Soybean Sudden Death Syndrome: Cultivar Reactions to Inoculation in a Controlled Environment and Host Range and Virulence of Causal Agent

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ABSTRACT

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Reactions of 27 soybean cultivars to inoculation with *Fusarium solani* form A, the cause of sudden death syndrome, were determined under growth chamber conditions. Significant differences in susceptibility occurred among the cultivars. Selected comparisons between cultivar reactions in the growth chamber and in the field indicated close correspondence, but some inconsistencies occurred. Of 21 nonwounded and wounded plant species inoculated in a host range test, the only nonwounded species developing symptoms were soybean and mung bean; green bean, lima bean, and cowpea became infected only after wound inoculation. In virulence tests, some of the 31 isolates tested differed; but most, 24, were equally virulent. Virulence was not related to substrate or geographic origin.

Sudden death syndrome (SDS) of soybean (*Glycine max* (L.) Merr.), a relatively new root and lower stem disease, occurs in Alabama (K. W. Roy, *unpublished*), Arkansas, Illinois, Indiana, Kentucky, Mississippi, Missouri, and Tennessee (4,5,7,9,12–14,17,18,20). It is especially prevalent in irrigated fields or in fields with high soil moisture (5,7, 12–14) and is often associated with high soil populations of the soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe (5,7,12,14,18). The major symptoms of SDS include root rot, crown necrosis, vascular discoloration of roots and stems, interveinal chlorosis and necrosis of leaves, defoliation, and pod abortion. Leaf symptoms are the most conspicuous phase of this disease (4,5,7, 9,12–14,17,18,20).

Progress has been slow in identifying cultivars that consistently express either resistant or susceptible reactions to SDS in the field. Although several reports (4,5,7,16,18–20) indicated specific levels of SDS susceptibility for different cultivars, there are conflicting results for many of these cultivars, the results depending on the year and/or location. A further complication which SDS researchers have generally experienced is that the disease may be present in a given field one year and be totally absent or too mild in one or more succeeding years to allow for screening. Apparently, occurrence of SDS and disease severity are greatly influenced by weather (5,6,7,10) and other factors. These problems led to speculation that environmentally controlled screening tests might be more effective and expeditious in identifying resistant or susceptible cultivars than screening in the field (3).

Roy et al (12) provided evidence that inoculation of seedling hypocotyls with a form of *Fusarium solani* (Mart.) Appel & Wollenweb. emend. W.C. Snyder & H.N. Hans., distinguished as form A (FSA), could be used to determine cultivar reaction to SDS. Further study (10) indicated that inoculation of the seedlings at 7–14 days of age and incubation at ca 27 C were optimal for obtaining infection of soybean seedlings with FSA and for symptom expression under growth chamber conditions.

After the completion of Koch's postulates, it was concluded that FSA was the cause of SDS (12–14), and confirmation soon followed (17). These findings are relatively recent, and consequently there is limited information regarding the host range (2) and virulence of FSA (17). Gray (2), apparently using Lim's inoculation technique (9), which entails wounding, found that soybean, mung bean, and green bean were susceptible to a strain of *F. solani* recovered from SDS-symptomatic soybean plants. He did not mention whether other plant species were inoculated. Rupe (17) observed differences in virulence among a limited number of FSA isolates from roots of SDS-symptomatic plants. Although isolates of FSA have been recovered from SCN cysts associated with the roots of symptomatic plants (11,15), the virulence of such isolates has not been determined.

Our objectives were to determine 1) reactions of selected soybean cultivars to SDS under growth chamber conditions, 2) the host range of FSA, and 3) the virulence of FSA isolates from soybean roots and SCN cysts.

MATERIALS AND METHODS

Reactions of soybean cultivars to inoculation. FSA, isolate 90-1, was recovered from surface-disinfested roots of SDS-symptomatic soybean plants at the Mississippi State University Plant Science Research Center. The inoculation technique used was a modification of the toothpick method (1). Excised tips (ca 1 cm long) of round toothpicks were soaked in nutrient broth and autoclaved twice for 20 min on two separate days. The toothpick tips then were aseptically placed at the periphery of 5-day-old colonies of FSA growing on potato-dextrose agar containing streptomycin B sulfate (100 mg/L) and aureomycin (20 mg/L).

