Location of Macrophomina phaseoli on Soybean Plants Related to Culture Characteristics and Virulence

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ABSTRACT

Single hyphal-tip isolations were made from 15 cultures of *Macrophomina phaseoli (Rhizoctonia bataticola)* isolated separately from the roots, stem, petiole, pod, and seed for each of three field-grown soybean plants. Root isolates from each of the three plants caused 80-100% mortality of wound-inoculated seedlings within 10 days or produced severe rosetting on surviving seedlings. Petiole, stem, and pod isolates caused 60-100, 30-60, and 10-20% mortality, respectively. After 4 days, sclerotia from cultures of root isolates ranged from 122-188 × 77-127 μ and stem isolates 84-100 × 60-71 μ. Growth rate and colony type varied among isolates from the same plant, and between isolates from different plants, on different media, and within incubation temperatures (15, 20, 25, 30, and 35 C) on potato-dextrose agar. At 30 C, all stem isolates produced fluffy growth, all root isolates were partially fluffy, and the remainder showed appressed growth. The maximum growth was at 35 C for pod and seed isolates and two isolates from root, petiole and stem; and 30 C was optimum for the other three isolates. Each isolate performed consistently in three separate trials. Phytopathology 63:934-936.

Additional key words: charcoal rot, seedling blight.

Variation has been reported among isolates of *Macrophomina phaseoli* (Maubl.) Ashby [*Rhizoctonia bataticola* (Taub.) Butler] pathogenic to soybean collected from different regions of the United States (2). Jain (3) and Khare et al. (4) reported variation among *R. bataticola* isolates from the same host plant, the urid bean. Variation has been reported among crown, root, and foliar isolates of *R. solani* from sugarbeet grown in different locations in Colorado (5). In these studies (3, 4, 5), the isolates were probably collected from different plants in the same field or from scattered fields. We investigated the variation among *M. phaseoli* isolates from different plant parts of a single, naturally infected soybean plant. A preliminary report of the work has been made (1).

Isolations of *M. phaseoli* were made from roots, stems, petioles, pods, and seeds of three naturally infected, soybean (*Glycine max* L.) plants. All isolations were made on Difco potato-dextrose agar (PDA) and single, hyphal-tip cultures were stored on PDA at 4 C. Growth rates and colony characteristics of each isolate on PDA were recorded at 15, 20, 25, 30, and 35 C; and at 25 C on soybean seed agar (SSA) (150 g seed/liter water, boiled for 15 min, strained through cheesecloth, and then 20 g Difco agar added), and Difco nutrient agar (NA). For each study, 4-mm culture disks were cut from the periphery of a 2-day-old PDA culture (25 C) and then transferred upright to the center of 9-cm culture dishes containing 15 ml of either PDA, NA, or SSA. Diameter of colonies was measured after 48 hr for all temperatures and after 72 hr for media. Sclerotia produced on PDA at 25 C were measured after 4 days.

Pathogenicity tests were made on 'Amsoy' soybean seedlings transplanted into vermiculite (Terralite brand) without nutrient solution and grown in an ISCO growth chamber, programmed at 30 C, 50% relative humidity, and 14-hr daylight. Ten 4-day-old seedlings were wound-inoculated at a point 1 cm below the cotyledonary node with inoculum from 7-day-old SSE broth cultures. Wounded, but noninoculated, seedlings served as controls. Seedling mortality and height in cm of surviving seedlings were recorded at 10 days after inoculation. One-cm pieces were cut in succession from hypocotyls above and below the point of inoculation of surviving seedlings to determine the approximate distance the fungus moved within hypocotyl tissues from the inoculation site.

Each experiment was performed for three subsequent subculturings for each hyphal-tip isolate, with three replications in each experiment, to determine any variability within each isolate.

Growth rates varied between each isolate, but not within any single isolate through three subculturings. Variation in growth rate at different temperatures and on different media among isolates from each plant was not comparable (Fig. 1). Most isolates produced maximum growth on PDA at 35 C and had a reduced rate with decrease in temperature except for stem isolates, which showed an optimum growth rate at 25 C on SSA (Table 1).

Colony characteristics, and average sclerotial size and shape, were consistent with each isolate source through three subculturings. Stem isolates produced fluffy growth, root isolates partially fluffy growth; all other isolates had appressed growth. Only seed and petiole isolates produced concentric alternating zones of sclerotia at all temperatures; this characteristic was more conspicuous with the seed isolates (Fig. 2). Seed and root isolates produced irregular-shaped sclerotia, whereas those of all other isolates were round to elliptical. Root isolates produced the largest sclerotia (122-188 × 77-127 μ) ones. Jain (3) reported that isolates of *R. bataticola* from urid bean roots produced the largest sclerotia and that isolates from seed produced the smallest.

