Phytotoxicity of Culture Filtrate from *Fusarium solani*, the Causal Agent of Sudden Death Syndrome of Soybean

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ABSTRACT

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An isolate of *Fusarium solani* that caused sudden death syndrome (SDS) of soybean was grown in a semidefined liquid medium. The phytotoxicity of cell-free fungal culture filtrates was determined on soybean calli grown on tissue culture medium amended with fungal culture filtrate. The mean calli brown rating increased as culture filtrate increased in the medium. There were significant ($P < 0.05$) positive correlations between SDS severity from field microplot data and calli browning due to culture filtrates. Seedlings of five soybean cultivars inoculated with the fungus under greenhouse conditions had significantly ($P < 0.05$) different foliar severity ratings and relative plant fresh weights. Sensitivity of calli of the same five cultivars to culture filtrate also had significantly ($P < 0.05$) different brown ratings and relative fresh weights. There were significant ($P < 0.05$) correlations between variables measured on inoculated soybean seedlings and calli sensitivity. Pathogenicity and phytotoxicity of *F. solani* isolates from different hosts were tested on soybean plants and calli, respectively. Isolates from hosts other than soybean did not cause SDS symptoms, and their culture filtrates had significantly lower toxicity to soybean calli than did that of soybean SDS-causing isolates.

Sudden death syndrome (SDS) of soybean (*Glycine max* (L.) Merr.) is a mid- to late-season disease (12). SDS symptoms include root rot, crown necrosis, vascular discoloration of roots and stems, interveinal chlorosis and necrosis of leaves, premature defoliation, and pod abortion (12). Interveinal chlorosis and necrosis of leaves are the most noticeable symptoms (23,28). They first appear as scattered, interveinal, chlorotic spots or blotches, usually on leaves from the upper nodes. The chlorotic areas may become necrotic or may enlarge and coalesce, forming interveinal chlorotic streaks. These streaks eventually become necrotic, and only the midvein and major lateral veins remain green (24). The causal agent, *Fusarium solani* (Mart.) Appel & Wollenw. emend. W.C. Snyder & H.N. Hans., has been isolated mainly from roots and only occasionally from above the crown (23,24). The fact that the fungus has not been isolated from leaves suggests that a toxin(s) translocated from the roots may be responsible for foliar symptoms.

A number of plant pathogens produce toxins in association with a number of different diseases (6,32). Race T of *Bipolaris maydis* (Nisikado & Miyake) Shoemaker (syn. *Helminthosporium maydis*), the causal agent of southern corn leaf blight, produces T-toxin, which acts specifically on mitochondria of susceptible cells (18). Segregation for virulence but not for pathogenicity was observed from a cross between race T (producing toxin) and race O (not producing toxin), suggesting that the toxin was a virulence factor (34). Toxins produced by *Alternaria alternata* (Fr.:Fr.) Keissl. (syn. *A. kikuchiana*) and *A. mali* Roberts were considered to be pathogenicity factors since mutations eliminating toxin production resulted in loss of pathogenicity (21,22). Pathogenicity and toxin production could be restored by another mutation. Numerous phytotoxins have been isolated from cultures of *Fusarium* spp. (2,4,15,20). Correlations between toxin formation in vitro and pathogenicity have been reported for *F. solani* f. sp. *pisi* isolates (15).

Culture filtrates from isolates of *F. solani* causing soybean SDS were reported to be toxic to soybean callus, cotyledons, germinating seeds, and plants (16). Variability in response of soybean calli and cotyledons to culture filtrates among fungal isolates and soybean cultivars was observed (16). A phytotoxin produced by *F. solani* isolates causing SDS was characterized as a polypeptide with an estimated molecular weight of 17,000 (13). In another report, isolates causing SDS produced a low-molecular-weight phytotoxin, monorden, which inhibited shoot and root growth, and caused leaf and stem necrosis of soybean plants (1).

The genetics of resistance in soybean to *F. solani* isolates causing SDS indicated that a single dominant gene (*Rfs*) in cv. Ripley controlled resistance (28). However, most cultivars are susceptible to root infection, and an extensive search for new sources of resistance in the soybean germ plasm has shown a wide range of foliar symptom development (G. L. Hartman, *unpublished*). In the search for sources of disease resistance, a toxin would be useful as an in vitro selection agent, providing it plays a significant role in disease development (31). This method has been successful in maize, where regenerated plants selected from maize calli resistant to the toxin produced by *H. maydis* race T were resistant to the toxin and the pathogen (8,9). In soybeans, plants regenerated from calli resistant to a toxin produced by *Septoria glycines* Hemmi delayed brown spot symptoms compared to the plants from cultivars that were the source of the regenerants (27).

