Reaction of Selected Soybean Genotypes to Isolates of *Fusarium solani* f. sp. *glycines* and Their Culture Filtrates

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ABSTRACT

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Four soybean plant introductions, PI 520.733, PI 567.374, PI 567.650B, and PI 567.659, and one soybean cultivar, Great Lakes 3202, were inoculated under greenhouse conditions with four isolates of *Fusarium solani* f. sp. *glycines*. Foliar disease severity rating was greatest on PI 567.659, followed by Great Lakes 3202, PI 520.733, PI 567.650B, and PI 567.374. There was no significant interaction between isolates and soybean entries for foliar disease severity ratings. Experiments also were conducted to determine if disease development and root colonization differed among entries. Root infection of the five entries did not differ ($P = 0.05$). Foliar disease progress curves increased faster for PI 567.659 and Great Lakes 3202 than for PI 567.374. The area under the disease progress curve (AUDPC) value for PI 567.374 was the lowest and differed ($P = 0.01$) from AUDPC values for Great Lakes 3202 and PI 567.659. There were no differences ($P = 0.01$) in length of taproot lesions, losses in root dry weight, and vascular stem length discoloration among the entries, and there was no correlation ($P = 0.05$) between these measurements and foliar AUDPC values. Cut seedling stems immersed in culture filtrate developed interveinal chlorosis on leaves of each entry within 2 days. Disease severity on cut seedlings of PI 567.374 was lower ($P = 0.01$) than on the other entries. There was a positive correlation ($r = 0.94$, $P = 0.05$) between AUDPC values of the five entries inoculated with the fungus and the cut seedling test using culture filtrate.

Sudden death syndrome (SDS) of soybean (*Glycine max*), caused by the soil-borne fungus *Fusarium solani* f. sp. *glycines* (15-17), has been reported from Argentina, Brazil, and the United States (4,24,25). Disease symptoms include mottling, interveinal chlorosis, and necrosis on the upper leaves at flowering, and also root rot, crown rot, vascular discoloration of stems, defoliation, and pod abortion (16,17). Disease is most severe in wet conditions and under irrigation (16), and decreases as soil moisture decreases (20).

Soybean cultivars and lines that exhibit various levels or resistance to *F. solani* f. sp. *glycines* have been reported based on tests conducted in the growth chamber, greenhouse, microplots, and field (6,12,18,23). Resistance to *F. solani* f. sp.

glycines was reported to be conditioned by a dominant gene (*Rfs*) in cv. Ripley (22) and quantitatively in cv. Forrest (7). Frequency of root infection and disease severity of *F. solani* f. sp. *glycines* were significantly less for Forrest than for cv. Essex based on roots sampled from field-grown plants, and it was concluded that root rot severity was more important than disease severity on leaves for predicting soybean resistance (14). In another study, foliar and root disease severity on cv. BSR 101 were not significantly correlated based on a growth chamber study (20). Resistance sources that prevent root infection have not been reported. The relationship of foliar disease severity to the degree of root colonization needs further study in both controlled conditions and field experiments.

Different inoculation methods have been reported for testing pathogenicity of *F. solani* f. sp. *glycines* and for evaluating soybean resistance to the pathogen (6,10,12,16,17). *F. solani* f. sp. *glycines* produces toxins in liquid medium, and their culture filtrates were reported toxic to soybean calli, cotyledons, germinating seeds, and plants (11). A phytotoxic polypeptide with a molecular weight of 17 kDa and a low molecular weight phytotoxin, monorden, were identified in culture filtrates of *F. solani* f. sp. *glycines* (2,9). Since toxins produced by *F. solani* f. sp. *glycines* cause foliar symptoms, it is conceivable that culture filtrates may be

used to screen cut seedlings for resistance to the foliar phase of SDS.

