Greenhouse and Field Evaluation of *Fusarium solani* Pathogenicity to Soybean Seedlings

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ABSTRACT

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Koch's postulates were completed with *Fusarium solani* under both greenhouse and field conditions. Root rot was the most prevalent and damaging symptom caused by the fungus. Five *F. solani* isolates tested were pathogenic when inoculated on soybean, and each was reisolated from inoculated, symptomatic plants. Isolates differed in virulence. Disease severity was not substantially changed when *F. solani* and soybean cyst nematode (*Heterodera glycines*) were inoculated on soybean in combination, as compared with *F. solani* or soybean cyst nematode alone. The fungus reduced yield under field conditions. Disease severity was greater with poor-quality seeds inoculated with *F. solani* than with inoculated high-quality seeds.

Additional keywords: *Glycine max*, seed quality

Seedling disease complex of soybean (*Glycine max* (L.) Merr.) causes annual economic losses in Mississippi (11) and other soybean-producing areas in the United States (1,19). *Fusarium* spp. are major fungal components of the complex (1,6,14,19,22), although their pathogenicity has generally been difficult to demonstrate and some species are considered secondary invaders (3,9,15, 21,23,24). The presence of nematodes in soil was reported to increase seedling disease severity and/or colonization of seedlings by soilborne fungi (5,14).

Fusarium solani (Mart.) Appel & Wollenw. emend. Snyd. & Hans. is frequently isolated from soybean seedlings, but there are conflicting reports regarding its pathogenicity (7,9,15,16,20,21,23,24). Results of Schlub et al (15,16) indicated that predisposition of soybean seeds by drought stress for 4–6 days was required for infection of seedlings. Only one study (24), conducted in the greenhouse, demonstrated that *F. solani* was capable of reducing soybean yield. There have been no reports of infection or reduced yield in the field.

This paper reports the completion of Koch's postulates with different isolates of *F. solani* in both greenhouse and field, the effect of *F. solani* isolate and inoculum concentration on disease severity, predisposing effects of nematodes and poor seed quality on infection of seedlings by *F. solani*, and effect of the fungus on yield.

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MATERIALS AND METHODS

Effect of *F. solani* isolate and inoculum level on disease severity in the greenhouse. Five single-spore isolates of *F. solani*, obtained from symptomatic roots of cv. Coker 156 soybean seedlings and designated K15, K37, B312, H216, and C39, were tested. Their identification was based on descriptions reported by Booth (2) and Nelson et al (12). Isolates were maintained on slants of modified Bilay's medium (2) at 5 C.

To produce inocula, isolates were grown at 24 C for 10 days in sterile sand-cornmeal cultures (250 cm^3 of dry sand, 14 cm^3 of cornmeal, and 100 ml of distilled water). A mechanical soil mixer was used to mix portions of the inocula with sterilized soil to obtain inoculum levels of 0.050%, 0.025%, and 0.0125% (w/w). The potting medium used in all tests was a 1:1 (v/v) mixture of sand and soil fumigated with methyl bromide. Infested and noninfested (control) soil each were distributed to 12.5-cm-diameter pots. Ten soybean cv. Bragg seeds were surface-disinfested in 1% sodium hypochlorite, rinsed in sterile water, and aseptically plated on Difco potato-dextrose agar (PDA). After 36 hr, germinated seeds free from microorganisms were placed on the soil surface in each pot and covered with 2.5 cm of the appropriate soil. Experimental units, each replicated four times, were arranged in a completely random design and plants were watered daily. Disease severity, assessed 3 wk after planting, was based on a 1–6 scale: 1 = no symptoms, 2–4 = increasingly severe root-hypocotyl necrosis, 5 = postemergence damping-off, and 6 = preemergence damping-off. To assure uniformity in experimental units, plants were randomly culled to five per pot before disease assessment. During the course of experiments, temperatures in the greenhouse ranged

from 16 to 25 C during the night and from 25 to 34 C during the day.

