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www.publish.csiro.au/journals/app *Australasian Plant Pathology*, 2004, **33**, 9–15

Phytotoxicity of *Fusarium solani* culture filtrates from soybeans and other hosts assayed by stem cuttings

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Abstract. *Fusarium solani* infects roots of a number of different plant species and some strains produce phytotoxins. *F. solani* f. sp. *glycines*, the causal organism of sudden death syndrome (SDS) of soybean (*Glycine max*), colonises soybean roots and produces toxin(s) that are translocated to leaves and cause intervienal chlorosis and necrosis. Several experiments evaluated the phytotoxicity of cell-free culture filtrates of *F. solani* f. sp. *glycines* by immersing cuttings of soybean seedlings into filtrates to determine what *in vitro* growth conditions alter the phytotoxicity, and to determine the specificity of toxicity of *F. solani* f. sp. *glycines* and other *F. solani* on cuttings of soybean and other legume species. Foliar disease severity ratings of soybean cuttings in cell-free culture filtrates diluted 25- and 50-fold were higher than when diluted 100-fold or more. Cell-free culture filtrates originating from cultures grown at 15, 20, and 25°C caused greater ($P = 0.05$) foliar disease severity ratings on cutting than when the fungus was grown at 30°C. Cell-free culture filtrates of *F. solani* isolates from cucumber (*Cucumis sativus*) and pumpkin (*Cucurbita pepo*) did not cause symptoms on soybean cuttings whereas filtrates of *F. solani* isolates obtained from other hosts caused some leaf chlorosis and/or necrotic spots. *F. solani* f. sp. *glycines* inoculated on eight legume species caused symptoms such as leaf chlorosis, defoliation, wilt or death on most of the species, but azuki bean (*Vigna angularis*), common pea (*Pisum sativum*) and vetch (*Vicia sativa*) were symptomless. Cell-free culture filtrates of *F. solani* f. sp. *glycines* caused foliar symptoms on all cuttings of legume species except for mung bean (*Vigna radiata*), although none of the symptoms matched the SDS symptoms observed on soybean foliage.

Additional keywords: culture filtrates, fungal toxins, *Glycine max*, sudden death syndrome.

Introduction

Sudden death syndrome (SDS) of soybean (*Glycine max*) is caused by the soilborne fungus *Fusarium solani* f. sp. *glycines* (Roy 1997; Roy *et al.* 1989; Rupe 1989). In addition to the United States, SDS has been reported in Argentina, Brazil and Canada (Anderson and Tenuta 1998; Nakajima *et al.* 1996; Ploper 1993). Yield losses of 20–46% were reported based on estimates of experimental plots in three fields in east-central Illinois (Hartman *et al.* 1995). Disease symptoms include mottling, mosaic, interveinal chlorosis and necrosis on the upper leaves at flowering as well as root rot, crown rot, vascular discoloration of stems, defoliation and pod abortion (Hartman *et al.* 1999). In addition to soybean, other hosts including cowpea, garden bean, limabean and mung bean have been infected with *F. solani* f. sp. *glycines* (Gray and Achenbach 1996; Gray *et al.* 1999).

Isolates of *F. solani* f. sp. *glycines* were shown to produce phytotoxins in a liquid medium (Jin *et al.* 1996*b*; Li *et al.* 1999). A phytotoxic polypeptide was identified from culture filtrates of an isolate of *F. solani* f. sp. *glycines* (Jin *et al.* 1996*a*). A low molecular weight phytotoxin, monorden, from cultures of *F. solani* f. sp. *glycines* isolates was also shown to cause leaf and stem necrosis on soybean cuttings (Baker and Nemec 1994). Although culture filtrate toxicity of the fungus has been reported, no fungal phytotoxins have been isolated from soybean plants with SDS symptoms, and it is possible that multiple toxins or other nontoxic mechanisms along with certain environmental conditions affect SDS foliar symptoms.

