Sudden Death Syndrome of Soybean: *Fusarium solani* as Incitant and Relation of *Heterodera glycines* to Disease Severity

K. W. Roy, G. W. Lawrence, H. H. Hodges, K. S. McLean, and J. F. Killebrew

Professor of plant pathology, assistant professor of plant pathology, professor of agronomy, graduate research assistant, and extension plant pathologist, Mississippi State University, Mississippi State 39762.

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ABSTRACT

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Two morphologically distinct forms of *Fusarium solani*, designated FS-A and FS-B, were isolated from soybean plants with symptoms of sudden death syndrome, a disease of unproven etiology. Form FS-A was isolated seven to 17 times more frequently from symptomatic than from asymptomatic plants, whereas FS-B was equally frequent on symptomatic and asymptomatic plants. In pathogenicity tests, FS-A caused the symptoms characteristic of sudden death syndrome; FS-B caused root rot but no other symptoms characteristic of the disease. Dual inoculation of soybean with FS-A and the soybean cyst nematode (*Heterodera glycines*) caused more severe foliar symptoms than those caused by FS-A alone, but the nematode was not required for infection of soybean by FS-A. It was concluded that FS-A is the primary causal agent of sudden death syndrome. FS-A caused a reduction in plant height, root dry weight, shoot dry weight, number of seeds per plant, and seed yield, but only in continuously irrigated plants as compared with periodically irrigated plants.

Additional keywords: *Fusarium* spp., *Glycine max*, soybean cyst nematode.

Sudden death syndrome (SDS) of soybean (*Glycine max* (L.) Merr.), a root and lower stem disease of unproven etiology, first was described in Arkansas in the early 1970s (9). In the 1980s, SDS also was found in Illinois (7,8,16), Indiana (8,9), Kentucky (5,9,16), Mississippi (7,9,18), Missouri, and Tennessee (8,9). The disease often is associated with high soil populations of the soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe (5,7–9,18), but the nature of this apparent relationship is unclear. SDS appears to be most frequent in irrigated soybeans or those growing in very moist soil (7–9).

The major symptoms of SDS include root rot, crown necrosis (9,16–18), vascular discoloration of roots and stems (6,7,9,16), interveinal chlorosis and necrosis of leaves (7,9,16,18), defoliation (6,8,9,18), and pod abortion (6,8,9). This disease has caused severe yield losses in localized areas of several states (6–9). Yield reductions of 20–70% (7) and 20–40% (8) have been reported.

The possible causal involvement of viruses, mycoplasmas, bacteria, and fungi in SDS has been investigated. Diagnostic techniques for viruses and mycoplasmas, including electron microscopy, were employed with negative results (6,7,9). However, several microorganisms have been isolated from or found associated with soybean plants having symptoms of SDS. Bacteria were found within xylem vessels of SDS-symptomatic soybean plants (9). A *Xanthomonas* sp. isolated from such plants was tested for pathogenicity by inoculating wounded roots of soybean seedlings with a highly concentrated bacterial suspension (J. J. Bozzola, *personal communication*). Some symptoms characteristic of SDS were produced on inoculated seedlings, and bacterial cells again were found within xylem vessels.

In 1986, Bozzola et al (3) reported that an unidentified fungus frequently was associated with collapsed veins of leaves on SDS-symptomatic plants. Another leaf-inhabiting fungus, *Leptosphaerulina briosiana*, was reported by Rosenbrock and Wyllie (14) to be associated with necrotic leaves of SDS-symptomatic soybean plants. The causal involvement of these fungi in SDS was not determined.

In 1986, Rupe and Weidemann (17) reported that a *Fusarium* sp. was the fungus most frequently isolated from roots of SDS-symptomatic plants, and, in 1987, Rupe (16) reported the isolation of *Fusarium solani* (Mart.) Appel & Wollenw. emend. Snyd. & Hans. from roots of 4–16% of SDS-symptomatic plants collected from Arkansas, Illinois, Indiana, and Kentucky. In both studies, *Fusarium* sp. (17) and *F. solani* (16) caused some, but not all, symptoms characteristic of SDS when soybean seedlings were inoculated in the greenhouse.

Koch's postulates have not yet been fulfilled for any microorganism associated with SDS. We report isolation of *F. solani* and other fungi from SDS-symptomatic and asymptomatic soybean plants, reproduction of SDS symptoms in

