Measurement of Soybean Resistance to Stem Canker Caused by *Diaporthe phaseolorum* var. *caulivora*

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ABSTRACT

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Two inoculation techniques were used to determine the relative resistance of soybean (*Glycine max*) cultivars to stem canker caused by *Diaporthe phaseolorum* var. *caulivora*. Sixty-day-old field-grown plants were inoculated by inserting toothpicks infested with the pathogen through the stem below the apical meristem or by spraying the plants with a water suspension of ascospores. Cultivars were rated for resistance on the basis of internal stem lesion development 42 days after toothpick inoculation. Ten cultivars, listed in order of increasing relative resistance, were rated for symptom development: J77-339 (susceptible), Forrest, Bedford, Bragg, Semmes, Jeff, Braxton, Centennial, Hood, and Tracy (resistant). The relative susceptibility of cultivars based on lesion size after inoculation with ascospores was similar to that found with the toothpick inoculation method. Either of the inoculation techniques can be used to evaluate the relative resistance of cultivars and breeding lines to this disease.

Diaporthe phaseolorum (Cke. & Ell.) Sacc. var. *caulivora* Athow & Caldwell (*D. p.* var. *caulivora*) (1) has been recognized as the cause of stem canker in soybean (*Glycine max* (L.) Merr.) since 1954 (10). Stem canker on soybeans became a serious disease in Canada (5) and in the north central region of the United States (2,3) in the early 1950s. This disease was identified in Mississippi in 1974 (12) and subsequently has been observed in other southern states from Texas to Florida.

Natural epiphytotics of the disease are sporadic, and reliable artificial inoculation techniques are needed to evaluate cultivars for resistance. After toothpick inoculation in greenhouse tests, Dunleavy (4) concluded that growth of the fungus in plant stems is directly proportional to cultivar susceptibility. Keeling's (6) toothpick inoculation of soybean seedlings in the greenhouse produced cultivar evaluation results similar to those resulting from natural infection in field plantings. Although the seedling test for stem canker resistance (6) identified resistant and susceptible cultivars, observations from several tests showed variation in disease severity among tests that was probably caused by varying environmental conditions in the greenhouse. Variation in disease severity makes evaluation of cultivar resistance difficult to ascertain from seedling tests. Keeling inoculated stems of field-grown plants 30 and 60 days after planting at about 8 cm above the ground and 10 cm below the apical meristem, respectively. The extent of lesion development agreed generally with ratings assigned the cultivars on the basis of the greenhouse seedling tests (6). These data support Dunleavy's (4) hypothesis that lesion development is directly proportional to cultivar susceptibility. Limited data were obtained in preliminary tests (6), and further research was needed to refine the technique.

The objective of this study was to establish a reliable method for determining the relative resistance of soybean cultivars. This paper reports the responses of soybean cultivars with different levels of resistance to the stem canker disease when inoculated by the toothpick method and with ascospores.

MATERIALS AND METHODS

Pathogen. Three isolates of *D. p.* var. *caulivora* were used. Two isolates were recovered from the highly susceptible cultivar J77-339 at Verona, MS. The third isolate was collected by F. A. Laviolette from soybeans in Indiana. Limited studies indicated that these isolates differed in pathogenicity to seedlings of different soybean cultivars (7).

Toothpick inoculum. Flat toothpicks were boiled for 30 min in each of three changes of distilled water, dried, and placed on-end in large-mouth glass vials

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4 × 11 cm. About 250 toothpicks were placed in each vial with enough potato-dextrose broth (20 ml) so that some fluid remained after the toothpicks became saturated. The vials were stoppered with foam plugs and autoclaved 15 min at 120 C. After cooling, the toothpicks were aseptically inoculated with 10-day-old fungus mycelium grown on potato-dextrose agar (PDA) and incubated at 21 C for 15 days before use (6).

Ascospore inoculum. Ascospores were produced by growing the fungus on acidified PDA (APDA) (20 ml per plastic petri dish [15 × 90 mm]) for 6 wk. The inoculated plates were maintained at 20–23 C in closed plastic bags to reduce desiccation. The cultures received 12 hr of alternating dark and light from two 20W General Electric F20T12-BLB fluorescent black-light bulbs placed 30 cm above the plates. Six-week-old cultures containing perithecia with mature ascospores were macerated in a blender to release the ascospores. The macerated cultures were strained through cheesecloth to remove lumps of the culture medium and mycelial fragments. Inoculum density was adjusted to 50,000 ascospores per milliliter by diluting with distilled water. One milliliter of the wetting agent polyoxyethlene-20 sorbitan monolaurate (Tween 20) was added per 4 L of suspension immediately before inoculation. Ascospores of isolate 5 only were used in the ascospore inoculation test. Isolate 3 and the isolate from Indiana did not produce the quantity of ascospores needed for this test.

