Relationship Between Field and Greenhouse Reactions of Soybean to *Fusarium solani*

P. A. STEPHENS, Former Graduate Research Assistant, and C. D. NICKELL, Professor, Department of Agronomy, University of Illinois, Urbana 61801; C. K. MOOTS, Asgrow Seed Co., Stonington, IL 62567; and S. M. LIM, Professor, Department of Plant Pathology, University of Arkansas, Fayetteville 72701

ABSTRACT

Stephens, P. A., Nickell, C. D., Moots, C. K., and Lim, S. M. 1993. Relationship between field and greenhouse reactions of soybean to *Fusarium solani*. Plant Dis. 77:163-166.

Field and greenhouse tests were conducted to determine the relationship between field and greenhouse reactions of soybean (*Glycine max* (L.) Merr.) to *Fusarium solani* (Mart.) Appel & Wollenw. emend. Snyd. & Hans., the causal organism of sudden death syndrome (SDS). Seedlings of 12 soybean cultivars, ranging from maturity group III to late-maturity group IV, were inoculated in the greenhouse with oat grains infested with *F. solani*. The same cultivars were evaluated for disease reaction in *F. solani*-infested microplots at Urbana, IL, and at infested field sites in central and southern Illinois. SDS leaf symptom severity for field-grown plants at the R6 growth stage and greenhouse leaf symptom severity at about 3 wk after inoculation were highly correlated (r = 0.60–0.91). Thus, inoculation of soybean seedlings with the oat grain culture in the greenhouse is a reliable method for evaluating reactions of soybeans to *F. solani*. In performance trials during 1990 and 1991 at four field sites infested with *F. solani*, no relationship was found between SDS severity and the agronomic traits yield, maturity, plant height, lodging, seed weight, and seed quality.

Sudden death syndrome (SDS) is a disease of soybean (*Glycine max* (L.) Merr.) caused by the soilborne fungus *Fusarium solani* (Mart.) Appel & Wollenw. emend. Snyd. & Hans. (13,14). SDS was first observed in Arkansas in 1971 by H. J. Walters and has since been reported in Mississippi, Missouri, Illinois, Tennessee, Kentucky, and Indiana (15).

In general, SDS first appears as mosaiclike symptoms on the uppermost leaves at flowering. Chlorosis and necrosis spread across the leaflets, while the leaf tissue around major leaflet veins remains dark green. Chlorotic leaf symptoms resemble the symptoms of another soybean disease, brown stem rot, caused by *Phialophora gregata* (Allington & D. W. Chamberlain) W. Gams. However, in contrast to the pith browning seen in plants infected with *P. gregata* (16), pith of plants infected with *F. solani* appears healthy (white). Xylem tissue of plants infected with *F. solani* also exhibits a reddish discoloration, primarily in the taproot and extending into the lower part of the stem. Like other strains of *F. solani*, this fungus is a cortex-rotting pathogen, and severely infected soybean plants exhibit blackened and rotted taproots with few remaining lateral roots.

Research was supported in part by the Illinois Soybean Operating Board and the Illinois Crop Improvement Association.

Accepted for publication 26 October 1992.

F. solani isolates that cause SDS symptoms grow slowly on potato-dextrose agar (PDA) and produce blue, slimy masses with large numbers of macroconidia and few if any microconidia. Little aerial mycelium develops. In addition, the PDA may stain a dark maroon (14).

Differences in susceptibility to SDS have been observed among soybean lines in the field; however, environmental variation makes field evaluation difficult (7,15). In a previous study, we used microplots to evaluate the reaction of 12 soybean cultivars to natural and inoculated infestations of *F. solani* (17). Leaf symptom severity for soybean cultivars in the inoculated treatments was not significantly different from that in the naturally infested treatments. We concluded that inoculation with *F. solani*-infested oats was a reliable alternative to the use of naturally infested soil when soybean cultivars are evaluated in the field for reaction to *F. solani*. We also concluded that under field conditions the most consistent measure of cultivar reaction to *F. solani* was symptom severity scored at the R6 growth stage (5).