Various phytotoxins are produced by plant pathogens and a variety of methods have been used to assay fungal toxins. One approach to evaluate phytotoxic activity is to apply the

 10.1071/AP03072 0815-3191/04/010009

phytotoxin to the living host at the site of action that produces a measurable response (Naef-Roth 1972). Translocatable phytotoxins of higher plants can be assayed using cuttings immersed in a solution that contains the toxin. For example, rough lemon citrus seedlings became chlorotic and wilted when their roots were suspended in a dihydrofusarubin solution obtained from filtrates of *F. solani* (Nemec *et al.* 1989).

Phytotoxins produced by other *Fusarium* species and other fungi have been tested on tomato and potato (Naef-Roth 1972). Tomato cuttings were tested for their reaction to culmomarasmin, fusaric acid, lycomarasmin and marticin produced by *F. culmorum*, *F. oxysporum* f. sp. *lycopersici* and *F. solani*. Alternaric acid produced by *A. tenuis* caused black patches on leaflets and dark necrotic lesions on stems and petioles of potato cuttings. Phytotoxins produced by *F. solani* f. sp. *glycines* in culture filtrate caused similar foliar symptoms on cuttings immersed in filtrate as symptoms reported in greenhouse or field-infected plants (Huang and Hartman 1998; Li *et al.* 1999). Soybean calli also were used to test toxicity of *F. solani* f. sp. *glycines* culture filtrates (Jin *et al.* 1996*b*). A viability stain of soybean suspension-cultured cells was used to test phytotoxicity of cell-free culture filtrates of different *F. solani* isolates (Li *et al.* 1999). In our study, the phytotoxicity of cell-free fungal culture filtrates was assayed using plant cuttings for a bioassay system. The objectives were to determine (i) what *in vitro* growth conditions alter the phytotoxicity of *F. solani* f. sp. *glycines*, and (ii) the specificity of culture filtrate phytotoxicity of *F. solani* f. sp. *glycines* and other *F. solani* on cuttings of soybean and other legume species.

Methods

Culture filtrate

An isolate of *F. solani* f. sp. *glycines* (Mont-1), available at the Soybean Pathogen Collection Center at the National Soybean Research Center and reported to cause soybean SDS (Gray and Achenbach 1996; Gray *et al.* 1999; Hartman *et al.* 1997), was maintained on Bilay's medium (Booth 1971) and subcultured on 2% water agar 3 weeks before inoculation. Two 4-mm-diameter mycelial plugs from the outer edge were transferred to 250 mL flasks containing 50 mL modified Septoria medium (MSM) (Jin *et al.* 1996*a*, 1996*b*). These were incubated under 12 h per day fluorescent light (60 $\mu E/m^2/s$ at 25°C for 12 days. The culture filtrates were filtered through cheesecloth, Whatman No.1 filter paper and a 0.22 μm Millipore membrane. Cell-free culture filtrates were diluted 50-fold by adding 20 mL of culture filtrate and 980 mL of 1 mM KH_2PO_4 solution into a 2 L flask except for the cell-free culture filtrates dilution and pH studies.

Stem cutting assay

Soybean seeds of susceptible cv. Great Lakes 3202 were sown in pasteurised soil (1 part sand:1 part soil) in 20 × 30 cm trays that were placed in a growth chamber under a 12 h photoperiod with a light intensity of 300 $\mu E/m^2/s$ at 28°C in the day and 22°C at night. After 3 weeks, seedlings were cut near the soil line just below the cotyledonary node. Stems of cut soybean seedlings were immersed (3 to 5 cm) in test tubes (15 cm long × 2.5 cm diameter) containing 50 mL of diluted culture filtrate. Cuttings were incubated under a 12 h photoperiod with a light intensity of 300 $\mu E/m^2/s$ at 25–28°C. Twenty millilitres of diluted culture filtrate was added at 3-day intervals. Disease severity ratings were recorded 10 days after immersion for each cut seedling based on a 1 to 5 scale where 1 = no symptoms; 2 = light symptom development with mottling and mosaic (1–20% foliage affected); 3 = moderate symptom development with interveinal chlorosis and necrosis (21–50% foliage affected); 4 = heavy symptom development with interveinal chlorosis and necrosis (51–80% foliage affected); and 5 = severe symptom development with interveinal chlorosis and necrosis and/or dead plants (81–100% foliage affected). Data were converted to percents of mid-values (Hartman *et al.* 1997; Huang and Hartman 1998). All of the experiments described below were repeated.