Cultivars. The soybean cultivars evaluated included: Bedford and Forrest (group V); Jeff, Centennial, Hood, Tracy-M, and the breeding line J77-339 (group VI); and Bragg, Braxton, and Semmes (group VII).

Inoculation techniques. Sixty days after planting seed in Boskey fine sandy loam soil at Stoneville, MS, plants were inoculated either by inserting a *D. p.* var. *caulivora*-colonized toothpick into the stem or by spraying the plants with ascospore inoculum. Twenty plants per replicate were inoculated by the toothpick technique. The colonized toothpick was inserted through the center of the first elongated internode below the apical meristem (6). A tight fit existed between the stem and the toothpick, and no sealing agent was used.

Plots inoculated with the ascospore suspension were furrow-irrigated for 4 hr beginning at 1:00 p.m. to provide a humid atmosphere, then plants were sprayed with the inoculum at 6:00 p.m. on the same day. Each meter (about 20 plants) of row received 160 ml of suspension, which contained 50,000 ascospores per milliliter.

The experimental design consisted of single-row plots, completely randomized and replicated three times. Data were analyzed statistically by comparing means using an analysis of variance. Differences in treatment means are based on a least significant difference comparison. Log-transformed data were used for the analysis of data from toothpick inoculations because the variances were not homogeneous between treatments (14).

Disease ratings. Plants inoculated by the toothpick technique were rated for disease response 42 days after inoculation. Stems were split with a knife to expose the internal stem tissue, and lesion development was measured from the point of inoculation toward the base of the plant.

Plants inoculated with an ascospore suspension were inspected periodically for disease development. Largest lesions were used in rating the plants for their disease reactions 50 days after inoculation. The following scale was used: 0 = no lesions; 1 = 1 mm or less; 2 = 1.1–3 mm; 3 = up to 6 mm; 4 = up to 10 mm (stems green); 5 = 10 mm and larger (stems chlorotic and dying) (Fig. 1).

RESULTS AND DISCUSSION

Forty-two days after toothpick inoculation, the stems of J77-339 above the point of inoculation were chlorotic and almost dead. Growth of susceptible stems above the inoculation site was inhibited and obtained only about 25% of the growth of inoculated stems of the resistant cultivars Tracy-M and Braxton. At this time, some plants of Forrest and Bedford were showing leaf symptoms (interveinal chlorosis) caused by destruction of stem tissue by the pathogen but were less severely affected than J77-339. Because the stems of susceptible plants were girdled and killed above the points of inoculation, only the lesion length below the point of inoculation was measured. Similar results were obtained when plants were inoculated with isolate 3 or 5 (Table 1). The Indiana isolate of *D. p.* var. *caulivora* did not incite a susceptible reaction in the cultivars tested, and the data are not presented. This isolate was virulent when used to inoculate seedlings in greenhouse tests (*unpublished*). Although not statistically significant in most comparisons, isolate 5 caused longer lesion development in all cultivars except Semmes. The reactions of the 10 cultivars to isolates 3 and 5 differed statistically only for Hood. With Hood, the lesions were 13 mm for isolate 3 and 33 mm for isolate 5. The pathogen was reisolated from lesions caused by toothpick inoculation. No lesions