The objectives of this study were to (i) study the relationship between calli sensitivity to culture filtrates and susceptibility of plants to inoculation by the fungus and (ii) determine the relationship between phytotoxicity and virulence of *F. solani* isolates from soybean and nonsoybean hosts on a susceptible soybean cultivar. A preliminary report on the phytotoxicity of *F. solani* culture filtrates was published (14).

MATERIALS AND METHODS

Culture filtrates. Isolate 269 of *F. solani* (provided by S. M. Lim and L. E. Gray, originating from Arkansas) was maintained in the dark at 23°C on an agar-solidified medium consisting of 1 g of KH_2PO_4, 1 g of KNO_3, 0.5 g of $MgSO_4 \cdot H_2O$, 0.5 g of KCl, 0.2 g of starch,

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0.2 g of sucrose, and 8 g of agar per liter. Liquid cultures were grown in modified Septoria medium (MSM) (13,26). Growth of cultures and procedures to produce and obtain filtrates were the same as previously reported (13).

Calli assay. Calli were induced from 14 soybean cultivars (Table 1). Twelve had been previously tested for their reaction to *F. solani* in field microplots (28). Seeds were dipped in 70% ethanol for 1 min, soaked twice in 15% commercial bleach for 8 min, and rinsed five times with sterilized water. The seeds were placed on MS basal medium (19) and incubated at 28°C under 16/8 h light/dark conditions (180 $\mu E \cdot m^{-2} \cdot s^{-1}$) for 10 days. Two-mm sections of hypocotyls cut near the cotyledons of germinating seeds were placed on MS medium amended with 0.5 mg of 6-BAP (6-benzylaminopurine) and 5 mg of NAA (naphthaleneacetic acid) (medium B) per liter and incubated for 3 to 4 weeks under the conditions described for seeds. The induced friable calli were subcultured biweekly.

Culture filtrates adjusted to pH 5.8 were filtered through a 0.22-µm Metricel sterile membrane, added to autoclaved medium B at four dilutions (1:100, 1:75, 1:50, and 1:25, vol/vol), and poured into 100-mm-diameter dishes. Sixteen pieces (approximately 2-mm-diameter) of calli from each cultivar were transferred to medium B. The experiment consisted of three dishes (replicates) for each cultivar in a completely randomized design. Dishes were incubated under 16/8 h light/dark (180 $\mu E \cdot m^{-2} \cdot s^{-1}$) conditions at 28°C. One week later, calli were visually rated on a 1 to 5 scale, where 1 = no browning, 2 = callus surface slightly brown, 3 = whole tissue brown, 4 = deeply brown with restricted growth, and 5 = deeply brown with no growth. This experiment was repeated once.

To quantify calli browning, 0.1 g of either yellow-green healthy calli or brown calli of cv. Asgrow A3427 was collected and ground in 4 ml of 80% acetone using a TenBroeck glass-glass tissue grinder (10.16 to 15.24 cm). The solution was centrifuged at 1,000 × *g* for 4 min. The supernatant was scanned for absorbance from 260 to 700 nm to determine the peak absorbance of the calli extract, which could be used in further studies.

To analyze the relationship between calli browning and absorbance of calli extract, different calli of cv. Asgrow A3427, representing brown ratings of 1, 2, 3, 4, and 5, were collected after being grown on culture filtrate amended medium B (4:100, vol/vol) for several days. The supernatant was scanned for absorbance at 330 nm. To analyze the relationship between concentrations of culture filtrate in medium B and absorbance of calli extract at 330 nm, cv. Asgrow A3427 calli were grown on medium B amended with culture filtrate (1:100, 2.5:100, 5:100, or 7:100, vol/vol) or not amended and incubated under 16/8 h light/dark cycles at 28°C for 5 days. There were five plates for each treatment, with 16 calli (pooled sample) on each plate arranged in a completely randomized design. The absorbance of the supernatant of the extracts was measured at 330 nm. This experiment was repeated once.