Dilution of cell-free culture filtrate

Cell-free culture filtrates were diluted 25-, 50-, 100-, 200-, 300-, 400-, 500-, 800- or 999-fold by adding 960, 980, 990, 995, 996.3, 997.5, 998, 998.8, or 999 mL of 1 mM KH_2PO_4 solution to 40, 20, 10, 5, 3.8, 2.5, 2, 1.3 and 1 mL of filtrate, respectively. This experiment was repeated once. Treatments were arranged in a randomised complete block design (RCBD) with three blocks using nine cut seedlings per dilution. In another experiment using the same dilutions and experimental design, 3-week-old seedlings were uprooted and immersed in culture filtrate.

pH adjusted culture filtrate

Twenty millilitres of cell-free culture filtrate from 12-day-old cultures were placed in a 2 L flask and diluted with 980 mL of either 1 mM KH_2PO_4 or 1 mM K_2HPO_4 solution. There were three flasks diluted with 1 mM KH_2PO_4 solution and the pH was adjusted to 4, 5 and 6, respectively, by using 1 M KH_2PO_4 and K_2HPO_4 solutions. Another two flasks were diluted with 1 mM K_2HPO_4 solution with pH adjusted to 7 or 8 with 1 M KH_2PO_4 and K_2HPO_4 solutions. Treatments were arranged in a RCBD with three blocks using nine cut seedlings per pH level.

Effects of culture conditions on mycelial growth, pH, and stem cuttings

Culture filtrates of *F. solani* f. sp. *glycines* isolate Mont-1 that were grown either on Czapek Dox broth (CDB), MSM, potato-dextrose broth (PDB), or V8 broth were collected from 12-day-old cultures by filtering through cheesecloth and Whatman No.1 filter paper. Mycelial mass with the filter paper was dried at 50°C for 12 h and weighed. Culture filtrate pH from each medium was measured. Cell-free culture filtrates (diluted 50-fold) obtained from fungal growth on different media were tested using stem cuttings arranged in a RCBD with three blocks using nine cut seedlings per medium.

The effect of temperature was tested by growing cultures either at 15, 20, 25 or 30°C in 250-mL flasks containing 50 mL MSM without light for 12 days. Cell-free culture filtrates obtained from fungal growth at different temperatures were tested using stem cuttings arranged in a RCBD as above.

The effect of pH was tested by growing cultures for 12 days in MSM with adjusted pH values of 4, 5, 6, 7, 8, or 9 by using 1 N HCl or 1 N NaOH solutions. Cell-free culture filtrates obtained from fungal growth at different pH values were tested using stem cuttings arranged in a RCBD as above.

The effect of incubation time was tested by growing cultures for 1, 2, 3 or 4 weeks in 250 mL flasks containing 50 mL MSM. Cell-free culture filtrates obtained from fungal growth at different ages were tested using stem cuttings arranged in a RCBD as above

The effect of light was tested by growing cultures under darkness and a 12 h photoperiod of 60 and 300 $\mu E/m^2/s$ for 12 days in MSM at 25°C. Cell-free culture filtrates obtained from fungal growth under

different light intensities were tested using stem cuttings arranged in a RCBD as above.

The effect of aeration was tested by growing cultures in 50 mL of MSM in 250 mL flasks in three different treatments. In treatment one, cultures were grown with a 0.22 μm Millipore membrane over the flask mouth. In treatment two, cultures were grown with two layers of aluminum foil sealed over the flask mouth. In treatment three, cultures were grown with 30 mL of sterilised mineral oil added into the flask immediately after adding the fungus and sealed with two layers of aluminum foil. Cell-free culture filtrates obtained from growing the fungus at different levels of aeration were tested on stem cuttings arranged in a RCBD as above.

