Severity of Foliar Symptoms and Root and Crown Rot of Soybean Inoculated with Various Isolates and Inoculum Rates of *Fusarium solani*

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ABSTRACT

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Soybean plants (cvs. Spencer and Ripley) were grown in Cone-Tainers infested with different rates of sudden death syndrome (SDS) isolates of *Fusarium solani*. Soil inoculum rate significantly affected severity of root rot and percent leaflets of inoculated plants with SDS symptoms with isolate Mont-1. Leaf symptoms of SDS on Ripley were significantly less than on Spencer. When SDS isolate Cora-7 was used, only the soil inoculum rate was significant for percent leaflets with SDS symptoms and root rot severity. Nine SDS fungal isolates differed in the amount of root rot and severity of leaf symptoms that they produced on inoculated Spencer and Ripley plants.

Soybean (*Glycine max* (L.) Merr.) sudden death syndrome (SDS) is caused by specific isolates *of Fusarium solani* (Mart.) Appel & Wollenweb. emend. W. C. Snyder & H. N. Hans (6,7). The pathogen was originally isolated from roots and crown tissue of symptomatic soybean plants (6,7). Since the first reports of this disease, considerable effort has been placed on evaluating soybean cultivars for resistance under field conditions (3,8,9,12). In addition, various methods have been used to inoculate soybean plants in the greenhouse to determine virulence of different isolates of the pathogen (5) or to evaluate soybean cultivar response to inoculation (11,12). Soybean cultivar response has been based either on stem lesion severity (5) or on severity of leaf symptoms (7,10,11). The relative virulence of a number of fungal isolates has been evaluated by a stem inoculation procedure (5). Although the fungus was originally isolated from root and tap root tissue of field-collected plants (6,7), little information is available describing development of the pathogen on soybean roots and taproot crown tissue under greenhouse conditions. Roy et al. (6) used a soil infestation technique to demonstrate that *F. solani* form FS-A isolates produced root and crown necrosis on inoculated plants.

The present work was undertaken to determine if root necrosis caused by SDS *F. solani* isolates develops into a root and crown rot on inoculated soybean plants, to assess the extent of damage caused by different SDS isolates of *F. solani*, and to determine the relationship between inoculum concentration and disease development.

MATERIALS AND METHODS

Fungal isolates and inoculum production. Fungal isolates were obtained from diseased soybean plants by the authors or from other researchers (Table 1). Each fungal isolate originated from a single spore and was maintained on slants of Bilay's medium (2) at 19°C. A transfer from the stock slant was made to a 10-cm plate of 5× Bilay's medium (minus glucose, pH adjusted to 6.8 before autoclaving). The plates were incubated at 27°C for 12 days, then used for inoculum production. Inoculum for each experiment was produced by growing a fungal isolate at 27°C for 10 days in sterile sand/cornmeal culture: 90 g of quartz sand, 30 g of cornmeal, and 60 ml of distilled water (4,6). Each flask was inoculated with an individual fungal isolate by transferring two 1 × 0.5 cm pieces of agar culture of the fungus to a sterile tube. One milliliter of sterile water was added to the tube and the agar pieces were macerated with a sterile glass grinder. An additional 3 ml of sterile water was added and the contents of the tube were transferred to a flask of sterile sand-cornmeal medium. A new batch of inoculum was prepared for each trial of an experiment.

Plant growth conditions. Unless mentioned otherwise, all plants were grown in a growth chamber programmed for a 12-h day temperature of 27°C, and a 12-h night temperature of 24°C with a light intensity during the day period of 300 $\mu E \cdot s^{-1} \cdot m^{-2}$. All soybean plants were grown in 3.8 × 21 cm tapered Cone-Tainers (Ray Leach Cone-Tainers, Stuewe & Sons, Inc., Corvallis, OR). The drain hole of each Cone-Tainer was plugged with a nonabsorbent cotton ball, then the tubes were prefilled with pasteurized soil/sand mix (1:1 vol/vol) to a depth of 13 cm. The Cone-Tainers were watered the day before the experiment was started to allow the soil to equilibrate. Soybean seeds were germinated and grown for 10 days in flats of heat-treated sand before seedlings were transplanted. After the plants were transferred into infested and noninfested soil (one plant per Cone-Tainer), the Cone-Tainer rack with tubes was placed in a large plastic pan of water (water depth maintained at 5 cm) for the duration of the experiment in order to maintain a constant soil moisture of 25% (weight basis).

Inoculum rate studies. Two experiments examined the effect of inoculum rate on symptom severity and root rot severity.

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Table 1. Origin of *Fusarium solani* isolates used in pathogenicity studies

Isolate designation	Source	Geographic origin
Mont-1	P. Stephens	Illinois
FSA1	J. Rupe	Mississippi
Cora-7	L. Gray	Illinois
Villaridge (VR)	L. Gray	Illinois
1101A	J. Rupe	Illinois
NRRL 22825 (NRRL)[a]	USDA/ARS	Indiana
AR269	J. Rupe	Arkansas
Ridgeway (RW)	L. Gray	Illinois

[a]USDA/ARS, Peoria, IL, culture collection number. This isolate was also previously designated as In-F2X-11A (1).