Comparison of foliar symptom ratings and calli sensitivity to culture filtrates. Fifty ml of clean sorghum seed was soaked in distilled water in a 250-ml Erlenmeyer flask overnight, drained, and autoclaved once at 121°C for 20 min. After overnight cooling, three 1 cm^2 agar pieces containing fungal mycelia of *F. solani* isolate 269 were added to the sterilized sorghum and incubated under continuous fluorescent light (60 $\mu E \cdot m^{-2} \cdot s^{-1}$). The flasks were shaken daily for 2 weeks. Five seeds each of cvs. Asgrow A3427, Chamberlain, Jack, Ripley, and Spencer were planted in each of two 10-cm-diameter pots containing sterilized soil and sand (1:1, vol/vol). The pots were arranged in a completely randomized design with two replicates (pots) per experiment. Seedlings were thinned to three plants per pot. Two-week-old seedlings were inoculated by placing either four infested or noninfested sorghum seeds next to the soil–stem interface and covering the seeds with soil. Three weeks later, foliar symptoms were recorded using the following scale: 1 = no foliar symptoms, 2 = mottling and mosaic symptoms on the younger leaves, 3 = interveinal chlorosis on younger and older leaves, 4 = necrosis on both younger and older leaves, and 5 = all leaves with interveinal necrosis, curled, and dry. Three plants from each pot were cut above the cotyledonary node and weighed together. Leaves from three plants per pot were removed and dried at 80°C for 2 days before weighing. The experiment was repeated once.

The sensitivity of calli of the same five cultivars to culture filtrate was evaluated

Table 1. Soybean sudden death syndrome severity on inoculated plants, brown rating of soybean calli from 14 cultivars grown on medium B amended with four dilutions of *Fusarium solani* culture filtrate for 1 week, and correlations between disease severity and calli brown rating

Cultivar	Disease severity[a]	Calli brown rating for different culture filtrate dilutions (vol/vol)[b]							
		1:100		1:75		1:50		1:25	
		Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Asgrow A3427	6.1	1.8	2.8	2.2	4.7	4.0	4.5	3.8	5.0
Asgrow A4595	6.0	1.0	1.8	1.2	1.8	2.8	4.2	4.2	4.7
Asgrow A4715	5.8	3.2	2.8	4.0	4.5	3.8	4.7	5.0	4.8
Bass	1.9	1.0	2.3	1.0	2.7	1.5	3.5	2.8	4.7
D83-4499	1.0	2.3	1.5	3.0	1.7	5.0	2.2	5.0	4.8
Hamilton	1.8	1.0	1.3	1.5	1.8	2.3	3.0	5.0	4.2
Jack	...[c]	1.0	1.7	1.0	1.3	1.0	3.8	4.0	4.3
Pioneer 3981	4.4	3.2	3.0	1.7	2.0	4.3	3.8	4.7	4.5
Pioneer 9461	5.3	1.2	2.7	2.0	3.3	3.5	3.7	5.0	4.8
Ripley	1.3	1.0	1.2	1.0	1.2	1.0	1.3	1.2	1.8
Spencer	7.0	1.2	1.5	3.2	3.7	2.5	3.3	5.0	4.8
Spry	...	1.2	1.0	1.0	1.2	1.8	1.5	2.7	3.0
TN4-86	1.5	1.8	1.7	2.0	2.3	2.3	3.7	4.5	4.8
Williams	3.8	1.3	1.5	2.2	1.5	1.5	3.2	3.8	4.8
Least significant difference (0.05)	2.4	1.6	1.2	1.5	0.9	1.9	1.4	1.6	0.9
Coefficient of variation (%)	...	59	38	48	23	42	25	23	12
Correlation coefficient		0.61	0.71	0.72	0.83	0.78	0.87	0.84	0.83

[a] Disease severity (1 to 10 scale, where 1 = no affected leaf area, 2 = 3%, 3 = 6%, 4 = 12%, 5 = 25%, 6 = 50%, 7 = 75%, 8 = 88%, 9 = 94%, and 10 = 97%) of plants inoculated with *Fusarium solani* in microplots at Urbana, IL (28).

[b] Brown rating: 1 = no browning, 2 = callus surface slightly brown, 3 = whole tissue brown, 4 = deeply brown with restricted growth, and 5 = deeply brown with no growth.