Twenty-seven soybean cultivars from maturity groups (MG) V to VII, including selected standard (check) susceptible or resistant cultivars (Table 1) were tested. The standard cultivars were selected based on cumulative evidence from our field tests and observations (J. Melgar and K. W. Roy, *unpublished*) and from those of other workers (7,18; E. A. Drummond, M. C. Hirrel, and G. Howard, *personal communications*). Asgrow 5403, Deltapine 105, Essex, Terra-Vig 505 (MG V), Terra-Vig 616 (MG VI), Braxton, and Coker 82M-170 (MG VII) were considered susceptible; Bedford (MG V), Deltapine 3623, Hartz 6200, and Leflore (MG VI) were considered resistant. Cultivars were grown in 10-cm-diameter plastic pots in sterile soil (50% soil, 50% sand, v/v) in a controlled-environment growth chamber at a temperature of 27 C and a 13-hr photoperiod (light intensity = 200 $\mu E \cdot m^{-2} \cdot s^{-1}$). Prior to planting, seeds of each cultivar were surface disinfested for 1 min in 1% NaOCl, plated on potato-dextrose agar, and incubated at 24 C for 72 hr. Seven germinated seeds free of microorganisms were planted in each of three replicate pots per cultivar. One week after planting, infested toothpick tips were inserted into seedling hypocotyls at a point midway between the soil line and the cotyledons. The wounds created were covered with petrolatum to prevent contamination and desiccation of inoculum. Control seedlings received noninfested tooth-

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pick tips. Pots were arranged in a completely randomized design; plants were irrigated daily to maintain a high level of soil moisture; and lesion development and plant death were monitored. After 2 wk, plants were harvested and rated for disease severity. Our preliminary tests had shown that it was not feasible to base severity ratings on foliar symptoms, because many seedlings died before such symptoms are normally expressed. Therefore, severity ratings were based on lesion length and plant death, using a 0–9 scale, where 0 = no lesion, 1 = lesion < 0.5 cm, 2 = lesion 0.5–1.0 cm, 3 = lesion 1.0–1.5 cm, 4 = lesion 1.5–2.0 cm, 5 = lesion 2.0–2.5 cm, 6 = lesion 2.5–3.0 cm, 7 = lesion > 3.0 cm, 8 = plant dying, and 9 = plant dead. This experiment was conducted three times. Data were subjected to analysis of variance and means were separated with the LSD test (21).

Table 1. Reactions of soybean cultivars to inoculation with *Fusarium solani* form A in growth chamber tests

Cultivar[y]	Maturity group	Disease severity rating[z]
Terra-Vig 616 (S)	VI	8.6 a
Coker 82M-170 (S)	VII	8.5 ab
Terra-Vig 505 (S)	V	8.1 ab
Asgrow 5403 (S)	V	7.8 abc
Braxton (S)	VII	7.8 abc
Ringaround 606	VI	7.7 abc
Deltapine 105 (S)	V	7.7 abc
Tracy-M	VI	7.6 abc
Hartz 6130	VI	7.6 abc
Terra-Vig 5452	V	7.6 abc
Asgrow 6520	VI	7.5 abc
Asgrow 5474	V	7.5 abc
Cordell	V	7.5 abc
Coker 237	VII	7.4 abc
Northrup King S64-23	VI	7.4 abc
Asgrow 6297	VI	7.2 abcd
Riverside 696	VI	7.2 abcd
Terra-Vig 515	V	7.2 abcd
Deltapine X3595	V	7.2 abcd
Deltapine 506	VI	7.1 abcd
Coker 485	V	6.9 abcd
Essex (S)	V	6.9 abcd
Bedford (R)	V	6.8 bcd
Asgrow 6785	VI	6.8 bcd
Leflore (R)	VI	6.2 cd
Hartz 6200 (R)	VI	6.1 cd
Deltapine 3623 (R)	VI	5.6 d

[y] Considered a standard (check) susceptible (S) or resistant (R) cultivar based on previous field observations and tests (7,18; J. Melgar and K. W. Roy, *unpublished*; E. A. Drummond, M. C. Hirrell, and G. Howard, *personal communications*).