Effect of *F. solani* alone and in combination with *H. glycines* on disease severity in the greenhouse. Isolates U32, H216, FS, and FSC of *F. solani*, either alone or in combination with soybean cyst nematode (*Heterodera glycines* Ichinohe), were tested for pathogenicity. An inoculum level of 0.050%, obtained as previously described, was used to inoculate Bragg seedlings. Nematodes were obtained by increasing a population of race 3 on Coker 156 soybean for 60 days in the greenhouse. Light brown to tan cysts were dislodged from roots with a stream of water and collected on nested 20- and 60-mesh sieves. Cysts were suspended in water, then immediately poured through a 850-μm-pore sieve nested on a 250-μm-pore sieve. They were then washed from the 250-μm-pore sieve into 20-ml glass test tubes with 10 ml of tap water and crushed with a modified Seinhorst cyst crusher (18) for 1 min (G. W. Lawrence, *unpublished*). The resultant suspension was passed through a 75-μm-pore sieve nested on a 28-μm-pore sieve to remove broken cysts and debris. Eggs and second-stage juveniles were standardized to a population of 686 eggs and 43 juveniles per milliliter. Five germinated and apparently healthy seeds, obtained as previously described, were placed on the surface of methyl bromide-fumigated soil in each pot and covered with 2.5 cm of the appropriate soil. Seven milliliters of the *H. glycines* suspension were pipetted into three 1 × 4 cm depressions in soil, and a thin layer of fumigated soil was added to prevent egg desiccation. Experimental units, each replicated seven times, were arranged in a completely random design, and plants were watered daily. Disease severity was assessed 3 wk after planting, using the previously described method. Sections of root tissue from inoculated, symptomatic plants and from noninoculated plants were surface-disinfested in 95% ethanol for 5 sec and 1% NaOCl for 1 min, rinsed in sterile water, then aseptically plated on PDA. Colonies of *F. solani* growing from tissue were determined during a 1-wk incubation period. This experiment was conducted twice, during which time greenhouse temperatures ranged from 15 to 21 C during the night and from 21 to 29 C during the day.

For each experiment, data were subjected to analysis of variance and means were separated using Duncan's multiple range test. Percentage stand data were transformed to arcsine before analysis.

Pathogenicity tests in the field. Field plots of soybean cv. Asgrow 5474 were established at the Mississippi State University Plant Science Farm, Starkville, in June 1985 and 1986. Asgrow 5474 is as susceptible to seedling infection by *F. solani* as Coker 156 (authors, *unpublished*). Isolates of *F. solani* were grown on a modified Bilay's medium (2) for 10 days at 24 C under a fluorescent light bank (160 Wm^{-2}) set at a 12-hr photoperiod. The isolates used were recovered from diseased root tissues of soybean seedlings. Preliminary tests indicated that inoculation of seeds with conidia produced high levels of seedling infection (authors, *unpublished*). Inoculum of each isolate was obtained by flooding plates with sterile distilled water, dislodging conidia with a camel's-hair brush, and adjusting inoculum to 1.5 × 10^6 macroconidia per milliliter using a hemacytometer. Seeds were placed in inflated plastic bags, the appropriate inoculum was added, and seeds were agitated for 1 min to obtain uniform coverage. Seeds similarly treated with sterile distilled water served as controls. Seeds were stored at 10 C until planted. Immediately before planting, subsamples of inoculated seeds were plated on PDA to verify viability of inoculum.

To determine the effect of different isolates on disease severity, isolates H216, B312, and C39 were compared in 1985 and H216, B312, C39, and U32 were compared in 1986. Field plots were three rows each 6 m long on 1-m centers and were established in a randomized complete block design, with four replications in 1985 and seven in 1986.

To determine the effect of seed quality on disease severity, poor-, intermediate-, and high-quality seeds, testing 60, 70, and 85% germination on PDA, respectively, were inoculated with isolate H216, the most virulent isolate in previous pathogenicity tests. The levels of seed quality were obtained by examining seeds with a 7× magnifier and separating seeds having fissured seed coats or dull appearance (poor quality) from those appearing healthy (high quality); the two seed lots were intermixed to obtain intermediate quality. Three-row plots 6 m long on 1-m centers were established in a randomized complete block design with a split-plot arrangement of treatments, each replicated four times. Inoculation and seed quality were main and subplots, respectively.

In both years, seedling numbers (stand), plant height, and disease severity were determined 6 wk after planting in each field test. Plant height and disease severity were determined from 25 randomly selected plants per replicate. Postemergence damping-off was monitored during the 6-wk period, and yield was determined for the center row in each plot at maturity. Disease severity was

Table 1. Pathogenicity of *Fusarium solani* isolates to 3-wk-old Bragg soybean seedlings in the greenhouse

Isolate	Disease severity index[y]	Root volume (cm^3)	Height (cm)	Stand (% of control)
K15	2.7 b[z]	8.0 ab	15.4 a	92 b
K37	2.8 b	7.6 ab	15.6 a	91 b
B312	2.9 b	8.6 a	15.1 a	92 b
H216	3.7 a	6.0 b	14.6 a	92 b
C39	2.6 b	9.0 a	15.0 a	95 b
Control	2.1 c	10.1 a	15.9 a	100 a