[c] Not tested.

by initiating and maintaining the calli as previously described. Calli of each cultivar were grown on three plates of medium B amended with filter-sterilized culture filtrates of isolate 269 (100:4, vol/vol) in a manner similar to that previously described. After 10 days, the calli fresh weight, brown rating, and absorbance at 330 nm of 80% acetone extracts of calli were measured. Calli fresh weight and absorbance of their extracts were converted to a percentage based on a control where calli were grown on medium B without culture filtrate. This experiment was repeated once.

Pathogenicity of *F. solani* isolates from different hosts and phytotoxicity of culture filtrates. Isolates of *F. solani* (obtained from L. E. Gray) that originated from either alfalfa (*Medicago sativa* L.), bean (*Phaseolus vulgaris* L.), lupine (*Lupinus albus* L.), pea (*Pisum sativa* L.), potato (*Solanum tuberosum* L.), or soybean were tested for their phytotoxicity and pathogenicity on SDS-susceptible cv. Asgrow A3427. One-week-old plants were inoculated in the greenhouse with the isolates in a completely randomized design with six replicates (plants). Inoculum production and inoculation were completed as previously described for inoculation of the five soybean cultivars. Dishes filled with water were placed below the pots to maintain soil water content. Foliar symptoms were rated on a 1 to 5 scale as described 2 weeks after inoculation. Each plant was cut above the cotyledonary node and weighed. The leaves of three plants per pot were combined and weighed, then dried in an 80°C oven for 2 days and weighed again. The experiment was repeated once.

Twelve 250-ml Erlenmeyer flasks, each containing 50 ml of MSM, were individually inoculated with a piece of agar (1.5 cm long, 0.5 cm wide) from each of the isolates and incubated without shaking in the dark at 23°C for 12 days. The cell-free filtrates were adjusted to pH 5.8 and incorporated into medium B (5:100, vol/vol). Sixteen pieces of calli per plate of cv. Asgrow A3427 were transferred to the culture filtrate–containing medium B with three plates (replicates) for each isolate. The calli fresh weight and absorbance of extract at 330 nm of calli were determined after 5 days. The experiment was repeated once.

Data analysis. The experimental data were analyzed by analysis of variance (25). Means were compared by least significant differences at $P < 0.05$ unless otherwise stated. Correlations between variables were tested on mean data. Data of repeated experiments were analyzed as repeated measures and combined if there was no interaction between experiment and treatment effects and if the error variances were homogeneous. Otherwise, results from both experiments are presented.

RESULTS

Calli assay. Calli grown on culture filtrate–amended medium browned after 2 to 3 days, and by 10 days calli on filtrate–amended medium were brown and shrunken (Fig. 1). There was a general trend for increased calli browning within each cultivar at higher levels of culture filtrate in the medium (Table 1). The mean calli brown rating increased from 1.9 to 2.4, 3.3, and 4.4, respectively, as culture filtrate increased from 1:100 to 1:75, 1:50, and 1:25 in the medium. Within each dilution, there was a significant ($P < 0.05$) difference in calli browning among the 14 cultivars. Compared with other cultivars, Ripley calli had lower brown ratings, especially when higher levels of culture filtrate were added to the medium. The data from the first experiment were more variable than those from the second, although the data in both experiments were less variable when more culture filtrate was added to the medium. There were significant ($P < 0.05$) positive correlations between calli browning and disease severity of *F. solani*–inoculated plants for each experiment at each dilution, with higher correlations occurring when more culture filtrate was added to the medium (Table 1).

There was a significant ($P < 0.01$) linear relationship between the absorbance at 330 nm of calli extracts and the brown rating of calli. There was also a significant ($P < 0.01$) linear relationship between the absorbance of calli extracts and the concentration of culture filtrate added to medium B (Fig. 2).

Comparison of foliar symptom ratings and calli sensitivity to culture filtrates. Data from the two plant inoculation experiments were combined, as there were no significant experiment or experiment × cultivar interactions. Initial leaf symptoms were observed as chlorotic spots on upper leaves of cvs. Asgrow A3427 and Spencer 2 weeks after inoculation, while initial leaf symptoms developed 2 to 3 days later on the other three cultivars. The foliar disease severity ratings and plant fresh weights as a percentage of the control were significantly ($P < 0.05$) different among the five cultivars (Table 2), while leaf dry weights were not