SDS symptoms have been produced on soybean plants inoculated with *F. solani* in the greenhouse (9–11,13,14); however, no information is available on the relationship between greenhouse and field reactions of soybean cultivars to *F. solani*. Our first objective in this study was to evaluate various greenhouse inoculation methods and to develop the greenhouse technique that best reproduced the cultivar reactions to *F. solani* previously observed in the field. Our second objective was to compare the reactions of 12 soybean cultivars to *F. solani* in the greenhouse with the reactions in infested microplots and field locations. A third objective was to evaluate the effect of SDS severity on the agronomic performance of the 12 cultivars at infested field locations in central and southern Illinois.

MATERIALS AND METHODS

We selected 12 soybean cultivars from a range of SDS-resistant to SDS-susceptible cultivars on the basis of previous field observations (17). These cultivars also ranged in maturity from mid-maturity group III to late-maturity group IV.

We define resistance to SDS as a delay in or lack of leaf symptom expression after an incubation period that causes advanced expression of symptoms in susceptible infected genotypes. Cultivars Bass (BSR 301 × Essex), Williams (2), Hamilton (12), Ripley (3), TN4-86 (1), and the experimental line D83-3349 (Bedford × [Forrest × (Peking × Centennial)]) made up the resistant class. The susceptible class consisted of cultivars Asgrow A3427 (Asgrow X3826 × Asgrow A3127), Pioneer P3981 (Williams × Cutler 71), Spencer (18), Pioneer P9461 ([Pioneer X351-29 × Asgrow A4268] × [Pioneer P9401 × Asgrow A3127]), Asgrow A4595 (Douglas × Asgrow A3127), and Asgrow A4715 (Asgrow A5474 × Asgrow A4595). Asgrow A4715, TN4-86, and D83-3349 were resistant to races 3 and 4 of *Heterodera glycines* (6).

To evaluate SDS reactions of the 12 cultivars under field conditions, we conducted studies in *F. solani*-infested microplots and at three infested field sites in 1990 and 1991. Microplots were established at the University of Illinois Agronomy and Plant-Pathology Farm in Urbana (17). The four microplot treatments were as follows: noninfested control (C), same soil as control but plants inoculated with *F. solani* isolate 269 (I), infested soil from Villa Ridge, IL (V), and infested soil from Stonington, IL (S). The results of these microplot experiments have been presented (17).

Field experiments were conducted at *F. solani*-infested sites at Stonington in central Illinois (1990–1991) and at Villa Ridge (1990) and Pulaski (1991) in southern Illinois. Cultivars were planted

at Stonington on 30 May 1990 and 5 May 1991, at Villa Ridge on 5 June 1990, and at Pulaski on 30 May 1991. Cultivars were planted in four-row plots in a randomized complete block design. Plots were 3 m long, with a 76-cm spacing between rows. The two center rows were harvested, and seed yield was adjusted for 13% moisture. Seven traits were evaluated: yield (kg/ha), plant height at harvest (cm), harvest maturity (date when at least 95% of the plants had mature brown pod color), lodging (scored on a scale from 1 = all plants erect to 5 = all plants prostrate), seed weight (cg), seed quality (visual appearance as affected by discolored or wrinkled seed, rated on a scale from 1 = good to 5 = poor), and SDS disease severity at the R6 growth stage. Disease severity was rated on the Horsfall-Barratt scale (8), where 1 = no affected leaf area, 2 = 3% of leaf area affected, 3 = 6%, 4 = 12%, 5 = 25%, 6 = 50%, 7 = 75%, 8 = 88%, 9 = 94%, and 10 = 97% of leaf area affected. In the analysis of disease severity, location and cultivar were treated as fixed effects. Mean values were calculated for the agronomic traits, and the correlation with disease severity was determined for each location.