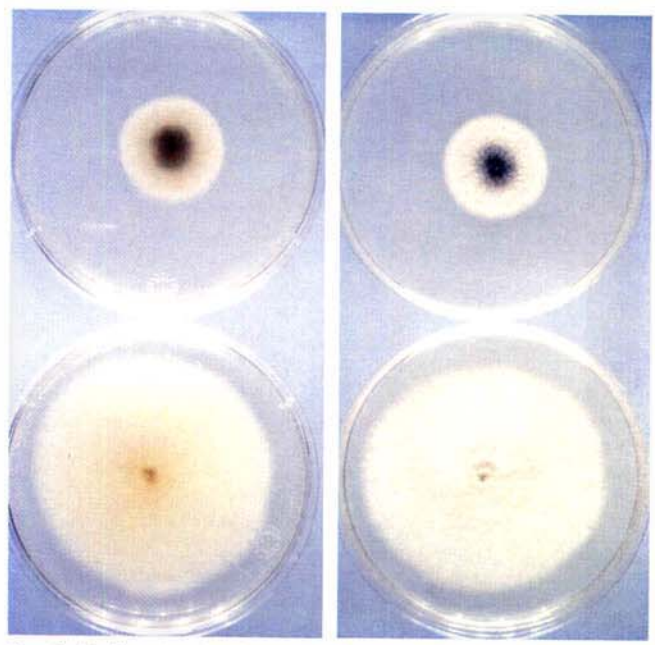

Fig. 1. Surface (top) and reverse (bottom) of 7-day-old colonies of *Fusarium solani* forms FS-A (left) and FS-B (right) on potato-dextrose agar.

soybean plants inoculated with a morphological form of *F. solani*, reisolation of the same fungus from diseased plants, morphological and cultural characteristics of the pathogen, and effects of irrigation and *H. glycines* on disease severity. A preliminary report of these findings was published (15).

MATERIALS AND METHODS

Isolation and identification of fungi from SDS-symptomatic and asymptomatic field-grown soybean plants. In September 1986, the natural occurrence of SDS in a field of irrigated cultivar Leflore soybeans at the Mississippi State University Plant Science Research Center in Starkville afforded an opportunity to investigate the etiology of the disease. The field was known to be infested with *H. glycines* (G. W. Lawrence, *unpublished*).

Soybeans had been planted on 15 June in 15 rows (replications) each 2 m wide by 16 m long. Each row contained an average plant density of three plants per 30 cm, with a row spacing of 17 cm. A drip-irrigated, 16-m-long ditch in the center and on either side of each row supplied water continuously, beginning with the V3 growth stage (4) until near plant maturity (growth stage R7).

At the R6 growth stage, 20 each of intact symptomatic and asymptomatic plants were randomly sampled from each row. One section each from the tip of the taproot, middle of the taproot, lateral roots, crown, and the stem 10, 20, and 30 cm above the crown was excised per plant. One section from the middle of a petiole and leaflet from each plant also was sampled. Samples were washed in running tap water for 20 min, then surface-disinfested for 5 sec in 95% ethanol and 1 min in 1% NaOCl, and rinsed in sterile water. A portion of the cortex from each root and stem section, except that from root tips, was aseptically peeled back with a scalpel. Underlying tissue from root and stem sections and tissue from root tips, petioles, and leaflets was plated on Difco (Difco Laboratories, Detroit, MI) potato-dextrose agar (PDA) amended with streptomycin B sulfate (100 mg/L) and aureomycin (2 mg/L) (PDASA). During 2 wk of incubation at 24 C, most fungi growing from tissues either were identified or were subcultured on PDASA for later identification. To induce sporulation, isolates of *Fusarium* spp. were subcultured on modified Bilay's medium (1). Identifications of *Fusarium* species were based on descriptions reported by Booth (2) and Nelson et al (13).

The population density of *H. glycines* in soil was determined 1 wk after irrigation was discontinued. Rhizosphere soil within each replication was sampled with a soil probe 2 cm in diameter to a depth of 12–15 cm. Nematodes were extracted from soil with a semiautomatic elutriator, and the fraction collected on the 38-μm-pore sieve was processed further by centrifugal flotation (sucrose specific gravity 1.13) (10). Cysts were collected on a 250-μm-pore sieve directly below an 850-μm-pore sieve that separated organic debris.

Cultural and morphological characteristics of forms of *F. solani*. Two isolates of *F. solani* with different colony types grew from soybean tissues during the bioassay of SDS-symptomatic and asymptomatic plants from the field. These were designated forms FS-A and FS-B. Seven isolates of each form were selected for further characterization.

Initial colonies growing from soybean tissues onto PDASA, i.e., wild-type colonies, and colonies subcultured on PDASA and PDA and incubated in darkness or in continuous fluorescent light (163 Wm^{-2}) were observed for colony characteristics. To determine growth rate, single-spore isolates from macroconidia were grown on PDA in darkness for 7 days at 24 C. Two measurements of colony diameter were taken at right angles and averaged for each of seven replicates per isolate.

To evaluate chlamydospore production, cubes of agar containing mycelium were taken from actively growing colonies on PDA, placed in sterile distilled water in petri dishes, and incubated in darkness or in light (163 Wm^{-2}) at 24 C.

Macroconidia from colonies grown on modified Bilay's medium in continuous fluorescent light (163 Wm^{-2}) for 1 wk at 24 C were used to determine spore dimensions. One hundred conidia were measured per isolate. Conidiogenesis and conidiophore morphology were determined from slide cultures containing squares of modified Bilay's medium.