[y] Disease severity was based on a 1–6 scale: 1 = no symptoms, 2–4 = increasingly severe root-hypocotyl necrosis, 5 = postemergence damping-off, and 6 = preemergence damping-off.
[z] Means within a column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

Table 2. Pathogenicity of *Fusarium solani* isolates alone or in combination with soybean cyst nematode, *Heterodera glycines* race 3, to 3-wk-old Bragg soybean seedlings in the greenhouse

Isolate and/or nematode treatment	Disease severity index[y]	Stand (% of control)	Root volume (cm^3)	Height (cm)
U32	2.7 c[z]	96 b	11.3 b	13.5 ab
U32 + *H. glycines*	2.8 bc	95 b	10.6 bc	12.5 ab
H216	3.5 ab	93 c	8.6 cd	12.4 b
H216 + *H. glycines*	3.0 bc	95 b	9.7 bc	13.5 ab
FS	2.5 cd	96 b	10.0 bc	13.0 ab
FS + *H. glycines*	2.4 cd	96 b	10.4 bc	12.7 ab
FSC	4.2 a	82 e	5.7 e	10.9 c
FSC + *H. glycines*	4.2 a	86 d	7.2 de	10.8 c
H. glycines	1.9 de	99 a	14.0 a	12.2 b
Control	1.5 e	100 a	14.5 a	13.9 a

[y] Disease severity was based on a 1–6 scale: 1 = no symptoms, 2–4 = increasingly severe root-hypocotyl necrosis, 5 = postemergence damping-off, and 6 = preemergence damping-off.
[z] Means are averages of two experiments (14 total replications). Means within a column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

based on a 1–5 scale: 1 = no symptoms, 2–4 = increasingly severe root-hypocotyl necrosis, and 5 = postemergence damping-off. In each experiment, sections of root tissue from 25 randomly sampled plants per replicate were surface-disinfested in 1% NaOCl and aseptically plated on PDA acidified with HCL (APDA), and colonies of *F. solani* growing from tissues were determined.

RESULTS

Effect of *F. solani* isolate and inoculum level on disease severity in the greenhouse. Isolates of *F. solani* caused root rot and preemergence damping-off and were reisolated from symptomatic root tissue plated on APDA (Table 1). Root rot was the most prevalent symptom. Lesions varied in color from light to dark brown, were often sunken, and seldom occurred above the soil line. Little postemergence damping-off was observed. All isolates were pathogenic as measured by disease severity index, root volume, and stand. Isolate H216 was the most virulent. Virulence of the other four isolates did not differ. None of the isolates had a significant effect on seedling height in these tests. Inoculum level had no effect on disease severity and did not interact with isolates ($P = 0.05$).

Effect of *F. solani* alone and in combination with *H. glycines* on disease severity in the greenhouse. *H. glycines* alone had no significant effect on disease severity index, root volume, or stand but reduced seedling height (Table 2). Each isolate of *F. solani* was pathogenic as measured by disease severity index, stand, and root volume. Isolates FSC and H216 caused significant height reductions. Combinations of H216 and *H. glycines* and of FSC and *H. glycines* caused less stand reduction than did H216 or FSC alone. Isolates H216 and FSC caused more severe symptoms and greater reductions in stand and root volume than did isolates U32 and FS. Isolate FSC caused significantly greater reductions in stand, root volume, and height than the other three isolates.

F. solani was recovered from more than 90% of the surface-disinfested, symptomatic plants but from less than 1% of the noninoculated plants.

Effect of different isolates on disease severity in the field. Each isolate of *F. solani* caused seedling disease in the field (Table 3). The isolates differed in virulence. In both years, isolate H216 was the most virulent and caused reduced stand, seedling height, and yield. In 1985, B312 was the only other isolate that reduced plant performance, causing a yield reduction. In 1986, isolates U32, B312, and C39 had no significant effect on stand, but each caused significant levels of disease on roots and hypocotyls. Isolate U32 reduced seedling height and yield, and isolate B312 reduced yield. In general, isolate C39 was the least virulent and did not significantly reduce plant performance.

In both years, *F. solani* was recovered from surface-disinfested tissues of more than 75% of the inoculated plants but from less than 20% of noninoculated plants.

Effect of seed quality on disease severity in the field. The mean squares for both main effects and the interaction were significant. As measured by each parameter, high-quality seeds were superior to intermediate- and poor-quality seeds (Table 4). Seedling disease was produced by *F. solani* on poor-, intermediate-, and high-quality seeds but reduced stand, seedling height, and yield only on poor-quality seeds. The interaction indicated that *F. solani* was more destructive on poor-quality seeds than on intermediate- and high-quality seeds, as measured by disease severity index and three other parameters.