Effect of culture filtrates of various isolates of F. solani *on soybean stem cutting*

Nine *F. solani* isolates (Li *et al.* 2000) were obtained from alfalfa (*Medicago sativum*), bean (*Phaseolus* spp.), cucumber (*Cucurbita* spp.), lupine (*Lupinus* spp.), pea (*Pisum sativum*), potato (*Solanum tuberosum*), pumpkin (*C. pepo*), soybean and tomato (*Lycopersicon esculentum*) and maintained on 2% water agar. Each isolate was grown in a 250 mL flask containing 50 mL of MSM by transferring two 4-mm-diameter mycelial plugs from the water agar colonies. The inoculated flasks were incubated under a 12 h photoperiod using fluorescent light (60 $\mu E/m^2/s$) at 25°C for 12 days. The culture filtrate of each *F. solani* isolate was filtered through cheesecloth, Whatman No.1 filter paper and a 0.22 μm Millipore membrane, diluted 50-fold with 1 mM KH_2PO_4 solution, and adjusted to pH 6. Three-week-old cuttings of cv. Great Lakes 3202 were immersed in test tubes containing culture filtrates of *F. solani* f. sp. *glycines* isolates Mont-1 and Fs-171 (Arkansas) or filtrates of the nine *F. solani* isolates and incubated under a 12 h photoperiod with a light intensity of 300 $\mu E/m^2/s$ at room temperature. There were three replications using nine cuttings per isolate. Disease symptoms were recorded daily from 3 to 10 days after stem immersion.

Reaction of selected legume species to F. solani *f. sp.* glycines *and its culture filtrate*

Nine legume species (see list Table 4) and two soybean entries, cvv. Great Lakes 3202 and Spencer, were tested for their reaction to *F. solani* f. sp. *glycines* and its culture filtrate. Isolate Mont-1 was maintained on Bilay's medium (Booth 1971) and subcultured on 2% water agar for 3 weeks before infesting sorghum grain. Sorghum grain, 200 cm^3, was soaked overnight in distilled water in each of three 1 L Erlenmeyer flasks, drained and autoclaved twice for 20 min at 121°C. Sorghum was infested by transferring ten 4-mm-diameter mycelial plugs from fungal colonies grown on water agar to each flask. Cultures on sorghum were incubated under a 12 h-photoperiod using fluorescent light (60 $\mu E/m^2/s$) for 10–14 days at 25°C. Five seeds of each of the different legumes were sown in a 20 × 30 cm tray (six entries per tray). Soil was infested using colonised sorghum grains placed ~3 cm below seeds following a previously reported technique (Hartman *et al.* 1997). After sowing seeds, trays were placed in a greenhouse at 25 ± 3°C and watered daily. There were three replications. Disease symptoms were recorded 14 to 21 days after inoculation. For culture filtrate immersion, ten seeds of three entries per tray were sown in a 8 × 12 cm tray. Trays were placed in a growth chamber under a 12 h photoperiod with a light intensity of 300 $\mu E/m^2/s$ at 28°C in the day and 22°C at night and watered daily. Three-week-old cuttings of each entry were immersed in test tubes (15 cm long × 2.5 cm diameter) containing culture filtrate of isolate Mont-1 as described previously or 50-fold dilution of the MSM medium. Cuttings were incubated under a 12 h photoperiod with a light intensity of 300 $\mu E/m^2/s$ at 25–28°C. Twenty millilitres of culture filtrate was added at 3-day intervals. There were three replications and nine cuttings per entry. Disease symptoms were recorded daily from 3 to 10 days after immersion.

Table 1. Foliar disease severity ratings of cut seedlings of soybean cv. Great Lakes 3202 10 days after stems were immersed in diluted culture filtrates of *Fusarium solani* f. sp. *glycines* when grown for 12 days in modified Septoria medium

Dilution	Disease severity ratings (%)[A]
25-fold	86 ± 4.2[B]
50-fold	78 ± 5.6
100-fold	55 ± 6.3
200-fold	50 ± 6.7
300-fold	31 ± 4.2
400-fold	14 ± 4.2
500-fold	8 ± 1.7
800-fold	0
999-fold	0

[A]Mean values of three plant samples per each of three replications per each of two experiments.
[B]Standard error of the mean.