The objectives of this paper were to: (i) compare soybeans resistant and susceptible to SDS by monitoring root infection, root and stem colonization, and disease severity on foliage; and (ii) evaluate the response of SDS-susceptible and -resistant soybean seedlings to culture filtrates of *F. solani* f. sp. *glycines*.

MATERIALS AND METHODS

Isolates, inocula, and plant entries.

Four *F. solani* f. sp. *glycines* isolates known to cause soybean SDS, Mont-1 (5,6), In-F2X-11A (5), Fs171 (20), and BH-F2-13 (20), were used. These cultures were maintained on Bilay's medium (3) and subcultured on 2% water agar for 3 weeks before infesting sorghum grain. Two hundred cm³ of sorghum grains were soaked in distilled water in a 1-liter Erlenmeyer flask overnight, drained, and autoclaved on 2 consecutive days for 20 min at 121°C. Each of the flasks containing sterilized sorghum grain was inoculated with an individual isolate by transferring 10 4-mm-diameter mycelial plugs from the water agar colonies. Cultures on sorghum grains were incubated under a 12-h photoperiod using fluorescent light (60 $\mu\text{Em}^{-2}\text{s}^{-1}$) for 10 to 14 days at 25°C. Four soybean plant introductions, PI 520.733, PI 567.374, PI 567.650B, and PI 567.659, and cv. Great Lakes 3202, reported to have different levels of resistance to foliar disease severity (6), were used in all experiments. For experiments where plants were inoculated with the fungus, noninoculated plants served as controls. The soil used in experiments was a pasteurized soil:sand mix (1:1 vol/vol) at pH 7.

Virulence of isolates. Five seeds of each entry were sown in an 8 × 12 cm tray. Soil was infested by placing colonized sorghum grains below seeds at planting (6). Five cm³ of infested sorghum grains was evenly distributed in each furrow after using a template to make three furrows that were 12 cm long, 2 cm deep, and 3 cm apart. About 800 cm³ of soil mix was added to cover the infested grains to a depth of 2 cm. Seeds were added to each furrow after reapplying the template to make a 2-cm-deep furrow directly over the inoculum. After sowing seeds and infesting soil, trays were placed in a greenhouse at 20 to 30°C and watered daily. The experi-

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mental design was a split plot with the four isolates as the main plot and five entries as the subplot, with three replications arranged in blocks. Foliar disease severity was rated for each plant 21 days after planting based on a scale of 1 to 5 where 1 = no symptoms; 2 = light symptom development with mottling and mosaic (1 to 20% foliage affected); 3 = moderate symptom development with interveinal chlorosis and necrosis (21 to 50% foliage affected); 4 = heavy symptom development with interveinal chlorosis and necrosis (51 to 80% foliage affected); and 5 = severe symptom development with interveinal chlorosis and necrosis and/or dead plants (81 to 100% foliage affected).

Root infection. Seeds of the five entries were sown in Cone-Tainers (Ray Leach Cone-Tainers, Stuewe & Sons, Inc., Corvallis, OR) and inoculated with *F. solani* f. sp. *glycines* isolate Mont-1 infested sorghum grains. There was one seed per Cone-Tainer. Three cm³ of infested sorghum grains was placed 2 to 3 cm below the seed in each Cone-Tainer. The Cone-Tainers were placed in a growth chamber under a 12-h photoperiod with a light intensity of 300 $\mu\text{Em}^{-2}\text{s}^{-1}$ at 28°C day and 22°C night. Plants were watered daily. To measure fungal colonization of roots, plants were removed from Cone-Tainers daily from 3 to 10 days. Roots were washed under tap water to remove soil particles, surface-sterilized in 1% NaOCl solution for 3 min, rinsed in sterile distilled water twice, and dried on filter paper for 1 min. Eight to 10 taproots from each sampling time were placed on a semiselective medium (8). Plates were incubated under a 12-h photoperiod with a light intensity of 60 $\mu\text{Em}^{-2}\text{s}^{-1}$ at 25°C. After 5 to 7 days, blue colonies were counted on the semiselective medium. There were three replications of each entry with 10 roots per replication for each sampling date.