By isolation source, the mortality of seedlings after wound-inoculation was: root, 80-100%; petiole, 60-100%; stem, 30-60%; seed, 30-40%; and pod, 10-20%. Symptoms varied from seedling death to stunting (2). Only root isolates produced rosetting of surviving seedlings. *M. phaseoli* was isolated from all surviving wound-inoculated seedlings, but not from controls 3-9 cm from the point of inoculation,

depending upon isolate source. There was no correlation between cultural growth rates and pathogenicity.

This is the first report of pathogenic variation among isolates of *M. phaseoli* from different parts of the same host plant, specifically soybean. Although variation between isolates from different plant parts of urid bean was reported (3, 4), it was not specified whether the isolates were obtained from different plant parts of a single plant or from different plants.

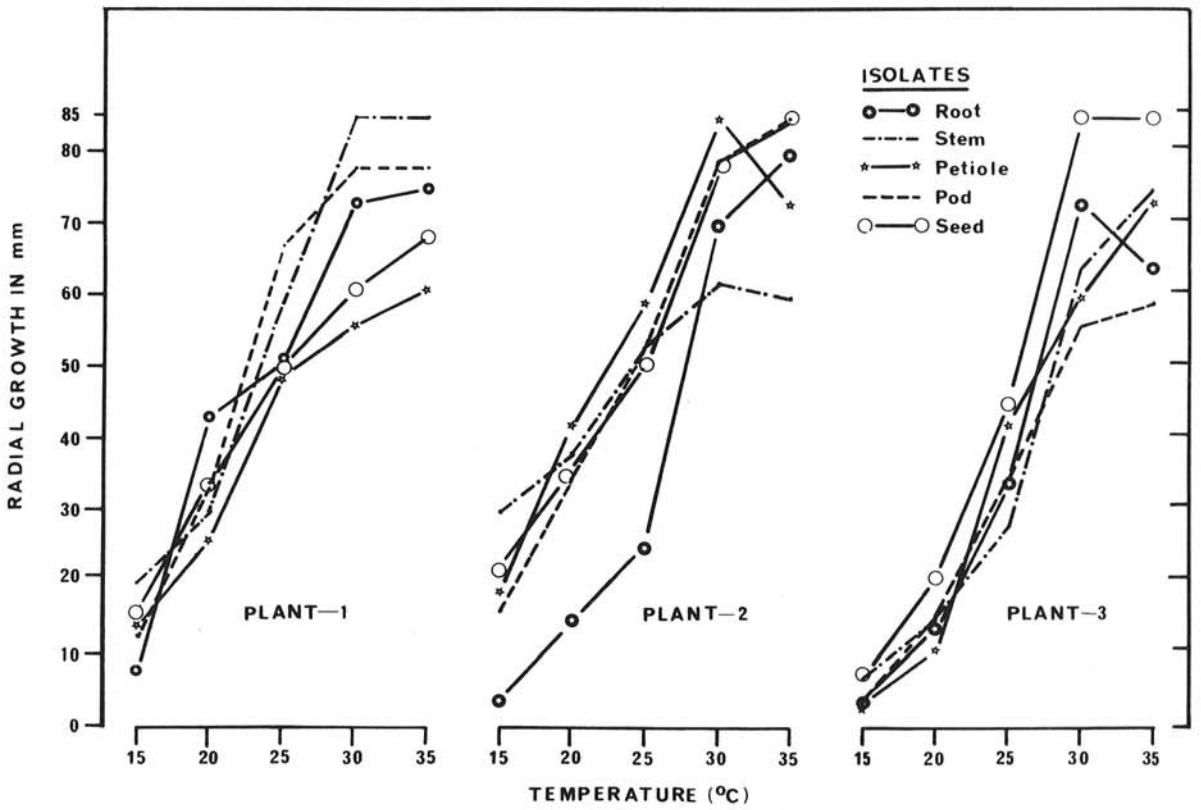

Fig. 1. Mean diameter (mm) of colony of *Macrophomina phaseoli (Rhizoctonia bataticola)* isolates from different parts of three soybean plants grown on Difco potato-dextrose agar for 48 hr at various temperatures. Means of three subsequent subculturings with three replications each.