Data analysis

Data were analysed by analysis of variance (SAS Institute, Cary, NC). All experiments were repeated and the data from both experiments were combined if there was no interaction between experiment and treatment and if error variances were homogeneous. Means were compared by least significant differences at $P = 0.05$.

Results

Dilution of culture filtrates

Soybean cuttings had foliar disease severity ranging from 8 to 86% depending on filtrate dilution (Table 1). Cuttings immersed in 25- and 50-fold-dilution culture filtrates had higher disease ratings than those in 100-fold or greater dilutions. A 25-fold dilution of MSM used as an experimental control caused < 10% leaf chlorosis on lower leaves of cuttings while the other MSM dilutions did not cause any visual symptoms. In the experiment using the same dilutions and experimental design, 3-week-old uprooted seedlings immersed in culture filtrate did not develop any foliar SDS symptoms.

pH adjusted culture filtrate

When cuttings were immersed in culture filtrates adjusted to pH 4, 5, 6, 7 and 8, foliar disease severity ratings did not differ ($P = 0.05$) and ranged from 72 to 82%.

Effects of various culturing conditions on mycelial growth, pH of culture filtrate, and symptom severity of cut seedlings

Mycelial dry weights were greater ($P = 0.05$) when the fungus was grown in PDB in both trials and MSM than in the other media (Table 2). The culture filtrate pH was highest ($P = 0.05$) when the fungus was grown in V8 juice broth (Table 2). Foliar disease severity ratings of cuttings were greater ($P = 0.05$) when cultures were grown in MSM and

Table 2. Mean mycelial dry weights of *Fusarium solani* f. sp. *glycines* and pH values of filtrates when grown in four media for 12 days and foliar disease severity ratings of soybean cuttings cv. Great Lakes 3202 10 days after stems were immersed in a 50-fold dilution of culture filtrate of each medium for two experiments

Medium	Mycelial dry weight (mg)	pH		Disease severity ratings (%)
		Initial[A]	Final	
Modified Septoria medium	503	4.5	7.2	73
Czapek Dox broth	208	7.5	6.1	66
Potato-dextrose broth	614	5.4	5.1	45
V8 juice medium	197	5.3	7.8	25
LSD ($P = 0.05$)	60		0.4	14

[A]Not tested for each replication.

CDB than in other media (Table 2). Mycelial dry weight was greatest ($P = 0.05$) when cultures were incubated at 25°C and least at 30°C (Table 3). The pH of culture filtrates ranged from 4.3 to 7.3 (Table 3). Foliar disease severity ratings of cuttings ranged from 70 to 75% for 15, 20 and 25°C, and were greater ($P = 0.05$) than those at 30°C (Table 3).

Mycelial dry weights were highest in both trials when cultures were initially started at pH 6 and were greater ($P = 0.05$) than weights obtained when grown at pH 8 and 9 in trial 1 and at pH 4, 8 and 9 in trial 2 (Table 3). The final pH of the culture filtrates ranged from 7.5 to 8.4 (Table 3). Foliar disease severity ratings of cuttings were greater ($P = 0.05$) when cultures were initially started at pH 4, 5 or 6 than at pH 7, 8 or 9 (Table 3).

Mycelial dry weights more than doubled from 1 to 2 weeks, but did not increase from 2 to 4 weeks (Table 3). The pH of culture filtrates ranged from 4.9 to 8.4 with the lower ($P = 0.05$) pH from 1-week-old cultures and no difference in pH at 2 to 4 weeks. Foliar disease severity ratings of cuttings were greater ($P = 0.05$) in filtrates from cultures grown for 2, 3, and 4 weeks than from cultures grown for 1 week (Table 3).

Mycelial dry weights and foliar disease severity ratings of cuttings were not different ($P = 0.05$) for the three light intensities. The pH of the culture filtrates ranged from 8.1 to 8.3.