In experiment 1, portions of the sand/cornmeal inoculum with isolate Mont-1 were mixed with the heat-treated soil/sand mix (1:1 vol/vol) to give inoculum rates of 5, 2.5, 1.2, 0.6, 0.3. and 0.0% (wt/wt). The infested soil was then used to fill the upper 8 cm of a Cone-Tainer around a transplanted Spencer or Ripley soybean seedling. In noninfested control tubes, sand/cornmeal was mixed with heat-treated soil/sand to give a final concentration of 5% (wt/wt). The plants were grown for 3 weeks after transplanting in a growth chamber. The treatment arrangement was a 6 × 2 factorial of six soil inoculum rates and two soybean cultivars with four replicates per treatment arranged in a completely randomized design. In experiment 2, the same soil inoculum rates and treatment arrangement were used with SDS isolate Cora-7. Both experiments were conducted two times and were denoted as trial 1 and trial 2.

Virulence of fungal isolates. In experiment 3, virulence of nine SDS fungal isolates (Table 1) was compared on Spencer soybean plants. A soil inoculum rate of 5% (wt/wt) was used in this experiment. There were four replicates of each isolate arranged in a completely randomized design. An additional experiment with the same fungal isolates, denoted as experiment 4, was done with Ripley as the soybean cultivar. Each experiment was conducted two times and denoted as trial 1 and trial 2.

Disease assessment. At the termination of each experiment, plants were removed from the Cone-Tainers and roots were washed to remove adhering soil and the roots were blotted with a paper towel to remove excess moisture.

The severity of SDS leaf symptoms for each plant was determined as a percentage of individual leaflets on each plant with SDS symptoms. The roots of each plant were rated for root rot on a scale of 1 to 5 (1 = healthy roots and tap root; 2 = <25% of lateral roots and tap root with necrosis; 3 = 25 to 50% of lateral roots and tap root with visible necrosis; 4 = 51 to 90% of lateral roots and tap roots with necrosis; and 5 = >90% of root system with necrosis, plants dead).

In the first inoculum rate study, samples of root tissue from inoculated and control plants were surface sterilized in 0.5%

Table 2. Root rot severity and percent leaflets with sudden death syndrome (SDS) symptoms of soybean plants (cvs. Spencer and Ripley) grown in Cone-Tainers infested with SDS isolates Mont-1 or Cora-7 at six soil inoculum rates for 3 weeks

		Mont-1 (experiment 1)				Cora-7 (experiment 2)			
		Root rot severity[c]		Leaflets with SDS symptoms (%)[d]		Root rot severity		Leaflets with SDS symptoms (%)	
		Soybean Cultivar							
Trial[a]	Soil inoculum rate (%) (wt/wt)[b]	Spencer	Ripley	Spencer	Ripley	Spencer	Ripley	Spencer	Ripley
Trial 1	5.0	4.0	3.8	93	53	5.0	5.0	100	100
	2.5	4.0	3.8	62	19	4.0	4.3	74	87
	1.2	4.0	3.8	40	0	4.0	4.0	47	44
	0.6	3.0	2.8	0	0	3.3	2.8	0	0
	0.3	2.0	2.8	0	0	2.8	2.8	0	0
	0.0	1.0	1.0	0	0	1.0	1.0	0	0
LSD ($P \leq 0.01$)		0.6		34		0.7		40	
Trial 2	5.0	4.3	4.5	93	90	5.0	4.5	87	100
	2.5	4.0	3.8	59	9	4.0	3.8	44	28
	1.2	3.5	4.0	43	9	4.0	4.0	7	19
	0.6	2.8	3.0	0	0	3.5	3.0	0	0
	0.3	2.8	3.0	0	0	3.3	3.0	0	0
	0.0	1	1	0	0	1.0	1.0	0	0
LSD ($P \leq 0.01$)		NS[e]		40		0.8		36	

[a] There were two separate trials of each experiment conducted with different batches of fungal inoculum.
[b] Inoculum rates were mixtures of heat-treated soil/sand and sand/cornmeal inoculum of the fungal isolate (wt/wt).
[c] Root rot severity score of 1 = healthy to 5 = severely diseased.
[d] Percentage of individual leaflets per plant with SDS symptoms.
[e] Not significant.

Table 3. Root rot severity and percent leaflets with suddent death syndrome (SDS) symptoms for two soybean cultivars in soil infested with different isolates of *Fusarium solani*

	Spencer (experiment 3)				Ripley (experiment 4)			
	Root rot severity[b]		Leaflets with SDS symptoms (%)[c]		Root rot severity		Leaflets with SDS symptoms (%)	
Fungal isolate	Trial 1[a]	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Mont-1	5.0	4.5	100	100	5.0	4.8	100	100
FSA1	4.5	4.8	100	100	4.5	4.5	100	100
Cora-7	4.8	5.0	90	90	5.0	4.5	100	100
VR	4.5	4.3	90	90	4.0	4.3	93	100
1101A	4.5	4.8	50	100	4.8	4.5	100	100
NRRL	1.8	2.8	0	0	2.0	2.0	0	0
K1	4.8	5.0	100	100	5.0	5.0	100	100
AR269	3.0	3.3	29	35	4.8	4.0	50	28
RW	5.0	5.0	100	100	5.0	4.5	100	100
None	1.0	1.0	0	0	1.0	1.0	0	0
LSD ($P \leq 0.01$)	0.8	0.9	45	17	0.6	0.8	12	22

[a] There were two separate trials of each experiment conducted with different fungal inoculum preparations for each trial.
[b] Root rot severity based on a score of 1 = healthy to 5 = severely diseased.
[c] Percentage of leaflets per plant with SDS symptoms.