Pathogenicity tests in the greenhouse. Forms FS-A and FS-B and juveniles and eggs of *H. glycines* race 3 were included in the first experiment. Treatments were: FS-A alone, FS-B alone, *H. glycines* alone, FS-A plus *H. glycines*, FS-B plus *H. glycines*, and the control (no fungus or nematode).

To obtain inoculum, FS-A and FS-B were grown at 24 C for 7–10 days in sterile sand-cornmeal culture (250 cm^3 of dry sand, 14 cm^3 of cornmeal, and 100 ml of distilled water). Portions of this inoculum were mixed with sterile soil, using a mechanical soil mixer to obtain an inoculum concentration of 0.05% (w/w). The soil medium was a 1:1 (v/v) mixture of sand and Marietta silty clay loam soil fumigated with methyl bromide.

Eggs and juveniles of *H. glycines* were obtained by increasing a population of race 3 on soybean cultivar Coker 156 for 60 days in the greenhouse. Light brown to tan cysts were dislodged from roots with a stream of water and collected on nested sieves with pore sizes of 850 and 250 μm. Cysts were suspended in water, then immediately poured through the 850-μm-pore sieve nested on a 250-μm-pore sieve. Cysts 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 (19) for 1 min. 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 that averaged 686 eggs and 43 juveniles per milliliter.

Soil infested with the fungi and uninfested (control) soil was distributed to 15-cm-diameter clay pots. Six Coker 156 soybean seeds, previously surface-disinfested in 1% NaOCl for 1 min, germinated on PDA for 36 hr, and determined to be free of microorganisms, were treated with *Rhizobium japonicum* and placed on the soil surface in each pot. Coker 156 is susceptible to both FS-A and race 3 of *H. glycines* (K. W. Roy and G. W. Lawrence, *unpublished*). Seven milliliters of the nematode suspension was pipetted into three depressions 2.5 cm deep and 2 cm wide, and a thin layer of fumigated soil was added to prevent desiccation of eggs and juveniles.

All treatments were replicated seven times, and pots were arranged in a completely randomized design on a greenhouse bench. To obtain uniformity in size and stage of growth, seedlings were culled to three per pot. Plants were irrigated continuously, beginning with the V3 growth stage. Foliar, stem, and root

TABLE 1. Frequency of isolation (%) of fungi from roots and crowns of cultivar Leflore soybean plants asymptomatic and symptomatic for sudden death syndrome in 1986[a]

Fungus	From asymptomatic plants	From symptomatic plants	Average
Fusarium solani (total isolates)[b]	69	95	82.0
F. solani (FS-B)[c]	64	76	70.0
F. solani (FS-A)[d]	7	51	29.0
F. equiseti	0	7	3.5
F. tricinctum	3	3	3.0
F. acuminatum	3	3	3.0
F. semitectum	0	2	1.0
F. oxysporum	2	0	1.0
Macrophomina phaseolina	58	44	51.0
Rhizoctonia solani	19	17	18.0
Gliocladium roseum	27	5	16.0
Phoma spp.	17	12	14.5
Phomopsis longicolla	7	17	12.0
Cercospora kikuchii	0	15	7.5

[a] Other fungi less frequently isolated (≤%): *Alternaria*, *Aspergillus*, *Bipolaris*, *Chaetomium*, *Cladosporium*, *Colletotrichum*, *Cylindrocarpon*, *Diaporthe*, *Myrothecium*, *Nigrospora*, *Paecilomyces*, *Penicillium*, *Trichoderma* spp.
[b] Forms FS-A and FS-B inclusive.
[c] *F. solani* form FS-B.
[d] *F. solani* form FS-A.

symptoms and seedling stand (at 3 wk) were recorded. To confirm Koch's postulates, sections of root, stem, and leaf tissue from seedlings and mature plants (R8) were bioassayed for FS-A and FS-B as described previously. This experiment was conducted twice. Unless otherwise indicated, data were subjected to analysis of variance and means were separated with Duncan's multiple range test. Error variances of the two trials were tested for homogeneity before sums of squares were pooled for the analysis. Percentage data were transformed to arcsine before analysis.

In a second experiment, the treatments were FS-A alone, *H. glycines* alone, FS-A plus *H. glycines*, and the control (no fungus or nematode). Coker 156 plants either were irrigated continuously beginning with the V3 growth stage (continuous irrigation treatment) or were watered periodically (periodic irrigation treatment). In the continuous irrigation treatment, soil was kept wet continuously by drip irrigation. In the periodic irrigation treatment, plants received sufficient water for optimal growth, but the soil surface was allowed to dry between irrigations. Increase of inoculum, inoculation, incubation regime, randomization of pots, and bioassay were as described above. Foliar, stem, and root symptoms, disease severity, plant height, root and shoot weight, number of pods and seeds per plant, and seed size and yield were recorded. Root volume was determined by water displacement. Sections of root, stem, and leaf tissue from seedlings and mature plants (R8) were bioassayed for FS-A as described previously. This experiment was conducted twice. Data analysis was as described in the first experiment.