Disease development on foliage, roots, and stems. Seeds of the five entries were sown in Cone-Tainers and inoculated with infested sorghum grains of *F. solani* f. sp. *glycines* isolate Mont-1 as described previously. The Cone-Tainers were placed in a

greenhouse, and plants were watered daily and grown at 20 to 30°C. The experiments were arranged in a randomized complete block design with four replications and four to six plants for each entry. Ten to 21 days after sowing, foliar disease severity was rated daily on a scale of 1 to 5 as previously described. At 21 days after sowing, plants were uprooted and stems severed at the soil line. Roots were washed, and discolored lesion lengths on the taproots were measured. After measuring root lesions, all roots were dried for 24 h at 50°C and weighed. Percentages of dry weight loss were calculated by using dry root weights from noninoculated soybean plants. Stems were split, and the length of discoloration inside the stem was measured. End portions of discolored stems were cut into 5 mm² pieces and placed on the semiselective medium.

Effects of culture filtrate. *F. solani* f. sp. *glycines* isolate Mont-1 was grown in a 1-liter flask containing 400-ml of a modified Septoria medium (MSM) (9). The inoculated flask was incubated under a 12-h photoperiod using fluorescent light (60 $\mu\text{Em}^{-2}\text{s}^{-1}$) for 10 to 14 days at 25°C. The 2-week-old culture was filtered through cheesecloth, Whatman no. 1 filter paper, and a 0.22 μm Millipore membrane. The culture filtrate was diluted 50-fold with 1 mM KH₂PO₄ solution and adjusted to pH 6.0. Three-week-old seedlings of the five soybean entries were cut off at the crown. Cut seedlings were transferred to test tubes (15 cm long $\times$ 2.5 cm diameter) containing the diluted culture filtrate or MSM. Tubes were incubated under a 12-h photoperiod with a light intensity of 300 $\mu\text{Em}^{-2}\text{s}^{-1}$ at 25 to 28°C. Twenty ml of culture filtrate was added at 3-day intervals. The experiment was arranged in a randomized complete block design with three plants per each of three blocks. Disease severity was rated daily on a 1 to 5 scale for 10 days after inoculation.

Data analysis. To compare foliar disease severity ratings, the scales were converted to percentages using the midpoint value (6,20) where 1 = 0%, 2 = 10%, 3 = 35%, 4 = 65%, and 5 = 90%. For multiple

disease severity ratings, areas under the disease progress curves (AUDPC) (21) were calculated using converted percentages from disease severity scales. The data were analyzed by analysis of variance (SAS Institute, Cary, NC). All experiments were repeated once. Data from only one experiment are presented unless there was a significant experiment by treatment interaction and unless the error variances were not homogeneous, in which case both experimental data are presented. Data from noninoculated controls were not included in the analysis. Means were compared by least significant differences at $P = 0.05$ or $P = 0.01$.

RESULTS

Virulence of isolates. All soybean entries had foliar symptoms 10 to 11 days after inoculation regardless of the isolate used. PI 567.374 had the lowest mean disease severity rating (24%), followed by PI 520.733 (38%) and PI 567.650B (42%) (Table 1). No significant interaction in foliar disease severity rating occurred among isolates and soybean entries.

Root infection. Discolored lesions occurred on taproots as early as 3 days after sowing. *F. solani* f. sp. *glycines* was isolated from taproots after 4 days and increased to 90 to 97% for all entries after 10 days (Fig. 1). There were no differences ($P = 0.05$) in recovery frequency of the fungus among the soybean entries from 3 to 10 days.