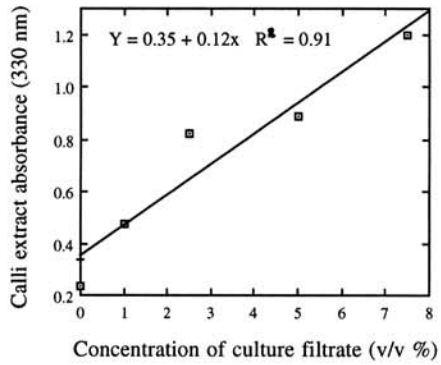

Fig. 2. Regression between the absorbance at 330 nm of acetone (80%) extracts of soybean cv. Asgrow A3427 calli and filtrate concentration 5 days after placing calli on medium B. Each data point is the mean of five replicates (16 calli from five plates) from two experiments.

Table 2. Severity of sudden death syndrome foliar symptoms and plant fresh weight as a percentage of the control for five soybean cultivars 2 weeks after inoculation with *Fusarium solani* under greenhouse conditions

Cultivar	Disease severity[a]	Plant fresh weight as % of control
Asgrow A3427	4.3[b]	28
Chamberlain	3.0	67
Jack	2.8	90
Ripley	1.9	80
Spencer	2.8	59
Least significant difference (0.05)	1.25	35
Coefficient of variation	28	35

[a] Rated on a 1 to 5 scale: 1 = no foliar symptoms, 2 = mottling and mosaic symptoms on the younger leaves, 3 = interveinal chlorosis on younger and older leaves, 4 = necrosis on both younger and older leaves, and 5 = all leaves with interveinal necrosis, curling, and dryness.

[b] Means based on three plants of each of two replicates in each of two experiments.

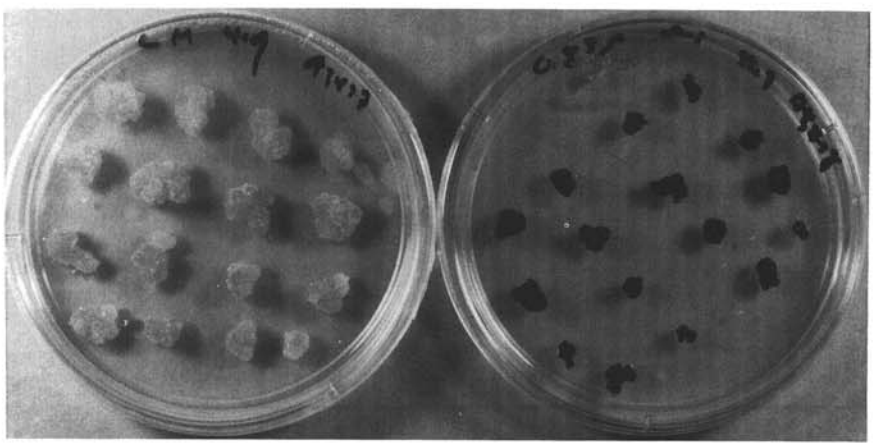

Fig. 1. Soybean cv. Asgrow A3427 calli grown on medium B amended with a modified Septoria medium (MSM) (left) and on culture filtrate of *Fusarium solani* grown on MSM (right) for 10 days.

(data not shown). Asgrow A3427 had significantly ($P < 0.05$) higher foliar ratings than the other cultivars and less plant fresh weight as a percentage of the control than all other cultivars except Spencer.

When calli of the same five cultivars were grown on culture filtrate–amended medium B, there were significant differences ($P < 0.05$) in calli browning and calli fresh weight as a percentage of the control, and there was an increase in extract absorbance over the control for each of the two experiments (Table 3). Calli from Asgrow A3427 browned after 2 to 3 days on culture filtrate–amended medium B, while Ripley calli were visually only slightly brown during the 10-day incubation.

The mean disease severity and plant fresh weight as a percentage of the control of inoculated plants in the greenhouse were significantly ($P < 0.05$) correlated to calli browning ($r = +0.89$ and $r = -0.78$), calli fresh weight as percentage of the control ($r = -0.75$ and $r = +0.46$), and increase in extract absorbance ($r = +0.94$ and $r = -0.74$), respectively, based on data from the second experiment.