In a third experiment, 10 Coker 156 seedlings in each of seven replicate pots per treatment were grown in methyl bromide-fumigated soil in 15-cm-diameter clay pots until they reached the V1 growth stage. Sterile sections of broom straw 0.5 cm long were placed in 3-day-old cultures of form FS-A for 5 days and allowed to become colonized. One colonized section was inserted midway into each seedling hypocotyl at a point 2.5 cm below the cotyledonary node and covered with petrolatum. Control seedlings received uncolonized straw sections. Treatments were arranged in a completely randomized design on a greenhouse bench. Leaves and hypocotyls were observed for symptoms during a subsequent 3-wk period, after which sections of hypocotyl and leaf tissue were bioassayed for FS-A as described previously. This experiment was conducted twice. Data were subjected to analysis of variance.

RESULTS

The effect of irrigation on disease severity was obvious within individual rows in the field. Plants growing near irrigation ditches were more frequently symptomatic than were those more distant from ditches. Populations of SCN in rhizosphere soil ranged from 20 to 218 cysts and juveniles per 500 cm^3 of soil.

Identity and frequency of isolation of fungi from SDS-symptomatic and asymptomatic field-grown soybean plants. *Fusarium* spp., including *F. solani*, *F. equiseti* (Corda) Sacc., *F. semitectum* Berk. & Rav., *F. oxysporum* Schlecht. emend. Snyd. & Hans., *F. tricinctum* (Corda) Sacc., and *F. acuminatum* Ell. & Ev., were the predominant fungi associated with both SDS-symptomatic and asymptomatic plants (Table 1). Of these, *F. solani* (FS-A + FS-B) was isolated most frequently. *Macrophomina phaseolina* (Tassi) Goid., *Rhizoctonia solani* Kühn, *Gliocladium roseum* Bainier, *Phoma* spp., *Phomopsis longicolla* Hobbs, and *Cercospora kikuchii* (Mat. & Tomoy.) Gardner comprised the

TABLE 2. Frequency of isolation (%) of *Fusarium solani* forms FS-A and FS-B from various tissues of cultivar Leflore soybean plants asymptomatic and symptomatic for sudden death syndrome

Form of *F. solani*	Type of plant	Lateral root	Taproot		
			Tip	Middle	Crown
FS-A	Asymptomatic	0	3	2	2
	Symptomatic	12	19	34	3
FS-B	Asymptomatic	15	59	14	3
	Symptomatic	22	58	20	7

TABLE 3. Colony diameter and macroconidial dimensions of two forms of *Fusarium solani* isolated from soybean plants

Form of *F. solani*	Colony diameter[a] (cm)	Size of macroconidia[b]			
		Length (μm)		Width (μm)	
		Mean	Range	Mean	Range
FS-A	2.6	51.8	40.7–61.6	4.6	3.9–4.9
FS-B	6.2	34.7	23.4–37.8	4.7	3.9–5.0

[a] Mean colony diameter of seven replicates of seven isolates of each form after growth on pototo-dextrose agar in darkness for 7 days at 24 C.
[b] Mean size based on 100 macroconidia for each of seven isolates per form after growth on modified Bilay's medium for 1 wk at 24 C under continuous fluorescent light (163 Wm^{-2}).

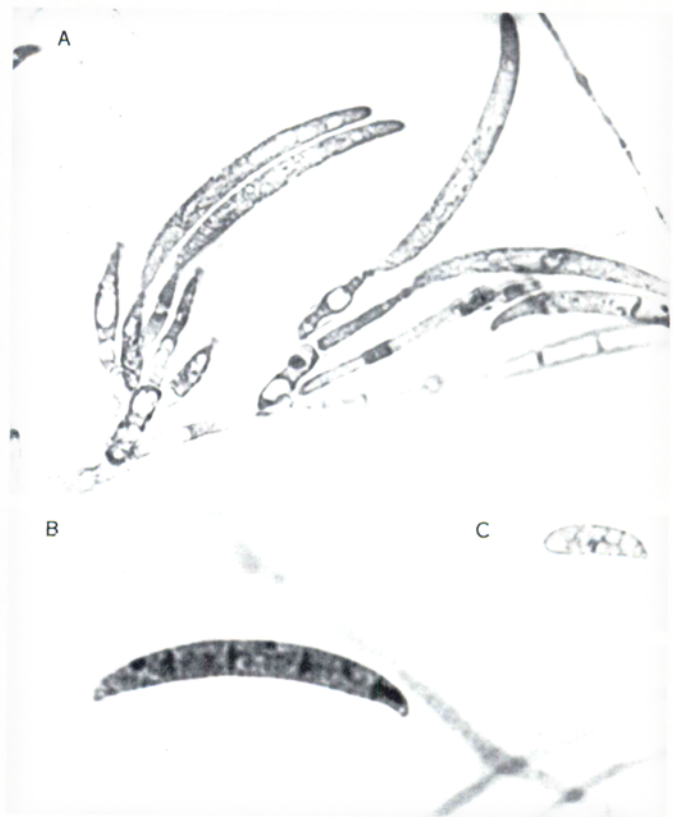

Fig. 2. Reproductive structures of *Fusarium solani* form FS-A. **A,** Macroconidiophores and developing macroconidia; **B,** mature macroconidium; **C,** microconidium (×825). Stained with cotton blue in lactophenol.