Mycelial dry weights increased ($P = 0.05$) when cultures were grown with two layers of foil sealed over the mouth of the flask compared with the other treatments (Table 3). The pH of the culture filtrates ranged from 4.1 to 8.0 with lower pH values occurring in the treatment of growth under mineral oil. Foliar disease severity ratings of cuttings recorded 10 days after stems were immersed in a 50-fold dilution of culture filtrate ranged from 47 to 55% and did not differ ($P = 0.05$) among the treatments (Table 3).

Effect of culture filtrates of F. solani *f. sp.* glycines *and* F. solani *non-SDS causing isolates on soybean cuttings*

Foliar symptoms of cuttings with stems immersed in fungal culture filtrates of isolates Mont-1 and Fs-171 included interveinal chlorosis and necrosis, leaf curling and defoliation of upper leaves. Irregular brownish necrotic spots 0.5 mm in diameter and general leaf chlorosis occurred on cuttings when they were immersed for 10 days in culture filtrates of *F. solani* isolates from alfalfa, lupine, and tomato. Cuttings immersed in culture filtrate of a *F. solani* isolate from pea had necrotic spots 0.5 to 2 mm in diameter without any associated chlorosis, whereas isolates from bean, potato and soybean had slight leaf chlorosis (< 3% of leaf area). There were no visible symptoms on cuttings immersed in culture filtrates of isolates from cucumber and pumpkin, or the control MSM.

Reaction of selected legume species to F. solani *f. sp.* glycines *and its culture filtrate*

Interveinal chlorosis and necrosis, defoliation of upper leaves and/or plant death occurred on inoculated seedlings of soybean cv. Great Lakes 3202 and cv. Spencer in the greenhouse and on cuttings (Table 4). Symptoms on seedlings and cuttings of alfalfa included lower leaf chlorosis and wilt. Inoculated cowpea plants wilted and defoliated, but cuttings immersed in filtrate only became chlorotic. Garden bean leaves dried and plants wilted. Lima bean seedlings had marginal leaf dryness of upper leaves when inoculated, but cuttings wilted. Inoculated mung bean plants were stunted, wilted and died; however, cuttings were symptomless. In contrast, azuki bean, common pea and vetch did not show any foliar symptoms when inoculated, but their cuttings were chlorotic and wilted when immersed in fungal culture filtrate.

Discussion

In vitro conditions that alter the phytotoxicity of culture filtrates of *F. solani* f. sp. *glycines* when grown under defined conditions are important in both optimising conditions for maximising phytotoxin production, and also for providing information related to foliar symptom expression in the field which can be sporadic. In addition, the bioassay system used to assess phytotoxicity is also important, as some bioassay systems may be more sensitive or selective than others. In the past, we have tested toxicity of *F. solani* f. sp. *glycines* culture filtrates using soybean calli, detached leaves and cotyledons

Table 3. Mean mycelial dry weights of *Fusarium solani* f. sp. *glycines* and pH values of filtrates when grown in modified Septoria medium under varying conditions of (a) temperature, (b) pH, (c) aeration, for 12 days or (d) for varying lengths of time; and foliar disease severity ratings of cut stems of soybean cv. Great Lakes 3202 10 days after stems were immersed in a 50-fold dilution of culture filtrate over two experiments

	Mycelial dry weight (mg)	Culture filtrate pH[A]	Disease severity ratings (%)
Temperature (°C)			
15	181	4.3	74
20	244	5.7	75
25	368	7.3	70
30	136	4.4	54
LSD ($P = 0.05$)	42	0.1	15
Medium pH			
4	337	7.7	71
5	368	8.1	76
6	387	8.2	73
7	378	8.4	64
8	328	7.8	57
9	220	7.5	51
LSD ($P = 0.05$)	45	0.1	8
Aeration			
Membrane filter	335	8.0	55
Sealed with foil	410	7.4	50
Mineral oil and foil	49	4.1	47
LSD ($P = 0.05$)	26	0.7	Not significant
Growing time (weeks)			
1	157	4.9	42
2	348	7.7	58
3	334	8.3	63
4	334	8.4	70
LSD ($P = 0.05$)	79	1.0	10

[A]pH was measured after collecting culture filtrate.

