A Seedling Test for Resistance to Soybean Stem Canker Caused by *Diaporthe phaseolorum* var. *caulivora*

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ABSTRACT

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Host-plant resistance to stem canker caused by *Diaporthe phaseolorum* var. *caulivora* in soybean (*Glycine max*) seedlings was detected in greenhouse tests. The reaction of 10-day-old seedlings to the disease was compared with disease development on artificially inoculated field-grown plants and with the occurrence of stem canker on plants subjected to natural disease development. Good agreement was found between the seedling response to artificial inoculation, the response of artificially inoculated field-grown plants, and the incidence of naturally occurring disease.

The stem canker disease of soybeans (*Glycine max* (L.) Merr.), which is caused by *Diaporthe phaseolorum* (Cke. and Ell.) Sacc. var. *caulivora,* was described in 1948 by Welch and Gilman (10). Stem canker, which is reported to be a serious disease of soybeans in Canada (7,9) and in the north-central region of the United States (1,3,4,6), was not observed in Mississippi until 1975 when infected plants were found in production fields and experimental plots in northeastern and east-central Mississippi. Since the first observation, the disease has occurred in varying levels of prevalence each year. The stem canker disease has been observed on the soybean cultivars Forrest, Lee 74, Mack, Bragg, Pickett 71, Davis, Essex, and the breeding line J77-339 in experimental plots grown at Verona, MS. No natural infection of the cultivars Tracy and Centennial has been observed. In 1979 and 1980, Tracy,

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Centennial, and J77-339 yielded approximately 2,700 kg/ha in disease-free environments (Stoneville, MS, and Jackson, TN). In the presence of stem canker at Verona, MS, the yield of J77-339 was only about 50% that of Tracy and Centennial.

Because the economic impact of the disease is potentially serious, and because the occurrence of natural epiphytotics is not dependable, a seedling test that may be done in the greenhouse to select for host resistance would be very desirable. This study was conducted to develop such a seedling test. To be of value, the seedling reaction must agree with the reaction of the cultivar when subjected to attack by the pathogen under natural field conditions.

On the basis of Dunleavy's (5) report that susceptibility of soybeans to stem canker is directly proportional to the growth rate of the pathogen in the soybean stem, field-grown plants of eight cultivars were inoculated to obtain a quantitative measure of their relative resistance. The relative resistance of cultivars obtained in this fashion was compared to the response of artificially inoculated greenhouse-grown seedlings and to their response when subjected to naturally occurring stem canker.

MATERIALS AND METHODS

Isolation of the pathogen. *D. phaseolorum* var. *caulivora* was recovered from cankers on soybean plants collected from plots of breeding lines and cultivars grown at Verona, MS. The pathogen was isolated as follows: small pieces of stem tissue taken from the margin of a canker were surface disinfested in 1% sodium hypochlorite for 1 min, rinsed in sterile water, and plated on acidified potato-dextrose agar. After incubation for 3 days at 21 C, hyphal tips were transferred to potato-dextrose agar slants for maintenance.

Preparation of inoculum. The inoculation technique used was a modification of the toothpick method (2,8,11). Flat toothpicks rather than the quill type were used, because they cause less mechanical damage to the soybean plant. The toothpicks were boiled for 30 min in each of three changes of distilled water, dried, and placed on end in large-mouth, 4 × 11-cm glass vials. Approximately 250 toothpicks were placed in each vial. Potato-dextrose broth was added to each vial so that the broth remained approximately 1.0 cm deep after the toothpicks became saturated. The vials were stoppered with foam plugs and autoclaved for 15 min at 120 C. After cooling, the toothpicks were inoculated with fungus mycelium and incubated at 21 C for 15 days before use. An isolate of the fungus recovered from a diseased Bragg soybean plant was used for all inoculations.

Cultivars tested. The soybean cultivars Tracy, CNS, D77-6046, D77-4912, D77-6103, Peking, Bragg, and J77-339 were selected for this study. Tracy has appeared to be very resistant or immune to stem canker in field plantings. CNS was included because it is in Tracy's parentage and is suspected of being its source of resistance. D77-6046, D77-4912, and D77-6103 were rated 1.7, 1.0, and 1.3, respectively, on a scale of 1 to 5 (1 = no stem canker, 5 = all plants dead 14 days prior to maturity) in experimental plots grown at Verona, MS, and are considered resistant to moderately resistant. Bragg and J77-339 are both susceptible. However, the incidence of stem canker observed in Bragg (4.0 rating) has been slightly less than in J77-339 (4.7 rating). Peking was included because it is in the parentage of nearly all breeding lines resistant to the soybean cyst nematode including J77-339, and was suspected as its source of susceptibility to stem canker.