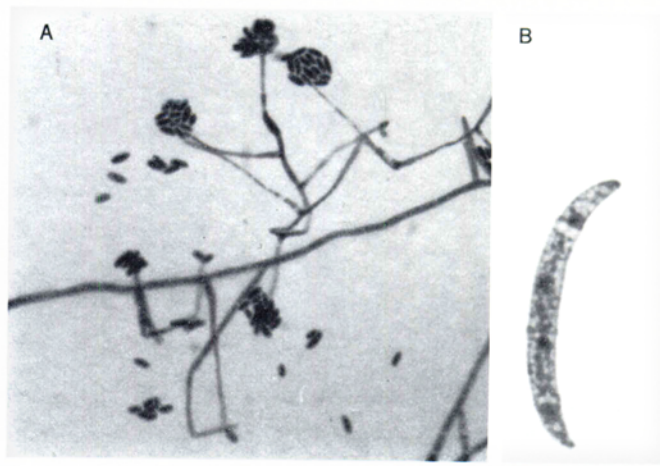

Fig. 3. Reproductive structures of *Fusarium solani* form FS-B. **A,** Simple and branched microconidiophores bearing false heads of microconidia (×200) **B,** macroconidium (×950). Stained with cotton blue in lactophenol.

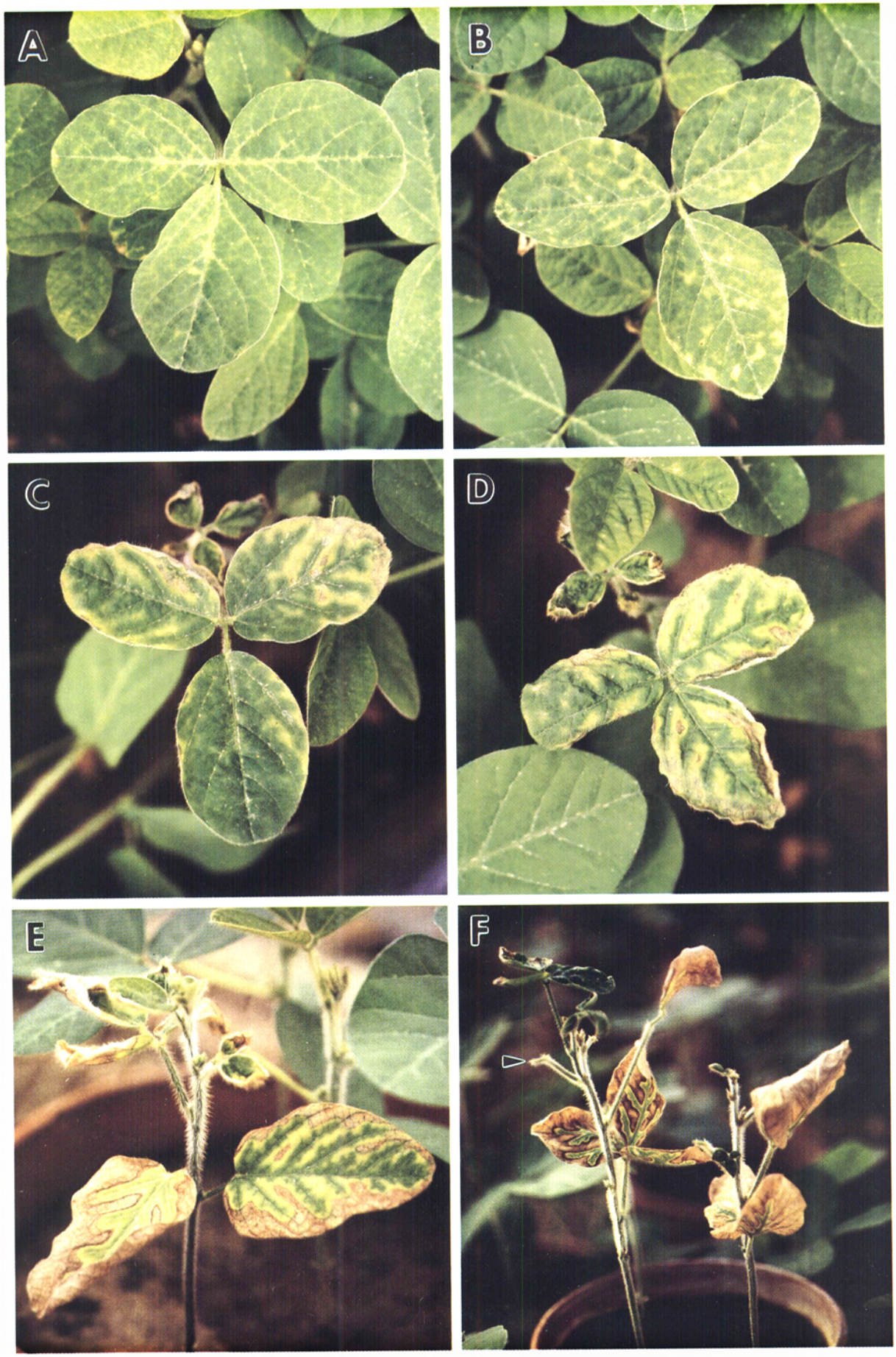

Fig. 4. Progression in severity of sudden death syndrome leaf symptoms on Coker 156 soybean after inoculation with *Fusarium solani* form FS-A: **A,** Chlorotic mottling (earliest symptom); **B–E,** interveinal chlorosis and incipient necrosis; **F,** interveinal necrosis and leaf abscission at juncture of petiole and leaflet (arrow).

remainder of the frequently isolated fungi. The frequency of isolation of FS-A from SDS-symptomatic plants was seven times greater than that from asymptomatic ones. Form FS-B, *P. longicolla*, and *C. kikuchii* also were more frequently isolated from symptomatic plants. *G. roseum* was more frequently isolated from asymptomatic plants.

Forms FS-A and FS-B were isolated more frequently from root than crown tissues (Table 2); neither was isolated from stem tissues above the crown. Frequency of isolation of FS-A in lateral roots and the tip and middles of taproots was up to 17 times greater in SDS-symptomatic than asymptomatic plants, whereas the frequency of FS-B in those tissues was similar for both symptomatic and asymptomatic plants.

Cultural and morphological characteristics of *F. solani* forms.