[z] All means are derived from three separate tests, with each test considered a replication in the ANOVA. Means followed by the same letter are not significantly different ($P \leq 0.05$) according to the LSD test. Disease severity was rated according to a 0–9 scale, where 0 = no lesion, 1 = lesion < 0.5 cm long, 2 = lesion 0.5–1.0 cm long, 3 = lesion 1.0–1.5 cm long, 4 = lesion 1.5–2.0 cm long, 5 = lesion 2.0–2.5 cm long, 6 = lesion 2.5–3.0 cm long, 7 = lesion > 3.0 cm long, 8 = plant dying, and 9 = plant dead.

Selected MG V and VI cultivars that produced reactions inconsistent with field reactions (18; J. Melgar and K. W. Roy, *unpublished*) were retested using the same methods and statistical analysis described above. For comparisons of relative susceptibility, selected cultivars exhibiting the least susceptibility and the most susceptibility in the main test (Table 1) were included.

Host range. Using Lim's technique (9), FSA (isolate 90-1) inoculum was increased on oat seeds. The seeds (100 ml) were soaked in tap water for 3 hr, then autoclaved twice for 40 min each time. Seeds were inoculated with 5 ml of a macroconidial suspension (10^6 conidia per milliliter), and the cultures were incubated for 3 wk at 24 C.

Twenty-one plant species (Table 2) representing six families were included in the test. The soybean cultivar used, Deltapine 105, is susceptible to SDS (4,5,7,16,18). Seeds of each species were surface disinfested and germinated for 72 hr as described previously. Ten germinated seeds free of microorganisms were planted in sterile soil in 15-cm-diameter plastic pots in the greenhouse. Two pots of each species were planted. Three weeks after planting, the epidermal layer around the root-hypocotyl transition zone of five of the plants in each pot was gently scraped before inoculation. None of the other five plants in each pot were wounded. Three infested oat seeds were then placed against, and evenly spaced around, the plants of each species. The pots of wounded and nonwounded control plants were treated with noninfested oat seeds.

In addition to the main host range test, 10-cm-diameter pots of sterile soil containing 10 plants each of the closely related species soybean, mung bean, green bean, lima bean, and cowpea were inoculated with infested toothpick tips as described previously. Another set of 10 plants per species received noninfested toothpick tips and served as the control.

Following inoculation, pots were arranged in a randomized complete-block design and observed for symptoms. Temperatures in the greenhouse ranged from 16 to 34 C. This experiment was conducted three times.

Virulence. Cultivar Deltapine 105 was grown in 10-cm-diameter plastic pots in sterile soil in a controlled-environment growth chamber under the conditions described previously. Seven plants were grown in each of four replicate pots. The 31 isolates of FSA tested were recovered from roots of SDS-symptomatic plants or from cysts of SCN (Table 3). Prior to inoculation, the isolates had been maintained in a 10% glycerol solution stored in liquid nitrogen.

Seedlings were inoculated 1 wk after planting using the modified toothpick method described above. Plants treated with noninfested toothpick tips served as controls. Pots were arranged in a completely randomized design; plants were irrigated daily; and lesion development and plant death were recorded 2 wk after inoculation. Disease severity was rated as described above. This experiment was conducted three times. Data were subjected to analysis of variance, and means were separated with the LSD test.

RESULTS AND DISCUSSION

Reactions of soybean cultivars to inoculation. Disease ratings ranged from 5.6 to 8.6 (Table 1). Deltapine 3623 was significantly less susceptible than the cultivars with the 15 highest ratings. Deltapine 3623, Hartz 6200, and Leflore, which had the lowest ratings, were significantly less susceptible than Terra-Vig 616, Coker 82M-170, and Terra-Vig 505, which had the highest ratings. Bedford, Asgrow 6785, Leflore, Hartz 6200, and Deltapine 3623 were significantly less susceptible than Terra-Vig 616. There were no significant differences in ratings among the three least susceptible cultivars, nor among the three most susceptible.