(Jin *et al.* 1996*a*, 1996*b*). In this study, we chose to use soybean cuttings as the bioassay system since they consistently reproduce SDS symptoms and probably provide a better measurement of the specific phytoxin(s) that causes SDS. Soybean cuttings were also used to test monorden toxicity produced by *F. solani* f. sp. *glycines* (Baker and Nemic 1994).

Culture filtrates from semi-defined media (CDB and MSM) were more toxic on cuttings than filtrates from the other media. Although nutritional factors were not investigated in our study, they can be important or even limiting factors in the production of toxins. For example, synthesis of toxins by *F. solani* f. sp. *pisi* depended mainly on the supply of ammonium-nitrogen and magnesium (Naef-Roth 1972). Additional research is needed to determine what chemical compounds may inhibit or stimulate phytotoxin production in *F. solani* f. sp. *glycines*.

In a soil temperature study, SDS foliar symptoms were more severe between 15 and 25°C than at 30°C (Scherm and Yang 1996). In our study, culture filtrates from *F. solani* f. sp. *glycines* grown at 15, 20 and 25°C increased foliar disease severity on cuttings more than filtrates obtained by growing the fungus at 30°C. Since both studies were measuring toxin activity (foliar severity), it appears that phytotoxin production, or at least its activity, is greater at moderate than at higher temperatures.

Differences in pH of culture filtrates adjusted just prior to immersing stems in filtrate did not affect filtrate toxicity on cuttings indicating that pH does not seem to play a role in altering toxin activity. However, the initial MSM pH may be important because we found more foliar disease on cuttings when MSM pH values were 4, 5 or 6 than when pH values were 7, 8 or 9. We did not monitor how fast pH values changed in the medium as the fungus grows, so initial and final pH values may not represent the pH values during the growth phase of the fungus.

The phytotoxicity of *F. solani* f. sp. *glycines* filtrate seems independent of light and aeration. For other fungi like *F. culmorum* and *Alternaria tenuis*, toxin production was influenced by aeration as *F. culmorum* produced more

Table 4. Foliar symptoms of some legume species and soybean 21 days after inoculation with *Fusarium solani* f. sp. *glycines* under greenhouse conditions, and of cut seedlings with stems immersed in a 50-fold dilution of culture filtrate of *F. solani* f. sp. *glycines* under laboratory conditions for 10 days

Name		Foliar symptom	
		Seedling	Cut seedling
Azuki bean	*Vigna angularis*	No symptoms	Leaf mosaic and dryness
Alfalfa	*Medicago sativa*	Lower leaf chlorosis and dryness, plant wilt and death	Leaf chlorosis, defoliation and plant wilt
Common pea	*Pisum sativum*	No symptoms	Leaf chlorosis and plant wilt
Cowpea	*V. unguiculata*	Plant wilt, defoliation and death	Lower leaf chlorosis
Garden bean	*Phaseolus vulgaris*	Dryness from leaf margin and plant wilt and death	Leaf dryness, defoliation and plant wilt
Lima bean	*P. lunatus*	Marginal dryness of upper leaves	Defoliation and plant wilt
Mung bean cvv. Berkum and Kiloga	*V. radiata*	Plant stunt, wilt and death	No symptoms
Vetch	*Vicia sativa*	No symptoms	Chlorosis and wilt
Soybean cvv. Great Lakes 3202 and Spencer	*Glycine max*	Interveinal chlorosis and necrosis, defoliation, and plant death	Interveinal chlorosis and necrosis, defoliation of upper leaves

culmomarasmin in shake than in still cultures (Naef-Roth 1972). In contrast, *A. tenuis* produced more tentoxin in still cultures than in shake cultures.

In our study, optimal conditions for culture filtrate toxicity of *F. solani* f. sp. *glycines* were different from optimal conditions for mycelial growth. The correlations between the dry weight of mycelia, final pH values of culture filtrates, and foliar disease severity of cut seedlings was not significant. This indicates that toxicity of the culture filtrate may not be influenced by fungal growth. Sonogo and Yang (2001) showed that a pH value of 8.2 increased culture growth 2.5-fold over a pH of 5.7. From our data, it was shown that the fungus can at least initially grow at a range of pH values; however, and as it grows, the pH value changes as the final pH is different from the initial pH value.