Cultivars also were evaluated in the greenhouse during the winter of 1990–1991. Plants in the greenhouse were inoculated with *F. solani* by the infested oat technique described by Lim (10). Oats were soaked in tap water overnight, then excess water was strained away. Approximately 150-cm^3 portions of the water-soaked oats were placed in 250-cm^3 Erlenmeyer flasks, and the flasks were capped and autoclaved for 40 min. After the oats had cooled, two 1-cm^2 agar plugs from the colony borders of *F. solani* plates were added to the sterilized oats. Inoculated oats were incubated in the dark at room temperature (24 C) for 3 wk before soybean inoculation. Flasks were shaken periodically to assure uniform fungal growth.

Isolates that corresponded to the microplot treatments were used to evaluate SDS reactions in the greenhouse. These isolates were 269 (obtained from J. C. Rupe, University of Arkansas), VR308, and ST90. VR308 and ST90 were isolated from the taproots of the soybean cultivar Spencer. Plants of Spencer exhibiting typical SDS symptoms were collected in the summer of 1990 from the field sites at Villa Ridge and Stonington. To isolate fungi, taproots were lightly scrubbed in 1 L of tap water to which several drops of commercial dishwater detergent had been added. Scrubbed root sections were then placed in a 250-ml beaker containing 6% household bleach and two drops of Tween 20. Root sections were stirred constantly for 3 min, then rinsed with sterile water and blotted on sterile paper towels. Thin cortical sections were sliced from the taproot with a scalpel and placed onto one-third-strength Difco PDA. This agar was prepared by adding 50-ppm tetracycline and 18 drops of Tergitol NP-10 (Sigma Chemical Co., St. Louis, MO) per liter to the cooled liquid just before it was poured into petri dishes. After 7 days at 24 C, *F. solani* colonies could be identified by their blue color (14) and morphology as described by Domsch et al (4). Isolates were maintained in the laboratory on one-third-strength PDA plus tetracycline. The isolate designated VR308 was collected from the Villa Ridge location, and ST90 was collected from the Stonington site. Periodically, isolates were reisolated from Spencer seedlings grown and inoculated in the greenhouse.

For greenhouse evaluation, seeds were germinated in sand in 10-cm-diam plastic pots. Seedlings at the late unifoliolate stage were removed, their roots were washed with water, and four uniform seedlings were selected. Seedlings were transplanted into 15-cm steamed clay pots containing 1:1 sand-soil mix, watered, and inoculated with *F. solani* within 48 hr. At the V1 growth stage, greenhouse plants were inoculated with three oat grains infested with *F. solani* and placed next to the taproot, 1–2 cm below the soil surface. Care was taken not to wound the taproot during inoculation. Plants were grown with a 14-hr photoperiod at 18–27 C, and each pot received 150 ml of water twice daily. Pots were fertilized weekly beginning on the day of inoculation with 150 ml of a solution that provided 98 mg of N, 89 mg of P_2O_5, 85 mg of K_2O, 0.12 mg of chelated Cu, Mn, and Zn, 0.05 mg of B, and 0.24 mg of chelated Fe per liter.

Inoculum for microplot treatment I was prepared in the same way as for the greenhouse test. However, plants in microplot treatment I were inoculated with about 15 infested oat grains per plant rather than three and were inoculated at the V7-9 growth stage.

SDS symptoms in the greenhouse were assessed as the percentage of chlorosis and necrosis (severity) on a pot basis every 3 days after symptom appearance and, as in the field experiments, were scored on the Horsfall-Barratt scale. Each greenhouse experiment included two replications, one isolate, and 12 cultivars, for a total of 24 pots. Experiments were repeated three times for each isolate. SDS symptom severity was analyzed with a randomized complete block design combined over experiments. In the analysis, isolates and cultivars were treated as fixed effects, and experiments were considered random. The relationship between microplot and field SDS symptom severity at R6 and greenhouse symptom severity was determined by correlation (r) analysis.