Since this is the first report of cultivar reactions to SDS under growth chamber conditions, results can only be compared with those based on previous field experiments or observations. Based on relative susceptibility, reactions of cultivars in the growth chamber, including those of standard susceptible or resistant cultivars (Table 1), generally agreed with results of our field observations and tests in which cultivars were classified as susceptible, resistant, or intermediate (moderately susceptible/moderately resistant) in their reaction to SDS (J. Melgar and K. W. Roy, *unpublished*). In our field tests involving MG V cultivars, Terra-Vig 505, Asgrow 5403, and Deltapine 105 were rated susceptible; Bedford was rated resistant; and Terra-Vig 5452, Cordell, Terra-Vig 515, Deltapine X3595, and Coker 485 were rated intermediate. In those involving MG VI cultivars, Terra-Vig 616 was rated susceptible; Asgrow 6785, Leflore, Hartz 6200, and Deltapine 3623 were rated resistant; and Northrup King S64-23, Asgrow 6297, and Riverside 696 were rated intermediate. In tests involving MG VII cultivars, Coker 82M-170 and Braxton were rated susceptible, and Coker 237 was rated intermediate. In addition to these consistencies, reactions in the growth chamber also were consistent with susceptibility classifications that other workers recorded in the field for Terra-Vig 505, Terra-Vig 515, Terra-Vig 616 (E. A. Drummond, *personal communication*), Deltapine 105, Bedford (7,18), Leflore (18), Asgrow 5403, Hartz 6200 (M. C. Hirrel, *personal communication*), Coker 82M-170 (G. Howard, *personal communication*), and Braxton (18).

There were some inconsistencies between the growth chamber ratings and

ratings that have been obtained in the field. With respect to MG V, Rupe et al (18) obtained low SDS severity ratings for Asgrow 5474 and high ratings for Essex. With respect to MG VI, they obtained low ratings for Ringaround 606 and Hartz 6130, and high ratings for Tracy-M and Deltapine 506. These ratings and a rating of susceptible that we assigned Essex and Tracy-M in the field (J. Melgar and K. W. Roy, *unpublished*) disagreed with the growth chamber ratings (Table 1). In our repeat trials comparing reactions of Asgrow 5474 and Essex with standard susceptible and resistant MG V cultivars, ratings for Terra-Vig 505, Asgrow 5474, Essex, and Bedford were 9.0, 8.1, 8.4, and 6.2, respectively. The ratings for both Asgrow 5474 and Essex were significantly different ($P \leq 0.05$) from the two standards, whereas the difference between them was insignificant. In repeat trials comparing reactions of Ringaround 606, Hartz 6130, and Deltapine 506 with standard susceptible and resistant MG VI cultivars, ratings for Terra-Vig 616, Ringaround 606, Tracy-M, Hartz 6130, Deltapine 506, and Deltapine 3623 were 8.8, 7.9, 7.9, 7.5, 7.4, and 4.7, respectively. The ratings for Ringaround 606, Tracy-M, Hartz 6130, and Deltapine 506 were significantly different ($P \leq 0.05$) from the two standards, whereas differences among the four cultivars were insignificant. Thus, standard susceptible and resistant MG V and VI cultivars produced anticipated reactions; Asgrow 5474, Ringaround 606, Tracy-M, Hartz 6130, and Deltapine 506 produced reactions consistent with the main test (Table 1); but Essex produced a higher rating than that produced in the main test.

It should be noted that Essex and other cultivars included in the present study have produced variable reactions to SDS in the field. The reactions Rupe et al (18) reported for Essex, Asgrow 5474, Bedford, Tracy-M, and Leflore in a 3-yr study did not agree with their prior field ratings for these cultivars. Moreover, the susceptibility rankings of these five cultivars, Asgrow 6520, Ringaround 606, Hartz 6130, and Deltapine 506 varied from year to year. In fact, it is a common observation that reactions of cultivars to SDS in the field may vary depending on the year, location (5,18; J. Melgar and K. W. Roy, *unpublished*; P. T. Gibson, D. E. Hershman, and M. C. Hirrel, *personal communications*), and planting date (5). The reasons for this variability are unclear, but both abiotic and biotic factors have been implicated. Expression of symptoms is influenced by temperature (5,7,10) and soil moisture (5,7, 12,14). Since SCN may exacerbate foliar symptoms (12,14), its presence, distribution, or absence in fields affected by SDS may influence cultivar reaction. Moreover, data suggest that SCN may be a

