.

This investigation was supported in part by a grant from the Kansas State Board of Agriculture/Soybean Commission.

Contribution 87-317-J, Kansas Agricultural Experiment Station, Manhattan.

Accepted for publication 7 May 1987 (submitted for electronic processing).

MATERIALS AND METHODS

During August 1986, soil and tissue samples were collected from 115 fields in the states of Alabama, Arkansas, Florida, Georgia, Illinois, Indiana, Kansas, Mississippi, Missouri, North Carolina, Ohio, South Carolina, and West Virginia. At a given site, four cylindrical soil cores about 5 × 13 cm were randomly taken from between plant rows. Four randomly selected maize stalk or soybean root samples also were obtained. Soil samples were mixed, and 5-g subsamples were wet-seived and treated with NaOCl as described by Campbell and Nelson (2). Tissue samples were surface-sterilized in 0.8% NaOCl for 1 min, blotted dry with paper toweling, and placed in paper bags. These tissue samples were dried in a forced-air oven for 24 hr at 28 C and then ground in a Wiley mill through an 850-μm (20-mesh) screen. A 100-mg subsample of milled tissue was placed in a 600-ml beaker containing 100 ml 0.525% NaOCl for 3 min. The contents of the beaker were then rinsed onto a 45-μm (325-mesh) screen and washed with water for 1 min. Samples were mixed with 100 ml of chloroneb-mercury-rose bengal agar (CMRA) (18) and poured into five petri dishes (100 × 15 mm). These cultures were incubated in the dark for 1 wk at 30 C. Colonies of *M. phaseolina* were transferred to chlorate medium (22,23), incubated in the dark for 1 wk at 30 C, and then scored for their chlorate phenotype: dense (chlorate-resistant), feathery (chlorate-tolerant), and restricted (chlorate-sensitive) (22). Because of the large number of isolates recovered from some samples, not all isolates could be tested on chlorate medium. To reduce bias when testing isolates from such samples for their chlorate phenotype, all colonies from a single CMRA plate were transferred to chlorate medium. If fewer than 25 isolates were recovered from the first plate, then all of the isolates from a second plate were transferred to chlorate medium. The process was continued until at least 25 colonies per sample had been transferred to chlorate medium.

Isolates representative of the chlorate phenotypes recovered from each sample were transferred from the chlorate medium to test tubes 13 × 100 mm containing 2-ml slants of potato-dextose agar (Difco). After 1 wk at 30 C, 5 ml of sterile 15% glycerol was added to each slant and sclerotia were scraped from the agar with a sterile pipet. About 2 ml of the resulting sclerotial suspension was transferred to a cryo-vial (Kew Scientific, Columbus, OH) and then frozen at −70 C. With this procedure, cultures of *M. phaseolina* remain viable for at least 15 mo and probably much longer.

A 2-yr crop rotation experiment was conducted at the Southeast Kansas Branch Experiment Station, Parsons, on a Parsons silty loam soil. For several years, the field had been planted to winter wheat; a 1-yr fallowing occurred just before the current study. During the first season of this study, soybean cultivar Douglas and maize cultivar Garst Blend 120 were planted in plots 6.1 × 12.2 m at a rate of about 349,000 and 58,000 seeds per hectare, respectively. In October, after harvest, crop residues were incorporated into the soil by disking. In May of the second year, maize and soybean were planted in plots 6.1 × 12.2 m to give four cropping sequences: maize after maize, soybean after maize, maize after soybean, and soybean after soybean. The design was a randomized complete block with four replicates. Soil and tissue samples were collected in September and assayed for *M. phaseolina* as described.

RESULTS

More than 2,100 *M. phaseolina* isolates from plant and soil samples collected from 13 states in the central and eastern United States were examined for their chlorate phenotype (Table 1). Isolates from soybean tissue generally were sensitive to potassium chlorate. Of the 388 isolates tested, 73.2 and 26.5% had a feathery and a restricted phenotype, respectively. Only one isolate collected from this material was chlorate-resistant and, as a result, grew in a dense pattern on the chlorate test medium. In contrast, 80.6% of the isolates from maize tissue had a dense phenotype; the remaining 19.4% were feathery. Although only a few samples were available from other hosts (Table 1), populations from alfalfa and sunflower were similar to those seen in soybean, whereas populations from sorghum and cowpea were similar to those from maize.