Inoculation of seedlings in the greenhouse. Seedlings in sand in 10-cm-diameter clay pots were inoculated 10 days after planting by inserting a toothpick overgrown with mycelium into a hole made with a dissecting needle in each hypocotyl 1 cm below the cotyledon. Inoculated plants were placed in a moist chamber at 100% RH for 4 days and then were placed on a greenhouse bench at approximately 24 C (28–30 C for short periods on sunny days) for an additional 6 days before rating for disease reaction. Forty plants of each cultivar and breeding line were inoculated in each of four experiments.

Inoculation of field-grown plants. The cultivars and breeding lines were seeded in sandy loam soil at Stoneville, MS, on 14 May 1980. Thirty days after seeding, 20 plants of each cultivar were inoculated between the second and third node at approximately 8 cm above the ground. Sixty days after seeding, an additional 20 plants were inoculated near the top of the plant approximately 10 cm below the terminal meristem. The inoculation technique used on field-grown plants was the same as that used to inoculate greenhouse-grown seedlings. The inoculated site on the plants was not protected with a sealing compound either in the greenhouse or field. The lengths of lesions on plants inoculated at the base were measured 60, 90, and 100 days after inoculation from the point of inoculation toward the top of the plant. In plants inoculated near the top, measurements were made 30, 60, and 70 days after inoculation from the point of inoculation toward the base of the plant. Plant stems were split to obtain internal lesion measurements at the time the final external lesion measurements were made. Periodic inspections were made to record the number of dead plants.

TABLE 1. Stem canker reaction of soybean seedlings[v]

Cultivar	Percentage of plants			Cultivar rating
	Resistant[w]	Intermediate[x]	Susceptible[y]	
Tracy	98 A[z]	1 A	1 A	Resistant
CNS	96 A	0 A	4 A	Resistant
D77-6046	86 AB	12 AB	2 A	Moderately resistant
D77-4912	76 B	19 BC	5 A	Moderately resistant
D77-6103	76 B	16 AB	8 A	Moderately resistant
Peking	70 B	18 BC	12 A	Moderately resistant
Bragg	39 C	23 BC	38 B	Moderately susceptible
J77-339	7 D	33 C	60 C	Susceptible

[v] Data are averages from four replications with 40 plants each.
[w] Resistant = no lesion development.
[x] Intermediate = lesion developed 5 mm or more up and down the hypocotyl from the point of inoculation.
[y] Susceptible = plant killed.
[z] Means not followed by the same letter are significantly different ($P = 0.05$) according to Duncan's multiple range test.

RESULTS

Response of seedlings to artificial inoculation. The reaction of greenhouse-grown soybean seedlings 10 days after inoculation with *D. phaseolorum* var. *caulivora* can be separated into three distinct groups. These groups include: plants with little or no disease development (resistant); plants that were dead (susceptible); and

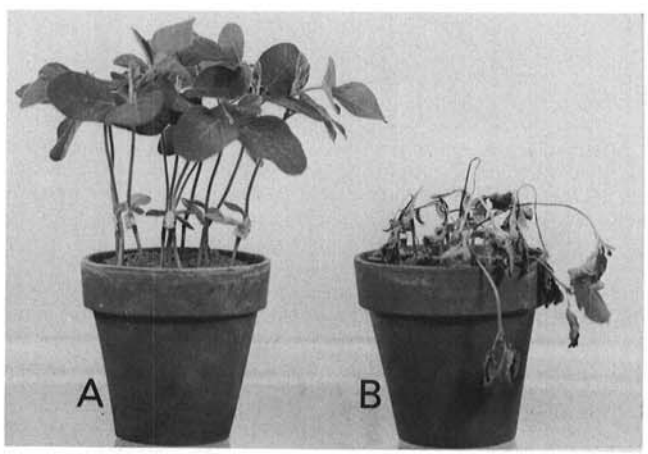

Fig. 1. Response of soybean seedlings to hypocotyl inoculation *Diaporthe phaseolorum* var. *caulivora:* **A,** resistant Tracy; **B,** susceptible J77-339. Plants were inoculated 10 days after planting.

TABLE 2. External and internal stem canker lesions length on field-grown soybean plants inoculated 8 cm aboveground[a]

Cultivar	External lesion length (cm) postinoculation			Internal lesion length (cm) postinoculation
	60 days	90 days	100 days	100 days
Tracy	0.1(0)[b]	0.1(0)	0.1(0)	0.3(±0.2)
CNS	0.1(0)	0.1(0)	0.1(0)	0.2(±0.1)
D77-6046	1.3(±1)	3.2(±4)	4.5(±6)	41.2(±22)
D77-4912	1.3(±1.4)	3.3(±4)	9.4(±5)	19.2(±17)
D77-6103	0.1(0)	1.3(±2)	5.0(±5)	41.9(±28)
Peking	0.1(0)	0.1(0)	...[c]	...[c]
Bragg	4.3(±2)	11.9(±10)[d]	11.0(±12)[d]	85.6(±32)[d]
J77-339	27.7(±14)	...[e]	...[e]	...[e]

[a] Plant inoculated 30 days after planting.
[b] Data based on 20 plants per cultivar, measurements taken from point of inoculation toward top of plants, figures in parentheses are standard deviations.
[c] All plants mature 100 days after inoculation.
[d] Data based on living plants; 28% dead at 90 days, 63% dead at 100 days.
[e] All plants dead 90 days after inoculation (killed by stem canker).