Pathogenicity of *F. solani* isolates from different hosts and phytotoxicity of culture filtrates. *F. solani* SDS isolates from soybeans caused SDS foliar symptoms; however, the isolates from nonsoybean hosts did not cause SDS foliar symptoms up to 2 weeks after inoculation (Table 4). The mean plant fresh weight with nonsoybean isolates was significantly ($P < 0.05$) greater than that with all SDS isolates for one or more experiments (Table 4). Leaf dry weight with nonsoybean isolates was significantly ($P < 0.05$) greater than with most SDS isolates (Table 4).

Browning of calli measured as absorbance of calli extracts was significantly greater ($P < 0.05$) for the soybean SDS isolates than for nonsoybean isolates (Table 5). This was also true for the differences in calli fresh weight, except in experiment 2, where several soybean isolates were not significantly ($P < 0.05$) different from the nonsoybean isolate from pea.

Table 3. Soybean calli brown rating, calli fresh weight as a percentage of the control, and increase in extract absorbance over the control (calli grown without culture filtrate) of five soybean cultivars grown on *Fusarium solani* culture filtrate amended medium B (4:100, vol/vol) for 10 days

	Calli brown rating[a]		Plant fresh weight as % of control		Increase of extract absorbance at 330 nm	
Cultivar	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Asgrow A3427	5.0	4.2	7	25	9.1	6.7
Chamberlain	4.0	3.8	9	16	4.8	3.3
Jack	3.7	2.0	27	38	3.8	4.5
Ripley	1.7	1.0	95	82	2.7	2.1
Spencer	...[b]	5	...	16	...	3.5
Least significant difference (0.05)	0.6	0.3	38	18	2.0	1.3
Coefficient of variation (%)	9	6	58	28	21	18

[a] Brown rating: 1 = no browning, 2 = callus surface slightly brown, 3 = whole tissue brown, 4 = deeply brown with restricted growth, and 5 = deeply brown with no growth.
[b] No data.

DISCUSSION

The results of this and other studies (13,16) indicate that *F. solani* isolates that cause soybean SDS produce a phytotoxin(s) in culture. In our study, culture filtrates of *F. solani* SDS isolates were toxic to soybean calli, as they decreased calli growth and caused calli to brown. Calli brown rating, absorbance of acetone extracts of calli (quantitative measure of browning), and calli fresh weight were all good measures of calli sensitivity to culture filtrates. The reaction of calli to culture filtrates was positively correlated to cultivar foliar severity ratings when inoculated with *F. solani* in field microplots (28). It appears that soybean resistance to *F. solani* may be due, at least in part, to insensitivity of plants to the fungal toxin.

The phytotoxicity of fungal-produced toxins has been measured by leaf infiltration (29) and by placing the toxin on wounded, detached leaves (30). In our study and in other reports, plant calli have been used to test sensitivity to culture filtrates produced by fungal pathogens. For example, calli derived from cultivars susceptible to soybean brown stem rot browned, and callus growth and cell viability decreased, more than for resistant cultivars when both were grown on a calli medium amended with culture filtrate of *Phialophora gregata* (Allington & D.W. Chamberlain) W. Gams. (10). When calli derived from tester lines of *Allium* spp. were challenged with the culture filtrate of *Pyrenochaeta terrestris* (Hansen) Gorenz, Walker, & Larson, the causal agent of pink root of onion, the degree of calli sensitivity corresponded to the in vivo tester line disease resistance classification (17).

In our study, only the soybean SDS isolates caused SDS foliar symptoms. Culture filtrates from nonsoybean isolates were less phytotoxic to calli than were filtrates

Table 4. Disease severity of sudden death syndrome foliar symptoms, plant fresh weight, and leaf dry weight of soybean cv. Asgrow A3427 2 weeks after inoculating with *Fusarium solani*

Isolate source	Isolate code	Foliar disease severity[a]		Plant fresh weight (g)		Leaf dry weight (g)	
		Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
None	No filtrate	1.0	1.0	2.2	2.0	0.38	0.34
Potato	MR3	1.0	1.0	2.1	1.9	0.36	0.32
Bean	S826	1.0	1.0	2.3	1.6	0.40	0.30
Lupine	S1177	1.0	1.0	2.9	2.1	0.44	0.38
Alfalfa	731	1.0	1.0	2.7	1.9	0.45	0.41
Bean	S738	1.0	1.0	2.0	1.4	0.34	0.27
Pea	S577	1.0	1.0	2.2	2.1	0.37	0.36
Soybean	269 (WA)	4.7 ± 0.3[b]	4.2 ± 0.3	0.5	0.5	0.13	0.09
Soybean	Ridgeway1-A	2.5 ± 0.4	3.7 ± 0.6	1.5	0.8	0.24	0.16
Soybean	149-2-1	4.5 ± 0.3	1.5 ± 0.5	0.5	1.6	0.50	0.30
Soybean	269 (O)	4.3 ± 0.2	2.0 ± 0.4	0.9	1.2	0.16	0.23
Soybean	Monticello	4.3 ± 0.7	2.5 ± 0.6	0.7	0.8	0.14	0.18
Soybean	269 (G)	3.8 ± 0.6	3.5 ± 0.8	0.7	0.9	0.18	0.20
Least significant difference (0.05)		...[c]	...[c]	0.68	0.54	0.18	0.11
Coefficient of variation (%)		32	48	37	32	52	33