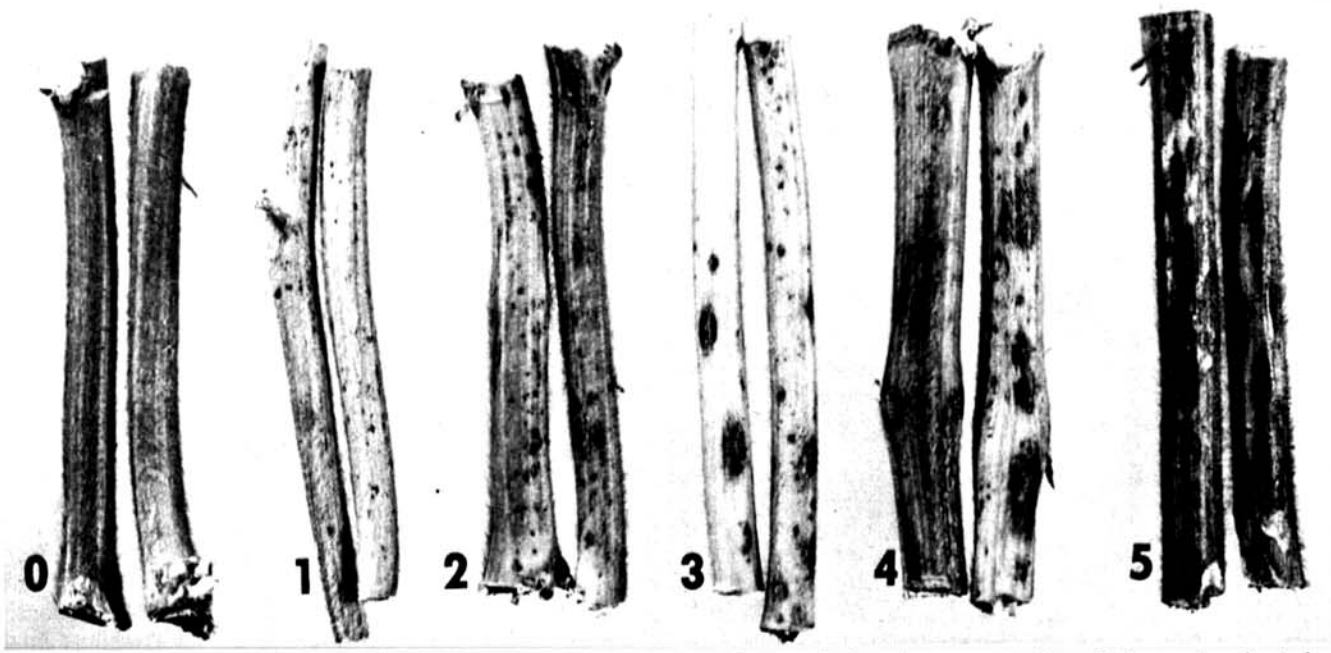

Fig. 1. Lesions formed on the stems of soybean after inoculation with ascospores of *Diaporthe phaseolorum* var. *caulivora*. Ratings are based on lesion size: 0 = no lesions, 1 = 1 mm or less, 2 = 1.1–3 mm, 3 = up to 6 mm, 4 = up to 10 mm (stems green), and 5 = 10 mm and larger (stems chlorotic and dying).

developed in stems of check plants receiving sterile broth-impregnated toothpicks. The toothpick inoculation (Table 1) produced lesions ranging from 5 mm in the resistant Tracy-M to 28.6 mm in J77-339. The response of Braxton did not differ significantly from that of Tracy-M. Lesion lengths in Hood, Centennial, Jeff, Semmes, Bragg, Bedford, and Forrest (Table 1) suggest increasing levels of susceptibility in the order listed. The order of their response was the same for both isolates.

The overall disease responses of these soybean cultivars were similar for ascospore and colonized-toothpick inoculations (Table 1). Small lesions (up to 1 mm) were produced on the resistant cultivars Tracy-M and Braxton. Progressively larger lesions developed on cultivars as their susceptibility increased. Large, rapidly expanding lesions killed all inoculated plants of J77-339 30–40 days before maturity. Lesion size on Semmes, Jeff, Centennial, and Hood increased between 35 and 50 days after inoculation but did not reach the size of those on J77-339, Forrest, Bedford, and Bragg. The pathogen was reisolated from lesions on plants inoculated with ascospores. Plants grown in control plots without inoculation remained healthy with no lesion developments that could suggest natural infection by the stem canker pathogen.

The cultivars used in this study represent a range in response to stem canker from very resistant to very susceptible. Based on previous tests and observations, the stem canker reaction of Tracy-M is very resistant, that of Bragg is moderately susceptible, and that J77-339 is very susceptible (6). In 1983, Tracy-M and Braxton were rated resistant, Centennial moderately resistant, Bedford and Jeff moderately susceptible, and Forrest and Bragg susceptible to naturally occurring stem canker in research plots at both Newton and Verona, MS. However, susceptible plants of Centennial were found in commercial production fields near Egypt, MS. Bedford (8) and Forrest (*unpublished*) were susceptible in previous field tests. The relative amount of stem canker development on some of these cultivars was reported by Weaver et al (15) when exposed to the pathogen in Alabama. The relative resistance exhibited by the cultivars used in this study agrees with the assigned resistance observed under natural disease development.