Isolates that belonged to each of the different phenotypic classes could be recovered in field soils regardless of the crop being cultivated in the field during the 1986 growing season. Most of the 1,380 isolates examined were chlorate-sensitive (55.9% feathery and 29.5% restricted). Only 14.9% of all the isolates examined were chlorate-resistant (dense phenotype).

To determine if host preference functions in either the *M. phaseolina*: maize or the *M. phaseolina*:soybean pathosystems, the frequencies of isolates with different chlorate phenotypes were determined in paired soil and tissue samples from several different locations. Soil samples collected from soybean fields averaged 2.1% dense isolates, 49.3% feathery isolates, and 48.6% restricted isolates (Table 2). Phenotypic frequencies of isolates in soybean tissue did not differ significantly from the soybean-soil isolate frequencies. Soils assayed from maize fields averaged 29.5% dense isolates, 57.9% feathery isolates, and 12.6% restricted isolates (Table 3). Maize tissue populations of *M. phaseolina* differed significantly from the maize-soil isolate frequencies, averaging 83% dense isolates, 17% feathery isolates, and 0% restricted isolates.

Several maize/soybean cropping sequences were evaluated for their influence on *M. phaseolina* populations (Table 4). When resident soil populations of this fungus were tested on chlorate medium, only feathery and restricted phenotypes were obtained (i.e., the samples were devoid of the dense phenotype commonly associated with maize). The relative frequencies of these two classes in the soil and the tissue populations were not significantly affected by the maize/soybean rotations. Cropping sequence did, however, alter the absolute amount of fungus found in both the soil and plant tissues (Table 4). Significantly fewer fungal isolates were recovered from soils planted continuously to maize than from soils planted continuously to soybean. The level of fungus was significantly higher in soybean tissue than in maize tissue (Table 4).

DISCUSSION

Charcoal rot is a stress-related disease that commonly attacks older plants grown under unfavorable environmental conditions. The causal agent of the disease, *M. phaseolina*, has a host range that encompasses 75 plant families and more than 500 species (9). With such a wide host range, the variability previously reported in sclerotial size and formation (26), pycnidial production (21), chrom-

Table 1. Chlorate phenotypes of *Macrophomina phaseolina* collected from the central and eastern United States

Source	Samples[a] (no.)	Isolates[b] (no.)	Chlorate phenotype		
			Dense	Feathery	Restricted
Soybean	140	388	0.3[c]	73.2	26.5
Soil[d]	84	1,380	14.6	55.9	29.5
Maize	27	263	80.6	19.4	0.0
Alfalfa	3	3	0.0	100.0	0.0
Sorghum	3	35	85.7	14.3	0.0
Sunflower	3	27	0.0	96.3	3.7
Cowpea	1	9	100.0	0.0	0.0

[a] Number of independent samples; in each of 96 soybean samples only one isolate was available for analysis.
[b] Number of isolates tested on chlorate medium.
[c] Percentages of isolates with this phenotype (22).
[d] Isolates included in this class are from soils from various crops.

ogenicity (7), and isolate aggressiveness (8) is not surprising. Even so, only one species is currently recognized (29). Several workers have attempted to group isolates based on size and shape of sclerotia and on pathogenicity (14,26). Schemes using sclerotial characters are quite artificial and usually difficult to use (9). Although identifying differences in pathogenicity would be useful, reliable inoculation procedures for comparing different *M. phaseolina* isolates on older host plants have not been established. Part of this difficulty may come from the need for stress to induce disease in older plants (12). Recently, we have used chlorate sensitivity to partition *M. phaseolina* into three physiologically distinct phenotypic groups (22). The results of the current study suggest that these groups are differentially capable of attacking different host plants.