Table 1. Sudden death syndrome severity scores[a] for 12 soybean cultivars evaluated in microplots and in the greenhouse

	Isolate of *Fusarium solani*			Microplot treatment[b]			Field location		
Cultivar	269	VR308	ST90	I	V	S	Pulaski	Villa Ridge	Stonington
Asgrow A4715	6.7	4.5	6.3	5.8	1.4	7.6	5.8	3.0	5.3
Spencer	6.8	7.3	7.8	7.0	4.8	6.8	4.3	4.8	3.5
Pioneer P3981	5.0	5.3	8.2	4.4	3.4	6.1	4.0	5.5	3.4
Asgrow A3427	6.0	7.3	8.0	6.1	2.9	6.3	3.3	3.8	2.4
Asgrow A4595	6.2	5.5	6.8	6.0	3.6	5.5	3.7	4.7	2.4
Pioneer P9461	6.5	5.8	6.5	5.3	3.5	4.4	4.0	4.2	2.8
Williams	2.5	2.2	4.3	3.8	1.7	3.5	1.7	2.0	1.8
Ripley	2.7	3.0	3.8	1.3	1.0	1.2	1.3	1.2	1.0
Hamilton	3.7	3.5	4.2	1.8	1.0	1.8	1.0	1.5	1.0
D83-3349	3.0	2.2	2.8	1.0	1.0	1.4	1.0	2.8	1.0
Bass	3.2	2.7	4.3	1.9	1.1	1.4	2.0	3.2	1.1
TN4-86	2.7	2.8	4.5	1.5	1.0	2.0	1.0	2.7	1.0
Mean	4.6	4.3	5.6	3.8	2.2	4.0	2.8	3.3	2.2
LSD (0.05)[c]		1.7			3.1			1.1	
LSD (0.05)[d]		NS			2.4			2.4	

[a]Leaf severity rated on Horsfall-Barratt scale. Greenhouse severity was rated about 3 wk after inoculation; severity in microplots and at field locations was rated at the R6 growth stage.
[b]I = oat-inoculated, V = Villa Ridge, S = Stonington.
[c]Least significant difference for cultivar within isolate, treatment, and location, respectively.
[d]Least significant difference for cultivar × isolate, treatment, and location, respectively. NS = not significant.

RESULTS AND DISCUSSION

Light leaf chlorosis could usually be recognized on greenhouse-inoculated seedlings 10 days after inoculation. Symptom severity was rated every 3 days after initial symptom appearance. The severity value used in the analysis of variance (ANOVA) was that for the rating date when the difference between the susceptible cultivar Spencer and the resistant cultivar Ripley in leaf symptom severity was maximum. This time was usually at about 3 wk; however, temperature affected the rate of symptom development in the greenhouse. Greenhouse temperatures below 21 C slowed symptom development.

Chlorosis and necrosis caused by *F.*

Table 2. Analysis of variance mean squares and F tests for 12 soybean cultivars evaluated in the greenhouse for reaction to *Fusarium solani*

Source of variation	df	Severity[a] (mean square)	F test[b]
Experiment (E)	2	2.7	NS
Isolate (I)	2	25.6	NS
I × E	4	9.0	**
Error a	9	1.8	
Cultivar (C)	11	60.0	**
E × C	22	2.0	NS
I × C	22	2.7	NS
I × E × C	44	2.3	NS
Error b	99	1.7	

[a]Scored as affected leaf area about 3 wk after inoculation.
[b]Significant at $P < 0.01$ (**) or not significant (NS).

Table 3. Analysis of variance mean squares and F tests for 12 soybean cultivars evaluated at four locations infested with *Fusarium solani*

Source of variation	df	Severity[a] (mean square)	F test[b]
Locations	3	12.0	NS
Blocks within locations	8	5.7	
Cultivars	11	19.1	**
Location × cultivars	33	1.8	*
Pooled error	88	1.0	

[a]Scored at the R6 growth stage.
[b]Significant at $P < 0.05$ (*) or $P < 0.01$ (**) or not significant (NS).

solani in the field were similar to greenhouse symptoms; however, greenhouse-inoculated seedlings did not always go through the mosaic leaf stage before becoming chlorotic. Mosaic leaf symptoms were rare on *F. solani*-susceptible cultivars but common on resistant cultivars. Leaves of susceptible cultivars such as Spencer developed characteristic interveinal chlorosis that progressed rapidly to interveinal necrosis. Resistant cultivars such as Ripley developed mosaic leaf symptoms and some chlorosis, but chlorosis developed more slowly than for the susceptible cultivars.