TABLE 4. Pathogenicity of *Fusarium solani* forms FS-A and FS-B on irrigated[a] cultivar Coker 156 soybean when inoculated on roots either alone or in combination with soybean cyst nematode, *Heterodera glycines* (SCN), in the greenhouse

Inoculation treatment	Seedling emergence (%)[b]	Root disease index[c]	Leaf disease index[d]
FS-A	55.6 bcd	3.3 a	3.9 a
FS-B	33.5 d	1.0 b	0.0
SCN	63.8 abcd	3.0 a	0.0
FS-A + SCN	75.0 abc	3.3 a	3.7 a
FS-B + SCN	50.0 cd	2.7 a	0.0
Control	80.5 a	1.7 b	0.0

[a] Plants were irrigated continuously, beginning with the V3 growth stage.
[b] Determined 3 wk after planting. Means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[c] 1 = no symptoms (necrosis) and 2–5 = increasingly severe root necrosis. Means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[d] 0 = no symptoms; 1 = 1–24% of plants with leaf symptoms (interveinal necrosis); 2 = 25–49% of plants with leaf symptom; 3 = 50–74% of plants with leaf symptoms; 4 = 75–100% of plants with leaf symptoms; 5 = 75–100% of plants with leaf symptoms and less than 50% defoliation; and 6 = 75–100% of plants with leaf symptoms and more than 50% defoliation. Because of the heterogeneity of variances, only the two treatments producing measurable variances were included in the analysis of variance. Means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

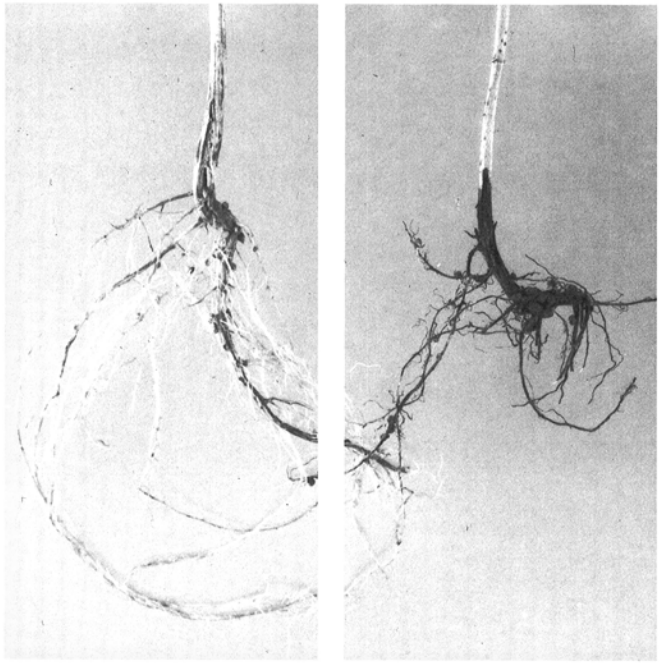

Fig. 5. Root and lower stem necrosis characteristic of sudden death syndrome on Coker 156 soybean 45 days after inoculation with *Fusarium solani* form FS-A. Control (left) and inoculated (right).