The plots receiving ascospore inoculation treatment were furrow-irrigated 4 hr (1:00–5:00 p.m.) before inoculation. This enhanced dew formation during the early evening hours that did not dry until about 9:00 a.m. the following day. The plants remained wet about 15 hr after inoculation. In other inoculation trials, plants in field plots have failed to become infected when a high-moisture environment was not provided either by rainfall or by irrigation. This apparent need to provide a high-moisture or wet environment to obtain infection after inoculation with ascospores is consistent with results obtained from inoculation experiments reported by Smith et al (13) and Ploetz and Shokes (11). The use of ascospores as inoculum also has the limitation that all isolates of *D. p.* var. *caulivora* may not be induced to produce ascospores. In an attempt to induce sporulation in 70 *D. p.* var. *caulivora* isolates recovered from soybean plants in 1983, I found that only 16 produced ascospores in quantities suitable for use as inoculum.

The measurement of cultivar resistance appears more precise when lesion length after toothpick inoculation is the basis for comparison rather than lesion size after ascospore inoculation. However, statistical comparisons tend to lump the cultivars into similar groups using either type of inoculation (Table 1). The toothpick inoculation technique for rating soybean cultivars for their relative resistance to *D. p.* var. *caulivora* could be a valuable tool to help soybean breeders evaluate lines for use as parental material where resistance of the Tracy-M type is not available or for making comparisons of resistance among advanced breeding lines.

Since 1974, when stem canker was first recognized in Mississippi, it has occurred at a level of severity that would permit the evaluation of cultivars for resistance over a wide geographic area only in 1980 and 1983.

Attractive features of the toothpick inoculation technique are the ease of use and the apparent lack of influence by environmental conditions. Several years' experience using the toothpick method of inoculation indicates that the cultivar response is affected little or none by the range of environmental conditions plants in field plots are normally subjected to.

Table 1. Response of soybean cultivars after inoculation with *Diaporthe phaseolorum* var. *caulivora* using colonized toothpicks or ascospores

Cultivar	Toothpick (mean lesion length [mm])[x] Isolate 3		Toothpick (mean lesion length [mm])[x] Isolate 5		Ascospores (isolate 5) (mean lesion rating)[y] 35 Days	Ascospores (isolate 5) (mean lesion rating)[y] 50 Days
J77-339	269 a	A[z]	286 a	A	5.00 a	5.00 a
Forrest	132 b	A	204 ab	A	2.13 bc	3.90 b
Bedford	124 b	A	168 ab	A	2.57 b	3.90 b
Bragg	109 bc	A	151 b	A	2.27 bc	3.53 b
Semmes	71 c	A	55 c	A	2.07 c	3.10 c
Jeff	37 d	A	52 c	A	0.97 de	2.23 d
Centennial	27 d	A	39 c	A	1.40 d	2.43 d
Hood	13 e	A	33 c	B	0.50 f	2.47 d
Braxton	7 ef	A	6 d	A	0.53 ef	0.77 e
Tracy-M	5 f	A	5 d	A	0.00 g	0.77 e

[x] Lesion length measured from point of toothpick insertion toward base of plant. Forty-two days after inoculation.

[y] Rating system: 0 = no lesion, 1 = 1 mm or less, 2 = 1.1–3 mm, 3 = up to 6 mm, 4 = up to 10 mm (stems green), and 5 = 10 mm and larger (stems chlorotic and dying) (Fig. 1).

[z] Means within a column sharing a lowercase letter in common are not significantly different ($P = 0.05$). Means between columns sharing an uppercase letter in common are not significantly different ($P = 0.05$) according to Waller-Duncan's multiple range test.

Pathogenic specialization on seedlings of differential soybean cultivars among isolates of the stem canker pathogen has been noted (5). However, significant differences in the responses of the cultivars used in this study to isolates 3 and 5 were observed on only the cultivar Hood.

Isolates of *D. p.* var. *caulivora* recovered from soybeans in Iowa and Indiana differ in their pathogenic behavior from isolates recovered from soybeans in Mississippi (*unpublished*). Isolates from both locations kill seedlings of the susceptible breeding line J77-339 in greenhouse tests. However, the isolate from Indiana used in this study did not incite disease development in any of the cultivars inoculated in field tests. Similar results were obtained earlier with an isolate from Ohio (8). I have not observed morphological differences between perithecia, asci, and ascospores of isolates from Ohio and Indiana and those from Mississippi. Perithecia formed in artificial culture by isolates from Mississippi are invariably single and randomly spaced. Perithecia formed in cultures of isolates from Ohio and Indiana are formed in clusters of three or more. Morgan-Jones and Backman (9,10) reported other differences between northern and southern isolates that include color, growth rate, temperature relations, and morphology of perithecia and ascospores.

The responses of soybean cultivars to inoculation using the toothpick method and ascospores show that either method may be used to make accurate evaluations for stem canker resistance.

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