Disease development on foliage, roots and stems. All entries showed mottling and mosaic symptoms on leaves 10 to 11 days after inoculation. Foliar disease severity developed rapidly on PI 567.659 and Great Lakes 3202, with severity ratings of 60 and 57%, respectively, 21 days after inoculation (Fig. 2). In contrast, the foliar disease severity rating on PI 567.374 was only 9% (Fig. 2). The AUDPC values for the five entries, based on foliar disease symptoms, ranged from 68 to 366 (Table 2). The AUDPC value for PI 567.374 was lowest and differed ($P = 0.01$) from values

Table 1. Sudden death syndrome (SDS) foliar severity on five soybean entries inoculated with four *Fusarium solani* f. sp. *glycines* isolates 21 days after inoculation under greenhouse conditions

Soybean entry	Disease severity (%) of isolates ^a				Mean
	Fs171	Mont-1	BH-F2-13	In-F2X-11A	
PI567.374	35.0	24.2	20.8	17.8	24.4
PI520.733	49.3	31.7	37.2	33.9	38.0
PI567.650B	60.3	35.8	39.6	33.8	42.4
Great Lakes 3202	72.7	57.7	57.4	49.9	59.4
PI567.659	83.0	79.0	55.3	52.4	67.4
Mean	60.1	45.7	42.1	37.6	46.3
LSD ($P = 0.05$) ^b	11.1				
LSD ($P = 0.05$) ^c	7.9				

^a Geographic origins of Fs171, Mont-1, BH-F2-13, and In-F2X-11A isolates were Arkansas, Illinois, Iowa, and Indiana, respectively.

^b Least significant difference among isolates.

^c Least significant difference among soybean entries.

Fig. 1. Mean isolation frequency of *Fusarium solani* f. sp. *glycines* from roots of five soybean entries 3 to 10 days after inoculating with *F. solani* f. sp. *glycines*. Vertical bars represent standard errors of the mean.

of Great Lakes 3202 and PI 567.659. The AUDPC values for PI 567.650B and PI 520.733 were not different.

Taproots of all entries showed dark brown lesions 21 days after inoculation, and lesion lengths ranged from 33 to 39 mm. There were no differences ($P = 0.01$) in lesion lengths among the five soybean entries. Losses in root dry weight ranged from 59 to 74% but were not different ($P = 0.01$). Discolored stems of all entries placed on the semiselective medium yielded fungal colonies with blue pigmentation. The lengths of stem discoloration from the soil surface ranged from 11 to 15 mm, without any difference ($P = 0.01$) among entries. The correlations of AUDPC values to taproot lesion lengths, root dry weight losses, and stem discoloration were not significant.

Effects of culture filtrate. Stems immersed in culture filtrate for 2 days had chlorotic and mosaic symptoms on lower leaves, and disease severity ratings ranged from 2 to 8% (Fig. 3). Foliar disease developed faster for PI 567.659 and Great Lakes 3202; upper leaves had interveinal necrosis 4 days after immersion in filtrate. Disease severity of PI 567.374 was lowest among all entries, with a 29% severity rating 10 days after immersion in filtrate (Fig. 3). The AUDPC value based on foliar symptoms for PI 567.374 was 109 and was lower ($P = 0.01$) than AUDPC values of the other entries, which ranged from 193 to 410 (Table 2). The correlation between AUDPC values based on seedling inoculations in the greenhouse trial and cut seedlings immersed in culture filtrate was $r = +0.94$, and the F value was significant at $P = 0.017$.

DISCUSSION

SDS foliar disease severity of the five soybean entries inoculated with the four *F. solani* f. sp. *glycines* isolates had similar disease severity rankings in the greenhouse

and in the cut-seedling assays, as reported previously (7). There was no interaction between the four isolates and the entries, but differences in isolate aggressiveness occurred, which agrees with previous reports (5,12).

F. solani f. sp. *glycines* colonizes soybean roots and lower stems, causing vascular discoloration. In our study, foliar disease severity was not correlated with taproot and stem colonization or losses in root dry weight. Root infection and coloni-

Fig. 2. Sudden death syndrome progress curves based on foliar severity ratings of five soybean entries 10 to 21 days after inoculating with *Fusarium solani* f. sp. *glycines*.