TABLE 3. External and internal stem canker lesion length on field-grown soybean plants inoculated 10 cm below top of plant[a]

Cultivar	External lesion length (cm) days after inoculation			Internal lesion length (cm) days after inoculation
	30	60	70	70
Tracy	0.1(0)[b]	0.1(0)	0.1(0)	2.5(±1.7)
CNS	0.1(0)	0.1(0)	...[c]	...[c]
D77-6046	2.2(±2)	10.2(±4)	15.0(±10)	27.5(±11)
D77-4912	1.8(±2.2)	11.3(±7)	14.0(±7)	20.8(±10)
D77-6103	0.1(0)	5.1(±5)	8.4(±5)	20.2(±10)
Peking	0.1(0)	0.1(0)	...[d]	...[d]
Bragg	7.5(±3.6)	23.7(±9)	36.0(±9)	47.0(±13)
J77-339	8.9(±4)	24.9(±4)	41.0(±8)	47.4(±6.8)

[a] Plants inoculated 60 days after planting.
[b] Mean of 20 plants per cultivar, measurement taken from point of inoculation toward base of plant, figures in parentheses are standard deviations.
[c] Inoculated stems killed by anthracnose.
[d] All plants were mature 70 days after inoculation.

plants with extensive lesion development (intermediate). The lesions on plants classed as intermediate extended more than 5 mm from the point of inoculation and occasionally extended the entire length of the stem. In most plants, lesion development ceased approximately 10 days after inoculation; however, in some the disease continued to develop until the plant was killed. Based on seedling reactions: Tracy (Fig. 1A) and CNS were rated resistant; D77-6046, D77-4912, D77-6103, and Peking were rated moderately resistant; Bragg was rated moderately susceptible; and J77-339 (Fig. 1B) was rated susceptible (Table 1).

Response of field-grown plants to artificial inoculation. Externally visible lesion development on field-grown plants of cultivars Tracy, CNS, and Peking did not extend more than 1 mm from the point of inoculation when inoculated either at the base of the plant or near the top (Tables 2 and 3). These cultivars were rated resistant.

When 30-day-old field-grown plants of D77-6046, D77-4912, and D77-6103 were inoculated near the base, the externally visible lesions averaged 4.5–9.4 cm in length after 100 days (Table 2), and 8.4–15 cm after 70 days when 60-day-old plants were inoculated 10 cm below the apical meristem (Table 3). These breeding lines were rated moderately resistant.

Lesion development on 60-day-old plants of Bragg and J77-339 inoculated 10 cm below the apical meristem was similar (36 and 41 cm, respectively) 70 days after inoculation (Table 3). However, when 30-day-old plants were inoculated near the base, Bragg had suffered 28 and 63% mortality at 90 and 100 days, respectively, after inoculation and all plants of J77-339 were dead within 90 days (Table 2). Bragg and J77-339 were rated susceptible.

Except for Tracy and CNS, the extent of internal disease development (as indicated by necrotic or discolored stem tissue) extended well beyond the externally visible lesion when plants were inoculated either at the base or near the apical meristem. However, the relative resistance of the cultivars remained unchanged from that determined by measuring the length of the external lesions.

DISCUSSION

The usefulness of artificial inoculation techniques in a program of breeding for resistance depends on how well the reactions of artificially inoculated plants agree with the response of plants of the same cultivars attacked by the pathogen under natural field conditions. In this study, the response of soybean cultivars and breeding lines Tracy, D77-6046, D77-4912, D77-6103, Bragg, and J77-339, both as seedlings grown in the greenhouse and as field-grown plants, to artificial inoculation agreed well with their observed response to natural infection.

Cultivar Peking, which had been suspected of contributing susceptibility to cultivars derived from it, did not appear to be susceptible in these tests. Seedlings of Peking were moderately resistant when artificially inoculated; 70% of the plants were rated resistant and 12% were rated susceptible. In the field, no disease developed in inoculated plants of cultivar Peking. The early maturity of cultivar Peking may account for this lack of disease in the field.

The good agreement between the response of soybean cultivars to natural infection by *D. phaseolorum* var. *caulivora* in the field and their response to toothpick inoculations with the pathogen show that seedling tests can be used to accurately evaluate soybeans for resistance to the stem canker disease. Use of this technique will result in a major saving of time, labor, and space in field plots. It also will circumvent the dependence on natural occurrence of the disease.

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