Severity scores were higher in the greenhouse than in the field for the resistant cultivars (Table 1). The greater severity of symptoms in the greenhouse could be the result of several factors, such as the earlier inoculation time in the greenhouse (V1) compared to the field (V7-9), reduced fungal competition for *F. solani* in greenhouse-steamed soil, or the restricted rooting volume for cultivars growing in clay pots, which concentrated the soybean roots in the zone of inoculation.

When cultivars were ranked by symptom severity, no significant differences among isolates could be found. Therefore, data for the three isolates and experiments were combined for analysis. In the combined ANOVA for the greenhouse study, F tests for cultivar and isolate × experiment were highly significant (Table 2). The isolate × experiment interaction resulted from cool temperatures that reduced symptom severity during one experiment involving isolate 269. The ANOVA for the infested field plots also indicated highly significant differences among cultivars for disease severity (Table 3). When the mean greenhouse severity values for each cultivar and isolate were correlated with microplot and infested-field R6 severity values (Table 1), a highly significant ($P < 0.01$) relationship was found (Table 4). Greenhouse evaluation with the oat inoculation technique accurately predicted cultivar reaction to *F. solani* in the field.

Isolates evaluated in the greenhouse did not differ (Table 2). A cultivar × treatment interaction found in the microplot studies (17) suggested that *F. solani* populations in treatments I and S were different from those in treatment V. The cultivar Asgrow A4715 was susceptible in treatments I and S but resistant in treatment V. Apparently, isolate VR308 does not possess the same genotype that was found in treatment V. VR308 was isolated from the field site at Villa Ridge. Our ability to find the same interactions in greenhouse evaluations as in the microplots would have improved if the greenhouse isolates had been collected from the microplots. Disease scores for the infested field sites (Table 1) indicated that the reaction for Asgrow A4715 contributed to the significant cultivar × location interaction and followed the same pattern as observed in the microplots. This further supports the hypothesis of differences in *F. solani* populations.

No relationship could be found between SDS disease severity at the infested field locations and any of the agronomic traits measured. Hershman et al (7) also found no correlation between yield or plant height and SDS. The finding that SDS did not affect yield was surprising because the disease in the susceptible

Table 4. Correlation coefficients (r)[a] between disease severity in microplots and field locations at R6 and greenhouse severity at about 3 wk after inoculation with *Fusarium solani*

Microplot treatment[b] or field location	Greenhouse isolate 269	VR308	ST90
I	0.91**	0.87**	0.90**
V	0.80**	0.88**	0.86**
S	0.90**	0.80**	0.90**
Pulaski	0.91**	0.72**	0.83**
Villa Ridge	0.71**	0.73**	0.78**
Stonington	0.81**	0.60*	0.73**

[a]Significant at $P < 0.01$ (**) or $P < 0.05$ (*).
[b]I = oat-inoculated with isolate 269; V = Villa Ridge, naturally infested soil; S = Stonington, naturally infested soil.

plots was obvious. Symptoms did not appear at Villa Ridge, the most severely diseased field site, until R5 and at the other three locations until R6. The fact that SDS did not significantly reduce yield in this study was probably the result of late symptom appearance. Early planting date and wet weather have been associated with increased occurrence of SDS (7). Excessive soil moisture delayed planting at the southern Illinois sites in both years. In addition, drought affected the Stonington site in both years and the Pulaski site in 1991. The expression of cultivar differences in reaction to *F. solani* was optimized in the microplots compared to the infested-field studies in that microplots were planted early (27 and 29 April) and were irrigated. In the microplots, initial SDS symptoms appeared at R1. Because the microplot study was not designed to measure yield, yield data were not collected, but we observed that plants of the cultivars susceptible to *F. solani,* such as Spencer and Asgrow A4715, had very few seed, whereas seed set appeared to be unaffected in resistant cultivars, such as Ripley and Hamilton. It appears that for SDS to affect yield, it must be severe early in plant development (before R5). Timing of symptom appearance will be an important factor in associating yield loss with SDS occurrence.