Fig. 3. Sudden death syndrome progress curves based on foliar severity ratings of cut seedlings from five soybean entries 1 to 10 days after immersing in cultural filtrate of *Fusarium solani* f. sp. *glycines*.

Table 2. Area under the disease progress curve (AUDPC) values of sudden death syndrome foliar severity ratings for five soybean entries inoculated with *Fusarium solani* f. sp. *glycines* under greenhouse conditions for 21 days and for cut seedlings immersed in its culture filtrate under laboratory condition for 10 days

Soybean entry	AUDPC	
	Seedling ^a	Cut seedling ^b
PI 567.374	68	109
PI 567.650B	118	193
PI 520.733	199	236
Great Lakes	237	374
PI 567.659	366	410
LSD ($P = 0.01$)	159	86

^a Seedlings were inoculated in the greenhouse by an infested-sorghum-grain method.

^b Cut seedlings were immersed in diluted culture filtrate (50:1, water/culture filtrate) of *F. solani* f. sp. *glycines* under a 12-h photoperiod of fluorescent light at $25 \pm 3^\circ\text{C}$ in the laboratory.

zation may not be as important as foliar disease severity when describing SDS resistance, although one report suggests using root disease severity as an indicator for *F. solani* f. sp. *glycines* SDS resistance (14). We find that assessment of roots is difficult and time-consuming, especially when there are many entries that need to be rated over time. Phytotoxins produced by *F. solani* f. sp. *glycines* (2,9) appear to cause foliar symptoms, and those symptoms have been used in most reports to describe resistance (6,12,18,23). Conditions that favor toxin production and subsequent foliar symptoms may be more important than knowing the conditions favorable for root infection and colonization (20).

Phytotoxins collected from fungal culture filtrates have been applied to plant cuttings to bioassay toxins. For example, cut seedlings of tomato immersed in toxin solutions including culmamarasmin, fusaric acid, lycomarasmin, and marticin produced by *F. culmorum*, *F. oxysporum* f. sp. *lycopersici*, and *F. solani*, and potato cuttings inoculated with alternaric acid produced by *Alternaria tenuis*, were used to bioassay toxins (13). Phytotoxin assays using cut seedlings were shown to be dependent on plant transpiration, which was assessed by using a 0.01% solution of crystal violet (19). Using the five soybean entries in our study, there was no difference in the movement of crystal violet in the cut seedlings immersed in a 0.01% solution (Y. H. Huang and G. L. Hartman, unpublished). In our tests, cut seedlings of Great Lakes had typical SDS symptoms using a 50-fold dilution of culture filtrate and were not injured by the same dilution of MSM. Using cut seedlings immersed in *F. solani* f. sp. *glycines* culture filtrate, disease severity was positively correlated to foliar disease severity of inoculated seedlings in the greenhouse. Diluted culture filtrate of *F. solani* f. sp. *glycines* caused typical foliar symptoms, while the culture filtrate of *F. solani* non-SDS isolates did not (Y. H. Huang and G. L. Hartman, unpublished). The cut-seedling technique may be another method to test toxicity of *F. solani* f. sp. *glycines* and to evaluate resistance.

Only a few reports have shown that phytotoxins can be detoxified and degraded in vivo (19). The detoxification of tab-toxin, a phytotoxic dipeptide, in transgenic tobacco has been reported to confer resistance to bacterial wildfire (1). The resis-

tance of pea and tomato to wilt diseases also has been demonstrated by the degradation of fusaric acid, an amino acid derivative, into a less phytotoxic pyridine derivative (19). To understand *F. solani* f. sp. *glycines*-SDS resistance mechanisms, other factors need to be understood, such as the conditions that favor phytotoxin production and how environmental, physiological, and biochemical factors influence toxin movement and potential degradation in plants.

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