On PDA, FS-A produced a grayish-white aerial mycelium, which was more appressed and much more sparse than that of FS-B (Fig. 1). A bluish color in the central part of the colony was most typical of wild-type colonies of FS-A. In contrast, FS-B produced a floccose, grayish white aerial mycelium, and a cream to tan color was imparted to the medium. Isolates of FS-A had a slower growth rate than isolates of FS-B (Table 3, Fig. 1). Growth rate varied little among isolates within a form. Usually, single, globose to subglobose, terminal and intercalary chlamydospores were produced within hyphae by both FS-A and FS-B.

Macroconidia of FS-A, formed on simple or branched conidiophores from monophialides (Fig. 2A and B), were longer than those of FS-B (Table 3, Fig. 2). Macroconidial production by FS-A was always more profuse than that of FS-B, but microconidia (Fig. 2C) were rare. On PDA, slimy pionnotal masses of macroconidia, ranging in color from cream to blue to blue-green to bluish purple, were produced in the central part of the culture by most isolates of FS-A. Microconidia predominated in isolates of FS-B. Microconidia were formed on long, unbranched or sparingly branched conidiophores from monophialides (Fig. 3A). As was the case with FS-A, macroconidia were formed on simple or branched conidiophores from monophialides, but they were seldom formed in pionnotes. Macroconidium morphology (Fig. 3B) was similar to that of FS-A. Macroconidia of FS-B were cream-colored in mass.

Pathogenicity tests in the greenhouse. In the first experiment, both FS-A and FS-B reduced seedling stand in continuously irrigated Coker 156 plants (Table 4). On older plants, both FS-A and *H. glycines* alone caused root rot, but only FS-A caused foliar symptoms characteristic of SDS. Combining *H. glycines* with FS-A or FS-B did not further reduce stand nor increase leaf or root disease severity. Both FS-A and FS-B were reisolated from roots and crowns of inoculated living seedlings and mature plants but not from leaves and rarely from the stem above the crown.

A general chlorotic mottling was the first leaf symptom detected about 3 wk after seedling emergence (Fig. 4A) in plants inoculated with FS-A. The chlorosis gradually became distinctly interveinal (Fig. 4B–D) within several days. These chlorotic spots gradually became necrotic (Fig. 4D–F) within 1–2 wk. In older plants, the more necrotic leaves abscissed at the juncture of the leaflet and petiole (Fig. 4F). Lateral roots and taproots infected with FS-A were severely necrotic (Fig. 5).

TABLE 5. Pathogenicity of *Fusarium solani* form FS-A on continuously irrigated (CI) or periodically irrigated (PI) cultivar Coker 156 soybean when inoculated on roots either alone or in combination with soybean cyst nematode, *Heterodera glycines* (SCN), in the greenhouse[a]

Inoculation treatment	Disease severity rating					
	Root disease index[b]			Leaf disease index[c]		
	CI	PI	Avg.	CI	PI	Avg.
FS-A	3.6	3.1	3.4 a	3.6	2.9	3.2 b
SCN	3.1	3.7	3.4 a	0.0	0.0	0.0
FS-A + SCN	3.6	4.0	3.8 a	3.8	4.3	4.1 a
Control	1.9	2.3	2.1 b	0.0	0.0	0.0
Average	3.1 a	3.3 a		1.9 a	1.8 a	

[a] In the continuous irrigation treatment, soil was kept wet continuously, beginning with the V3 growth stage, by drip irrigation. In the periodic irrigation treatment, plants received sufficient water for optimal growth, but the soil surface was allowed to dry between irrigations.
[b] 1 = no symptoms (necrosis) and 2–5 = increasingly severe necrosis. Main effect means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[c] 0 = no symptoms; 1 = 1–24% of plants with leaf symptoms (interveinal necrosis); 2 = 25–49% of plants with leaf symptom; 3 = 50–74% of plants with leaf symptoms; 4 = 75–100% of plants with leaf symptoms; 5 = 75–100% of plants with leaf symptoms and less than 50% defoliation; and 6 = 75–100% of plants with leaf symptoms and more than 50% defoliation. Because of the heterogeneity of variances, only the treatments producing measurable variances were included in the analysis of variance. Main effect means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

In the second experiment, both FS-A and *H. glycines* alone caused root rot; combinations of the two caused no more root rot than either alone (Table 5). As in the first experiment, only FS-A caused foliar symptoms characteristic of SDS, but these were more severe when FS-A was combined with *H. glycines*. Irrigation had no significant effect on the severity of root and leaf symptoms.