Table 2. Chlorate phenotypes of *Macrophomina phaseolina* recovered from soybean field soil and tissue samples

	Chlorate phenotype[a]					
	Soil			Tissue		
Sample location	D	F	R	D	F	R
Williamsburg, MO	0[b]	15	50	0	21	3
Wright City, MO	1	9	24	0	22	2
Marine, IL	0	2	8	0	2	14
Greenup, IL	0	3	17	0	3	4
Belleville, IN	0	1	13	0	10	10
Climax, NC	0	18	8	0	4	0
Buie, NC	4	26	4	1	29	0
Morgan, GA	0	45	7	0	3	14
Kusciusko, MS	4	25	1	0	1	16
Holcomb, MS	0	10	28	0	4	26
W. Helena, AR	0	15	16	0	10	10
Grays, AR	0	16	14	0	10	0
Tightwad, MO	0	22	14	0	11	14
Totals	9	207	204	1	130	113
Chi-square[c]	3.12 (ns)					

[a] Phenotype of isolates when grown on 120 mM potassium chlorate; D = dense (chlorate-resistant), F = feathery (chlorate-tolerant), and R = restricted (chlorate-sensitive).
[b] Number of isolates with this phenotype (22).
[c] Chi-square was calculated by using two phenotypic classes, maize (dense) and soybean (feathery + restricted), to compare soil and tissue populations; ns = not significantly different ($P = 0.05$).

Table 3. Chlorate phenotype distribution of *Macrophomina phaseolina* recovered from maize field soil and tissue samples

	Chlorate phenotype[a]					
	Soil			Tissue		
Sample location	D	F	R	D	F	R
Booneville, MO	2[b]	12	12	24	0	0
St. Elmo, IL	0	3	11	0	6	0
Ramseur, NC	3	9	0	7	28	0
Raeford, NC	3	25	0	7	0	0
Walterboro, SC	10	0	0	71	0	0
Luray, SC	0	15	0	0	3	0
Guyton, GA	15	25	0	34	1	0
Broadhurst, GA	11	6	0	29	0	0
Troy, AL	10	11	0	23	2	0
Totals	54	106	23	195	40	0
Chi-square[c]	122.13***					

[a] Phenotype of isolates when grown on 120 mM potassium chlorate; D = dense (chlorate-resistant), F = feathery (chlorate-tolerant), and R = restricted (chlorate-sensitive).
[b] Number of isolates with this phenotype (22).
[c] Chi-square was calculated by using two phenotypic classes, maize (dense) and soybean (feathery + restricted), to compare soil and tissue populations; *** = significantly different ($P < 0.005$).

Table 4. Influence of maize/soybean rotation on number and phenotype of isolates of *Macrophomina phaseolina* in soil and tissue populations in 1986

	Fungus level							
	Soil				Tissue			
Crop sequence[a]	cfu[b]	D	F	R	cfu[c]	D	F	R
Soybean/soybean	68.3	0[d]	53.7	46.3	27.3	0	74.2	25.8
Maize/soybean	56.5	0	82.4	17.6	24.0	0	60.3	39.7
Soybean/maize	44.8	0	56.8	43.2	3.5	0	41.4	58.6
Maize/maize	37.5	0	67.0	33.0	1.3	0	42.5	57.5
LSD ($P = 0.05$)	21.5	···	ns[e]	ns	15.7	···	ns	ns

[a] Cropping sequence used in 1985–1986.
[b] Colony-forming units per 5 g of soil.
[c] Colony-forming units per 100 mg of tissue.
[d] Percentage of isolates with chlorate phenotype: D = dense, F = feathery, and R = restricted.
[e] ns = Not significantly different ($P = 0.05$).