TABLE 1. Variation in colony diameter of *Macrophomina phaseoli (Rhizoctonia bataticola)* isolates from different parts of three soybean plants, on three media[a] at 25 C after 72 hr

Isolate Source	Colony diam (mm) of isolates from Plant No. 1 PDA	SSA	NA	MEAN	Plant No. 2 PDA	SSA	NA	MEAN	Plant No. 3 PDA	SSA	NA	MEAN
Root	80[b]	52	50	61	76	57	32	55	72	61	33	55
Stem	54	67	43	55	57	85	24	55	51	85	22	52
Petiole	63	50	43	52	70	64	35	56	85	71	38	65
Pod	49	42	34	42	51	52	27	43	76	62	25	54
Seed	56	50	44	50	85	76	41	67	85	73	41	66
Mean	63	50	43		68	67	32		74	70	32	
	For isolates		For media		For isolates		For media		For isolates		For media	
$LSD_{0.5}$	1.70		1.31		1.31		1.03		1.35		1.11	
$LSD_{0.1}$	2.29		1.77		1.77		1.38		1.83		1.49	

[a] PDA = Difco potato-dextrose agar; SSA = soybean seed extract agar; and NA = Difco nutrient agar.
[b] Growth in mm; mean of three subculturings with three replications in each.

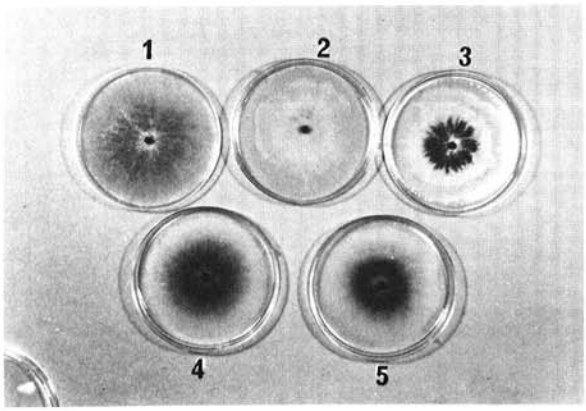

Fig. 2. Variation in cultural characteristics of *Macrophomina phaseoli (Rhizoctonia bataticola)* isolates from 1) stem, 2) petiole, 3) seed, 4) root, and 5) pod of a single soybean plant (potato-dextrose agar).

Also, these researchers (3, 4) studied only one set of isolates from the original culture. In our studies, three sets of single-tip isolates were studied for three succeeding subculturings, which reduced variation within isolates due to random field selection.

M. phaseoli appears to be highly variable, which may reflect the polykaryotic character of the fungus mycelium (T. D. Wyllie, *personal communication*). This may, at least in part, help explain the differences in pathogenicity and cultural characteristics reported by various workers.

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Treatment of Peanuts with Dimethyl Sulfoxide and its Effect on Aflatoxin Production by Aspergillus flavus

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ABSTRACT

Peanut seeds (cultivar 'Early Runner') were treated with different concentrations of dimethyl sulfoxide (DMSO) to study its effect on the production of aflatoxins by *Aspergillus flavus*. Treatment of peanut seeds with 2.5% DMSO or higher concentration prior to inoculation with *A. flavus*, caused an inhibition of normal conidial pigmentation and approximately 62%-64% inhibition in aflatoxin production.

Phytopathology 63:936-937.

Studies on aflatoxin control have concentrated on either the detoxification of aflatoxin or control of the organisms producing aflatoxins (4,5). The treatment of plant tissue to prevent formation of aflatoxins by *Aspergillus* spp. has received little attention. In 1967, Davis & Diener (3) found that p-aminobenzoic acid (PABA) inhibited both production of aflatoxins and growth of *Aspergillus parasiticus* var. *globosum* Speare in liquid culture. They also reported that aflatoxin production was reduced 50% in peanuts soaked in PABA and reduced 30% in peanuts sprayed with PABA.

In 1969, Bean et al. (2) reported that aflatoxin-producing strains of *A. flavus* Link grown on medium containing dimethyl sulfoxide (DMSO), produced white instead of normal green conidia. They also reported that white conidia were killed more rapidly by exposure to ultraviolet irradiation than were green conidia. In 1971,

TABLE 1. Aflatoxin production by *Aspergillus flavus* after 7 days' growth on shelled or unshelled peanut seeds (cultivar 'Early Runner') soaked 30 min with dimethyl sulfoxide (DMSO) before inoculation

Treatment % DMSO	Aflatoxin produced (μg per culture)	
	Shelled peanut seeds	Unshelled peanut seeds
0	104[x] ab	342 ab
0.625	235 a	452 a
1.25	87 b	282 bc
2.5	40 b	124 cd
5.0	24 b	48 d
10.0	12 b	26 d
20.0	10 b	28 d

[x] Values represent avg μg aflatoxin in three cultures. Values followed by the same letters are not significantly different at the 1% levels for Duncan's multiple range test.