Phytotoxins were reported from isolates of *F. solani* from bean, pea, potato and tomato (Cucuzza *et al.* 1992; Jeffries *et al.* 1984; Kern 1972). Likewise, Jin *et al.* (1996*b*) showed that *F. solani* isolates from alfalfa, bean, lupine, pea and potato did not cause SDS symptoms on inoculated soybeans but their culture filtrates were toxic to soybean calli. In another study, culture filtrates from various isolates were shown to be toxic to soybean suspension cells and differentially to soybean cuttings (Li *et al.* 1999). Using soybean cuttings, culture filtrates of *F. solani* isolates from cucumber and pumpkin did not cause any symptoms, and none of the other *F. solani* isolates caused typical SDS symptoms, but a few (those from alfalfa, lupine, pea and tomato) caused leaf chlorosis and/or necrotic spots. Although culture filtrates of *F. solani* isolates from bean and potato caused browning of soybean calli, they caused less than 3% leaf chlorosis on soybean cuttings. It is possible that soybean calli may be more sensitive than cuttings to culture filtrates. The ability of *F. solani* f. sp. *glycines* to incite SDS leaf symptoms provides a system to assay filtrates or their fractions for toxins and has been used to separate resistant and susceptible reactions in soybean (Huang and Hartman 1998). Interestingly, in our study we did not find whole seedlings with roots attached a good assay system to observe foliar symptoms since foliar symptoms did not develop, indicating the lack of movement of the toxin using this system.

Soybean plants either inoculated with *F. solani* f. sp. *glycines* or cuttings immersed in culture filtrate will display leaf symptoms of interveinal chlorosis and necrosis. Mung bean was reported as a host of *F. solani* f. sp. *glycines* and symptoms included root and lower stem discoloration, leaf chlorosis, defoliation and stunting (Melgar and Roy 1994). Similar symptoms, stunting and wilting, occurred on mung beans inoculated with infested sorghum grains in our study; however, mung beans were symptomless when cuttings were immersed in culture filtrate. In contrast, foliar symptoms were not observed on azuki bean, common pea and vetch when inoculated with *F. solani* f. sp. *glycines*, but leaf chlorosis or dryness and plant wilt occurred on cuttings of these species when immersed in culture filtrate. Cowpea, garden bean and lima bean also have been reported as hosts by using infested toothpick tips (Melgar and Roy 1994), but not by using a non-wounding infested oat grain method. However, in our study, seedlings of alfalfa, cowpea, garden bean and lima bean had leaf chlorosis, dryness, defoliation and wilt when inoculated with infested sorghum grain or when their cut seedlings were immersed in culture filtrates. More studies are needed to determine the host range of *F. solani* f. sp. *glycines* including a broader range of hosts along with differentiation between root and foliar symptoms as it seems that some hosts are sensitive to the toxin(s), based on foliar symptoms, whereas others are not.

In summary, filtrate phytotoxicity increased when *F. solani* f. sp. *glycines* was grown in MSM at a pH of 4–6, and 15 to 25°C for longer than 1 week. Soybean cuttings with stems immersed in culture filtrates of *F. solani* f. sp. *glycines*

isolates had interveinal chlorosis and necrosis, leaf curling and defoliation of upper leaves; when immersed in culture filtrates of *F. solani* isolates from other hosts, either no symptoms or symptoms not normally associated with SDS foliar symptoms were observed. It appears that the phytotoxin produced by *F. solani* f. sp. *glycines* is a pathotoxin(s), since the foliar symptoms appear without the fungus and it may be somewhat specific since other plant species tested in our bioassays were either not sensitive or did not have typical SDS foliar symptoms as observed in soybean.

Acknowledgements

We thank Ron Warsaw for technical assistance and the Illinois Soybean Check-Off Board and the United Soybean Board for partial support of this research.

Footnote: Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the USA Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

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Received 24 December 2002, accepted 15 July 2003