By examining multiple isolates from each of several maize and soybean fields, the frequency of isolates with different chlorate phenotypes was estimated for both soil and tissue populations of *M. phaseolina*. Populations from maize soils contained representatives from all three phenotypic classes; 29.5, 57.9, and 12.6% of the isolates had dense, feathery, and restricted phenotypes, respectively. If all of the isolates within the soil population were equally capable of colonizing the maize tissue, then the composition of the soil and tissue populations should be similar. As shown in Table 3, however, the populations recovered from maize tissue samples differed significantly from those recovered from maize soil samples. Although a mixed inoculum population was available in the maize soils, the high levels of the dense phenotype recovered from maize tissue suggests that members of the *M. phaseolina* population with a dense phenotype preferentially colonize maize. In soybean fields, soil and tissue samples were quite similar in their phenotypic composition, with an approximately 1:1 ratio of feathery and restricted individuals and virtually no members in the dense class (Table 2). It should be noted that no members from the restricted class were recovered from maize tissues but that relatively high levels of the feathery phenotype were recovered from the maize tissue obtained from Ramseur, NC (Table 3). Plants in this area of North Carolina were severely drought-stressed. High levels of stress may alter host nitrogen pools (27), possibly making maize a more suitable host for feathery isolates of the charcoal rot fungus and overriding the host preference that is normally observed.

Cropping sequence may influence *M. phaseolina* levels in the soil. In a field where the resident population was composed solely of isolates with the feathery and restricted phenotypes (commonly associated with soybean), significantly fewer fungal propagules were found in soils after a second year of maize than after a second year of soybeans (Table 4). Soils subjected to maize/soybean and soybean/maize rotations had intermediate levels of *M. phaseolina*. None of the four rotation schemes, however, significantly altered the proportions of feathery and restricted phenotypes obtained from the soil. In a soil devoid of the dense phenotype, about

10 times more fungal propagules were isolated from soybean tissue than from maize tissue (Table 4). Thus, isolates of *M. phaseolina* with either the feathery or the restricted phenotype appear better able to attack and colonize soybean.

Under extreme stress, feathery isolates in the population also may attack maize. Studies to determine how stress might influence *M. phaseolina* host preference in maize and soybeans are needed. Long-term crop rotation studies are currently under way to determine if altering the populations of the different chlorate phenotypes might reduce losses from charcoal rot. We anticipate that cropping soils with soybeans should select against *M. phaseolina* isolates with a dense phenotype. It may be necessary to manipulate both the absolute number of fungal propagules and the relative frequencies of the isolates with different phenotypes to effectively control this disease.