Table 2. Plant species used in determining the host range of *Fusarium solani* form A

Family Scientific name	Common name	Cultivar tested
Fabaceae		
Vicia villosa Roth	Hairy vetch	Vantage
Phaseolus vulgaris L.	Garden bean	Derby
Glycine max (L.) Merr.	Soybean	Deltapine 105
Phaseolus lunatus L.	Lima bean	Willa Lima
Vigna unguiculata (L.) Walp.	Cowpea	Purple Hull
Medicago sativa L.	Alfalfa	Delta
Trifolium pratense L.	Red clover	Kenland
Vigna radiata (L.) R. Wilcz.	Mung bean	Berken
Poaceae		
Zea mays L.	Corn	Pioneer 3165
Sorghum bicolor (L.) Moench.	Grain sorghum	522 DR.
Cucurbitaceae		
Cucurbita pepo L.	Zucchini	Dark Green
Cucurbita pepo L.	Squash	White Bush Scallop
Cucumis sativus L.	Cucumber	Long Green Improved
Citrullus vulgaris Schrad.	Watermelon	Black Diamond
Cucurbita pepo L.	Pumpkin	Jack-o-Lantern
Cucumis melo L.	Cantaloupe	Rocky Ford
Solanaceae		
Capsicum frutescens L.	Pepper	California Wonder
Lycopersicon esculentum Mill.	Tomato	President
Malvaceae		
Gossypium hirsutum L.	Cotton	Deltapine 20
Abelmoschus esculentus (L.) Moench.	Okra	Clemson Spineless
Asteraceae		
Helianthus annuus L.	Sunflower	Striped Sunflower

Table 3. Virulence of 31 isolates of *Fusarium solani* form A (FSA) inoculated on seedlings of soybean cultivar Deltapine 105

Isolate code	Substrate origin	Geographic origin	Disease severity rating[z]
FSA-2	Root	Mississippi	9.0 a
LEE-2	Root	Arkansas	9.0 a
CLAY	Root	Arkansas	9.0 a
HOPKINS-1	Root	Kentucky	9.0 a
HOPKINS-2	Root	Kentucky	9.0 a
LEE-1	Root	Arkansas	9.0 a
ALA-2	Root	Alabama	8.9 a
POUNDS-FSA-N	SCN cyst	Tennessee	8.9 a
JO-1	Root	Illinois	8.8 a
OWENS-1	Root	Kentucky	8.8 a
POINSET-1	Root	Arkansas	8.8 a
POUNDS-1	Root	Tennessee	8.8 a
CROSS-1	Root	Arkansas	8.7 ab
NF-2	Root	Mississippi	8.7 ab
MK-FSA-N	SCN cyst	Kentucky	8.7 ab
90-1	Root	Mississippi	8.7 ab
HOPKINS-FSA-N	SCN cyst	Kentucky	8.7 ab
FSA-1	Root	Mississippi	8.6 abc
SCOTT-1	Root	Indiana	8.6 abc
MK-2	Root	Kentucky	8.6 abc
JW-1	Root	Illinois	8.6 abc
OWENS-2	Root	Kentucky	8.4 abc
POINSET-FSA-N	SCN cyst	Arkansas	8.2 abcd
90-2	Root	Mississippi	8.2 abcd
CLAY-FSA-N	SCN cyst	Arkansas	7.9 bcd
MK-1	Root	Kentucky	7.8 cd
CLARK-1	Root	Mississippi	7.5 de
GIBSON	Root	Illinois	6.9 ef
NF-1	Root	Mississippi	6.8 ef
JW-FSA-N	SCN cyst	Illinois	6.6 ef
NF-6	Root	Mississippi	6.5 f

[z] All means are averages of two tests with four replications per test. Error variances were tested for homogeneity before pooling data. Means followed by the same letter are not significantly different ($P \leq 0.05$) according to the LSD test. Disease severity was rated according to a 0–9 scale, where 0 = no lesion, 1 = lesion < 0.5 cm long, 2 = lesion 0.5–1.0 cm long, 3 = lesion 1.0–1.5 cm long, 4 = lesion 1.5–2.0 cm long, 5 = lesion 2.0–2.5 cm long, 6 = lesion 2.5–3.0 cm long, 7 = lesion > 3.0 cm long, 8 = plant dying, and 9 = plant dead.