The greenhouse oat inoculation technique described here produced SDS symptoms on soybean seedlings that appeared similar to field symptoms. Data for 12 soybean cultivars indicate that greenhouse leaf symptom severity about 3 wk after inoculation is a good predictor of field reaction to *F. solani.* Greenhouse inoculation would be a useful tool for soybean breeders in the initial evaluation of soybean lines for resistance to *F. solani.*

ACKNOWLEDGMENTS

We thank Paul Gibson, Southern Illinois University, Carbondale, for providing the Villa Ridge and Pulaski locations.

LITERATURE CITED

1. Allen, F. L., Panter, D. M., and Young, L. D.

1988. Registration of 'TN4-86' soybean. Crop Sci. 28:1026.
2. Bernard, R. L., and Lindahl, D. A. 1972. Registration of 'Williams' soybean. Crop Sci. 12:716.
3. Cooper, R. L., Martin, R. J., McBlain, B. A., Fioritto, R. J., St. Martin, S. K., Calip-DuBois, A., and Schmitthenner, A. F. 1990. Registration of 'Ripley' soybean. Crop Sci. 30:963.
4. Domsch, K., Gams, W., and Anderson, T.-H. 1980. Compendium of Soil Fungi. Academic Press, New York.
5. Fehr, W. R., Caviness, C. E., Burmood, D. T., and Pennington, J. S. 1971. Stage of development descriptions for soybeans, *Glycine max* (L.) Merr. Crop Sci. 11:929-931.
6. Golden, A. M., Epps, J. M., Riggs, R. D., Duclos, L. A., Fox, J. A., and Bernard, R. L. 1970. Terminology and identity of infra-specific forms of the soybean cyst nematode (*Heterodera glycines*). Plant Dis. Rep. 54:544-546.
7. Hershman, D. E., Hendrix, J. W., Stuckey, R. E., Bachi, P. R., and Henson, G. 1990. Influence of planting date and cultivar on soybean sudden death syndrome in Kentucky. Plant Dis. 74:761-766.
8. Horsfall, J. G., and Barratt, R. W. 1945. An improved grading system for measuring plant diseases. (Abstr.) Phytopathology 35:655.
9. Killebrew, J. F., Roy, K. W., Lawrence, G. W., McLean, K. S., and Hodges, H. H. 1988. Greenhouse and field evaluation of *Fusarium solani* pathogenicity to soybean seedlings. Plant Dis. 72:1067-1070.
10. Lim, S. M. 1991. A technique for inoculating soybeans in the greenhouse with *Fusarium solani.* (Abstr.) Phytopathology 81:1238.
11. Lim, S. M., and Jin, H. 1991. Pathogenic variability in *Fusarium solani* isolated from soybeans with sudden death syndrome symptoms. (Abstr.) Phytopathology 81:1236.
12. Nickell, C. D., Thomas, D. J., and Stephens, P. 1990. Registration of 'Hamilton' soybean. Crop Sci. 30:1364.
13. Roy, K. W., Lawrence, G. W., Hodges, H. H., McLean, K. S., and Killebrew, J. F. 1989. Sudden death syndrome of soybean: *Fusarium solani* as incitant and relation of *Heterodera glycines* to disease severity. Phytopathology 79:191-197.
14. Rupe, J. C. 1989. Frequency and pathogenicity of *Fusarium solani* recovered from soybeans with sudden death syndrome. Plant Dis. 73:581-584.
15. Rupe, J. C., Gbur, E. E., and Marx, D. M. 1991. Cultivar responses to sudden death syndrome of soybean. Plant Dis. 75:47-50.
16. Sebastian, S. A., and Nickell, C. D. 1985. Inheritance of brown stem rot resistance in soybeans. J. Hered. 76:194-198.
17. Stephens, P. A., Nickell, C. D., and Lim, S. M. 1993. Sudden death syndrome development in soybean cultivars differing in resistance to *Fusarium solani.* Crop Sci. 33:63-66.
18. Wilcox, J. R., Roach, M. T., and Abney, T. S. 1989. Registration of 'Spencer' soybean. Crop Sci. 29:830-831.