LITERATURE CITED

1. Arst, H. N., and Cove, D. J. 1969. Methylammonium resistance in *Aspergillus nidulans*. J. Bacteriol. 98:1284-1293.
2. Campbell, C. L., and Nelson, L. A. 1986. Evaluation of an assay for quantifying populations of sclerotia of *Macrophomina phaseolina* from soil. Plant Dis. 70:645-647.
3. Correll, J. C. 1986. Differential growth response of *Fusarium oxysporum* and *Fusarium solani* on a medium containing potassium chlorate. (Abstr.) Phytopathology 76:1145.
4. Correll, J. C., Puhalla, J. E., and Schneider, R. W. 1986. Identification of *Fusarium oxysporum* f. sp. *apii* on the basis of virulence, colony size, and vegetative compatibility. Phytopathology 76:396-400.
5. Correll, J. C., Puhalla, J. E., and Schneider, R. W. 1986. Vegetative compatibility groups among nonpathogenic root-colonizing strains of *Fusarium oxysporum*. Can. J. Bot. 64:2358-2361.
6. Cove, D. J. 1979. Genetic studies of nitrate assimilation in *Aspergillus nidulans*. Biol. Rev. 54:291-327.
7. Crall, J. M. 1948. Charcoal rot of soybean caused by *Macrophomina phaseoli* (Maubl.) Ashby. Ph.D. dissertation. University of Missouri, Columbia. 148 pp.
8. Dhingra, O. D., and Sinclair, J. B. 1973. Location of *Macrophomina phaseoli* on soybean plants related to culture characteristics and virulence. Phytopathology 63:934-936.
9. Dhingra, O. D., and Sinclair, J. B. 1978. Biology and Pathology of *Macrophomina phaseolina*. Universidade Federal de Vicçosa, Brazil. 166 pp.
10. Garraway, M. O., and Evans, R. C. 1984. Fungal Nutrition and Physiology. John Wiley & Sons, New York. 401 pp.
11. Garrett, R. H., and Amy, N. K. 1978. Nitrate assimilation in fungi. Adv. Microbiol. Physiol. 18:1-65.
12. Ghaffar, A., and Erwin, D. C. 1969. Effect of soil water stress on root rot of cotton caused by *Macrophomina phaseoli*. Phytopathology 59:795-797.
13. Gordon, T. R., Correll, J. C., and McCain, A. H. 1986. Host specificity and vegetative compatibility in *Verticillium albo-atrum*. (Abstr.) Phytopathology 76:1111.
14. Haigh, J. C. 1930. *Macrophomina phaseoli* (Maulb.) Ashby and *Rhizoctonia bataticolia* (Taub.) Butler. Ann. R. Bot. Gard. Penadeniya 11:213-249.
15. Klittich, C. J. R., Leslie, J. F., and Wilson, J. D. 1986. Spontaneous chlorate-resistant mutants of *Gibberella fujikuroi* (*Fusarium moniliforme*). (Abstr.) Phytopathology 76:1142.
16. Marzluf, G. A. 1981. Regulation of nitrogen metabolism and gene expression in fungi. Microbiol. Rev. 45:437-461.
17. McClure, P. R., and Israel, D. W. 1979. Transport of nitrogen in the xylem of soybean plants. Plant Physiol. 64:411-416.
18. Meyer, W. A., Sinclair, J. B., and Khare, M. N. 1973. Biology of *Macrophomina phaseoli* in soil studied with selective media. Phytopathology 63:613-620.
19. Papa, K. E. 1986. Heterokaryon incompatibility in *Aspergillus flavus*. Mycologia 78:98-101.
20. Pate, J. S. 1973. Uptake, assimilation, and transport of nitrogen compounds by plants. Soil Biol. Biochem. 5:109-119.
21. Pearson, C. A. S. 1982. *Macrophomina phaseolina*, causal organism of charcoal rot of soybean. I. Laboratory tests for resistance. II. Implications of conidia in epidemiology. III. Antimicrobial activity of toxin(s). M.S. thesis. Kansas State University, Manhattan. 59 pp.
22. Pearson, C. A. S., Leslie, J. F., and Schwenk, F. W. 1986. Variable chlorate resistance in *Macrophomina phaseolina* from corn, soybean, and soil. Phytopathology 76:646-649.
23. Pearson, C. A. S., Leslie, J. F., and Schwenk, F. W. 1987. Nitrogen source utilization by chlorate-resistant and chlorate-sensitive isolates of *Macrophomina phaseolina*. Trans. Br. Mycol. Soc. In press.
24. Puhalla, J. E. 1985. Classification of strains of *Fusarium oxysporum* on the basis of vegetative compatibility. Can. J. Bot. 63:179-183.
25. Puhalla, J. E., and Spieth, P. T. 1985. A comparison of heterokaryosis and vegetative incompatibility among varieties of *Gibberella fujikuroi* (*Fusarium moniliforme*). Exp. Mycol. 9:39-47.
26. Reichert, I., and Hellinger, E. 1947. On the occurrence, morphology, and parasitism of *Sclerotium bataticolia*. Palest. J. Bot. 6:107-147.
27. Schoeneweiss, D. F. 1975. Predisposition, stress, and plant disease. Annu. Rev. Phytopathol. 13:193-211.
28. Solomonson, L. P., and Vennesland, B. 1972. Nitrate reductase and chlorate toxicity in *Chlorella vulgaris* Berjeerinick. Plant Physiol. 50:421-423.
29. Sutton, B. C. 1980. The Coelomycetes: Fungi Imperfecti with Pycnidia, Acervuli, and Stromata. Commonw. Mycol. Inst./Assoc. Appl. Biol., Kew, Surrey, England. 696 pp.