predisposing factor with some, but not all, cultivars (5,18). Variability in reactions also could be due in part to genetic heterozygosity within cultivars. This is suggested in the present study by the variable reaction of Essex between the main test (Table 1) and the repeat trial, because this variability occurred even though the environment and growing conditions in the growth chamber, and the inoculum, were the same across tests. Heterozygosity would not be unexpected, because soybean cultivars presumably have not undergone repeated exposure to FSA and selection for resistance to SDS in the field. Variability of reactions within cultivars has been observed for stem canker of soybean (8).

The variability of cultivar reactions in the field suggested that the use of environmentally controlled screening tests might be more effective in identifying resistant or susceptible cultivars (3). Our results indicate that the growth chamber method of screening is a valid alternative to field screening. At the very least, it could serve as an adjunct to field screening or a means of prescreening and selecting cultivars and lines for field screening. Although there were some discrepancies between cultivar reactions in the chamber and those in the field, it can be argued that reactions obtained in the chamber are more indicative of genetic differences among cultivars, because unlike many field situations, the impact of factors potentially capable of modifying reactions were either not present (SCN) or minimized (temperature and soil moisture) in the growth chamber.

Host range. Of the 21 species tested using the oat seed inoculation technique (Table 2), nonwounded soybean and mung bean plants developed dark brown root and lower stem lesions, then foliar symptoms. Many plants died within 2–3 wk after inoculation. The root necrosis extended 5–7.5 cm above the soil line. Leaf symptoms on soybean consisted of interveinal chlorosis and necrosis, whereas those on mung bean consisted of a general chlorosis. Defoliation occurred on plants from both species but was more severe on soybean plants. Both soybean and mung bean were stunted. None of the other species became infected, even when they were wounded prior to inoculation.

The susceptibility of mung bean to FSA agrees with Gray's report, notwithstanding that he presumably wounded plants prior to inoculation (2). Gray also reported the development of symptoms on green bean. However, in the present study, since neither nonwounded nor wounded inoculated green bean plants produced symptoms, we cannot confirm his results with the oat seed inoculation technique. Possibly the strain of *F. solani* Gray used for the inoculations differs from FSA.

Each of the five species inoculated using the modified toothpick method, i.e., soybean, mung bean, green bean, lima bean, and cowpea, developed brown to dark brown cortical lesions extending up and down the stem from the point of inoculation. Soybean, mung bean, and green bean, the most affected, produced foliar symptoms within 2 wk after inoculation; and many of the soybean and mung bean plants and some of the green bean plants died within 4 wk after inoculation. Some soybean, mung bean, and green bean plants died before producing foliar symptoms. Leaf symptoms on soybean were typical for SDS and included interveinal chlorosis, interveinal necrosis, and defoliation; those on mung bean and green bean consisted of a general chlorosis.

Results of this study indicate that FSA is physiologically specialized, with a host adaptation limited to a few species in the Fabaceae. Because wounding of soybean and mung bean was not required for infection, these species are apparently more compatible hosts for FSA than are green bean, lima bean, and cowpea.

Virulence. Some isolates differed in virulence as determined by lesion size and number of plants killed (Table 3); but most, 24 of 31, were equally virulent. Seven isolates with the numerically lowest virulence ratings differed significantly from 12 isolates with the numerically highest virulence ratings. Such differences occurred both for isolates from roots and for those from cysts. Neither substrate origin nor geographic origin of isolates appeared to be related to virulence. Although Rupe (17) found differences in the virulence of FSA isolates, his isolate sample was much smaller (11 isolates), less diverse (three geographic locations and isolates from roots only), and therefore perhaps less representative of the FSA field population than the sample used in the present study.

Our results suggest that there is variation in virulence within FSA populations isolated either from soybean roots or from SCN cysts. This should be considered when selecting FSA isolates for experiments involving artificial inoculation of soybean plants.

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