[a] Rated on a 1 to 5 scale: 1 = no foliar symptoms, 2 = mottling and mosaic symptoms on the younger leaves, 3 = interveinal chlorosis on younger and older leaves, 4 = necrosis on both younger and older leaves, and 5 = all leaves with interveinal necrosis, curling, and dryness.
[b] Standard error of treatment mean.
[c] Not tested due to lack of equal error variance terms among severity ratings.

Table 5. Reaction of soybean cv. Asgrow A3427 calli grown for 5 days on medium B amended with *Fusarium solani* culture filtrates (100:5, vol/vol) from different isolates

		Difference in absorbance of calli extracts[a]		Fresh weight (g)	
Isolate source	**Isolate code**	**Exp. 1**	**Exp. 2**	**Exp. 1**	**Exp. 2**
None	No filtrate	0.12	0.22	0.85	0.43
Potato	MR3	0.15	0.37	0.43	0.25
Bean	S826	0.09	0.37	0.62	0.23
Lupine	S1177	0.11	0.20	0.79	0.41
Alfalfa	731	0.59	0.37	0.57	0.26
Bean	S738	0.75	0.36	0.25	0.41
Pea	S577	0.33	0.52	0.25	0.15
Soybean	269(WA)	1.24	0.74	0.12	0.14
Soybean	Ridgeway 1-A	1.22	1.52	0.13	0.08
Soybean	149-2-1	1.20	1.37	0.13	0.09
Soybean	269 (O)	...[b]	1.29	...	0.09
Soybean	Mont	1.37	1.19	0.11	0.10
Soybean	269 (G)	1.43	1.20	0.11	0.09
Least significant difference (0.05)		0.17	0.17	0.10	0.06
Coefficient of variation (%)		14	12	16	18

[a] Difference = absorbance at 330 nm of extract of calli grown on *Fusarium solani* culture filtrate amended medium – absorbance of extract of calli grown on control medium.
[b] No data.

of soybean isolates. These isolates also did not produce a 17,000 molecular weight polypeptide (13). For brown stem rot of soybeans, it was shown that the culture filtrate from nonpathogenic isolates of *P. gregata* did not affect the growth of either susceptible or resistant calli (10). In the case of brown leaf spot of rice, when the virulent isolate of *Helminthosporium oryzae* Breda de Haan lost its ability to produce a toxin because of repeated subculturing, it also became nonpathogenic (30). These reports show that phytotoxins produced by plant pathogens can contribute to pathogenicity and are important in symptom development. The results of our study suggest that a toxin produced by *F. solani* SDS isolates causes SDS foliar symptoms. At this time, it is not known what role, if any, this toxin plays in the development of root infections. Further studies are needed to determine the actual mechanism of toxin action in SDS foliar symptom development.

The correlation between soybean cultivar susceptibility and the toxicity of culture filtrates suggests that filtrates could be used to screen in vitro for disease resistance. Tissue culture systems can provide a means of rapid screening for disease resistance when a toxin is involved in disease development. For example, alfalfa cell lines screened for resistance to culture filtrate of *F. oxysporum* f. sp. *medicaginis* (J.L. Weimer) W.C. Snyder & H.N. Hans. produced regenerants resistant to the fungus (11). In addition, soybean plants resistant to *S. glycines* were regenerated from organogenic calli resistant to toxic fungal filtrates (27). Since the soybean regeneration system is well developed (3,5,7,33), we are using *F. solani* culture filtrates to screen embryogenic cultures for resistance and produce regenerants of popular soybean cultivars with SDS resistance in a relatively short time.

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