NaOCl for 4 min and plated onto potato dextrose agar plates amended with 30 µg of tetracycline per ml. After 10 days at 27°C, root tissue was examined for growth of *Fusarium* colonies. This was done to determine if the fungus could spread from infested soil tubes to the noninfested soil, since all of the plants were maintained in a plastic container of water during the experimental period.

Data analysis. Data from each experiment were subjected to analysis of variance (ANOVA) with a Statistica Package for the Macintosh computer (Statsoft Corp., Tulsa, OK). The data from the two trials of each experiment were combined to determine if there was significant experiment × treatment interaction or if the data could be pooled. Treatment means were compared by least significant difference (LSD).

RESULTS AND DISCUSSION

Inoculum rate studies. In experiments 1 and 2, there was a significant experiment × treatment interaction, so the data from the two trials from each experiment were not pooled but were analyzed separately and are presented as trials 1 and 2 (Table 2). Ripley was the primary source of the significant experiment by treatment interaction. There was a highly significant ($P \leq 0.001$) main effect of inoculum rate on percent plant leaflets with SDS symptoms and root rot severity in experiment 1 trial 1 but only percent plant leaflets with symptoms was significant ($P \geq 0.001$) in trial 2 (Table 2). There was no significant interaction of cultivar by inoculum rate for percent leaflets with SDS symptoms or root rot severity in both trials of experiment 1. Root rot severity between Spencer and Ripley in both trials of experiment 1 was not significantly different. A significant ($P \geq 0.01$) cultivar effect for percent symptomatic leaflets was observed in both trials, with Ripley having fewer leaflets with symptoms than Spencer at high inoculum rates with isolate Mont-1. Ripley has been reported to carry a single gene for resistance to *F. solani* (10) based on severity of leaf symptom development on inoculated plants. In previous studies (11,12), soybean plants were inoculated by a different technique than that used in the present experiment. *Fusarium* was not isolated from any of the control plants in this experiment, indicating that the fungus does not readily move from infested soil tubes to the control tubes.

In experiment 2, trials 1 and 2, when isolate Cora-7 was used, inoculum rate was the only significant ($P \geq 0.001$) source of variation for percent leaflets with SDS symptoms and root rot severity (Table 2). The results from both experiment 1 and 2 showed that Ripley does not express any resistance to root rot caused by *F. solani,* compared with a highly susceptible cultivar such as Spencer. Leaf symptom development on inoculated plants may possibly be a distinct phase of the disease that is separate from root rot development.

These experiments demonstrate the impact of soil inoculum rate on the severity of leaf symptom development and root rot severity for soybean seedlings exposed to SDS isolates of *F. solani*. This is the first reported work in which soil inoculum rate has been evaluated for SDS development on soybean. Further work needs to be done with more isolates and soybean cultivars in order to develop a clear understanding of the progression of root rot, onset of leaf symptom development, and soybean cultivar differences in SDS susceptibility.

Virulence of fungal isolates. All fungal isolates tested on Spencer and Ripley soybeans caused a root and crown rot (Table 3). Fungal isolates differed significantly ($P \geq 0.01$) in their effect on percent leaflets with SDS symptoms and root rot severity (Table 3). Although a high soil inoculum rate was used in this study, the results showed that there are differences in the severity of root rot resulting from the use of different isolates of *F. solani*. Although the isolates were not compared on Spencer and Ripley soybean seedlings at the same time, two SDS isolates, NRRL22825 and AR269, caused significantly less leaf symptoms ($P \geq 0.01$) and produced less root rot than the other isolates in both experiments.

Although Ripley has been reported to be resistant to SDS (10,11), this cultivar developed some leaf symptoms in infested soil. Symptoms usually were not as severe as those on Spencer. In previous work (10, 11) different *F. solani* isolates and a crown inoculation technique with infested oats as inoculum was used. The severity of leaf symptoms on Ripley in the present experiment is probably due to inoculation methodology and virulence of the fungal isolates. Possibly there are differences between soybean cultivars in crown tissue susceptibility that limit either pathogen development or expression of leaf symptom development. Further work needs to be done to compare inoculation methods and inoculum rates in order to clearly define soybean cultivar reactions.

Results from the present experiments showed that SDS *F. solani* isolates cause a root and crown rot on inoculated soybean plants. The root rot phase of SDS has received little attention and needs to be further investigated. The Cone-Tainer system for growing soybean plants should be useful for simultaneously evaluating numerous SDS *F. solani* isolates, since a large number of isolates can be evaluated at one time under a closely controlled environment in a growth chamber.

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