Percentage reisolation of *F. solani* from surface-disinfested tissues of inoculated and noninoculated plants was similar to that in previous field tests.

DISCUSSION

Koch's postulates were fulfilled with five *F. solani* isolates under both greenhouse and field conditions. This is the first demonstration of pathogenicity and subsequent yield reduction by this fungus on soybean in the field.

F. solani was pathogenic on soybeans in some studies (7,16,20,24) and nonpathogenic in others (9,15,21,23). Strain differences, which are suggested by the differential virulence of *F. solani* isolates tested herein, may at least partially explain these conflicting reports. This is supported by comparative results of our study and of Klag et al (9). Klag et al used similar inoculation methods for pathogenicity trials with *F. solani* in the greenhouse, yet none of their several isolates tested was pathogenic. Nonpathogenic strains of *F. solani* (2,10) and other *Fusarium* species (3) are known to occur on other hosts.

Nematodes and fungi can act synergistically to increase the incidence and/or severity of certain soilborne diseases of soybean (5,13,14). Therefore, it was surprising that when *F. solani* was

Table 3. Pathogenicity of *Fusarium solani* isolates inoculated on Asgrow 5474 soybean seeds in the field in 1985 and 1986

	Disease parameter[y]			
Isolate	Seedling disease severity index	Stand (% of control)	Seedling height (cm)	Seed yield (kg/ha)
		1985		
C39	3.8 ab[z]	77 ab	21.4 a	2055 a
B312	3.7 ab	84 ab	21.0 a	1822 b
H216	4.2 a	65 b	16.4 b	1578 b
Control	3.3 b	100 a	23.0 a	2138 a
		1986		
H216	4.1 a	82 b	13.2 b	849 b
U32	3.9 b	96 a	13.0 b	916 b
B312	3.8 b	96 a	13.8 ab	936 b
C39	3.8 b	93 a	13.9 ab	1058 a
Control	3.5 c	44 a	14.5 a	1078 a

[y] Disease severity index, stand, and plant height were obtained 6 wk after planting. Disease severity was based on a 1–5 scale: 1 = no symptoms, 2–4 = increasingly severe root-hypocotyl necrosis, and 5 = postemergence damping-off.
[z] Means within a column and year followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

Table 4. Effect of different levels of seed quality on pathogenicity of *Fusarium solani* inoculated on Asgrow 5474 soybean seeds in 1986

	Disease parameter[y]			
Seed quality	Seedling disease severity index	Seedling numbers (stand)	Seedling height (cm)	Seed yield (kg/ha)
Poor	4.7 a[z]	16 d	16.9 c	578 d
Control	4.1 b	26 c	23.3 b	1097 c
Intermediate	4.1 b	41 b	26.3 ab	1636 b
Control	3.8 c	45 b	28.0 a	1878 b
High	3.3 d	80 a	29.6 a	2350 a
Control	3.1 e	84 a	28.8 a	2245 a

[y] Disease severity index, stand (per 6 m of row), and plant height were obtained 6 wk after planting. Disease severity was based on a 1–5 scale: 1 = no symptoms, 2–4 = increasingly severe root-hypocotyl necrosis, and 5 = postemergence damping-off.
[z] Means within a column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

combined with *H. glycines*, seedling stand was significantly greater than that which occurred when either isolate was used alone. Even though this effect on stand was inconsistent with the effect on disease severity, seedling height, and yield, it warrants further investigation.

Results of Schlub et al (15,16) indicated that predisposition of soybean seeds by drought stress for 4–6 days was required for infection of seedlings grown in soil infested with *F. solani*. We obtained high levels of infection without predisposing seeds, but disease severity was negatively associated with increased seed quality. Some evidence (8,17) indicates that exudates from seeds are at least in part responsible for increased infection of soybean seedlings by certain soilborne fungi. Seeds with fissured seed coats or other kinds of damage express greater amounts of exudates than healthy seeds (4,17). Exudates are known to contain amino acids, sugars, and other nutrients utilized by fungi for growth (4,8,17). Thus, a likely explanation for the association of higher levels of *F. solani* infection with poor-quality seeds is that the growth of *F. solani* was enhanced by exudates from such seeds.

Even though stand was reduced in some instances by some *F. solani* isolates, the slight reductions were of secondary importance compared with root rot. Data indicate that the yield reductions observed resulted from early, and presumably prolonged, colonization of roots by *F. solani*.

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