Form FS-A reduced plant height, root dry weight, and shoot dry weight, but only in continuously irrigated as compared with periodically irrigated plants (Table 6). *H. glycines* reduced root dry weight, but to a lesser degree compared with FS-A and only in periodically irrigated plants.

Form FS-A and *H. glycines* alone reduced the number of pods per plant, pod dry weight, number of seeds per plant, and seed yield, but they had no significant effect on the number of seeds per pod and seed size (Table 7). The fungus reduced the number of seeds per plant and seed yield only in continuously irrigated as compared to periodically irrigated plants. Reduction in seed yield due to *H. glycines* also occurred only in continuously irrigated plants. Form FS-A caused a greater reduction in the number of pods, the number of seeds, and seed yield per plant than did either *H. glycines* or FS-A + *H. glycines*, but only in continuously irrigated plants. The fungus was reisolated from roots and crown of inoculated living seedlings and mature plants but not from leaves or the stem above the crown.

In the third experiment, the hypocotyl inoculation technique was used. In both the initial and the repeat trial, all Coker 156 seedlings inoculated with FS-A developed dark brown lesions surrounding the point of inoculation within 1–2 wk after inoculation. Interveinal necrosis occurred on the leaves of all plants within 2 wk after inoculation, and all plants were killed 1–2 wk later. All control seedlings remained healthy for the duration of the test. The fungus was reisolated from hypocotyls but not from leaves.

DISCUSSION

The first of Koch's postulates is to establish the constant association of a pathogen with a disease. Of the 25 fungal taxa isolated from field-grown soybean plants in 1986, only 11 were isolated from SDS-symptomatic plants with a frequency greater than 1%. Of these 11, only *F. solani, P. longicolla,* and *C. kikuchii* were of potential etiological significance, because they were isolated more frequently from symptomatic than asymptomatic plants. *P. longicolla* and *C. kikuchii* can be eliminated as potential causes of SDS, as the symptoms associated with infection of soybeans by these fungi (1) are not among those characteristic of SDS (6–9,16–18). The predominance of *F. solani* on roots and crowns of symptomatic plants strongly implicated this species in the eitology of SDS.

It was shown that isolates of *F. solani* from the 1986 bioassay comprised two morphological forms. Forms FS-A and FS-B are pathologically distinct as well. The latter is a seedling pathogen capable of reducing stand (11,12, and Table 4); it also colonizes older plants (11,12, and Table 1) and is capable of reducing yield (12). Other than root rot, FS-B did not cause symptoms characteristic of SDS.

In the 1986 bioassay, the most important finding establishing an association of SDS with a pathogen was isolation of FS-A with an 7- to 17-fold greater frequency from root and crown tissues of SDS-symptomatic plants than from those of asymptomatic plants. This association is supported by other evidence. Form FS-A was isolated in relatively high frequency from roots of SDS-symptomatic plants from three soybean fields surveyed in 1985 and 1987 (average frequency of isolation, 38% in 1985 and 64% in 1987) (K. W. Roy and G. W. Lawrence, *unpublished*). Further, isolates of *F. solani* (16) and *Fusarium* sp. (17) that originated from SDS-symptomatic plants and that caused some of the symptoms of SDS in studies by Rupe (16) and Rupe and Weidemann (17) possibly are similar or identical to our form FS-A.

The possible causal involvement of other microorganisms in SDS has been investigated. Diagnostic techniques for viruses, mycoplasmas, and bacteria were employed with negative results until *Xanthomonas* sp. was found within xylem vessels of SDS-symptomatic plants (9). When this bacterium was inoculated on

TABLE 6. Effect of inoculating continuously irrigated (CI) or periodically irrigated (PI) cultivar Coker 156 soybean with *Fusarium solani* form FS-A either alone or in combination with soybean cyst nematode, *Heterodera glycines* (SCN), on plant height and root and shoot weight in the greenhouse[a]

Inoculation treatment	Plant height (cm)[b]			Dry weight (g)					
				Root			Shoot		
	CI	PI	Avg.	CI	PI	Avg.	CI	PI	Avg.
FS-A	26.8 b	20.4 cd	23.6	1.9 cd	2.8 cd	2.4	5.6 cd	7.9 cd	6.8
SCN	35.1 a	17.6 d	26.4	5.3 ab	0.9 d	3.1	9.1 ab	4.6 d	6.9
FS-A + SCN	25.9 bc	21.0 cd	23.4	3.2 bcd	2.2 cd	2.7	8.0 bcd	7.1 cd	7.6
Control	34.1 a	20.8 cd	27.4	6.7 a	3.3 bc	5.0	11.1 a	7.9 cd	9.5
Average	30.2	20.0		4.3	2.3		8.5	6.9	

[a] In the continuous irrigation treatment, soil was kept wet continuously, beginning with the V3 growth stage, by drip irrigation. In the periodic irrigation treatment, plants received sufficient water for optimal growth, but the soil surface was allowed to dry between irrigations.
[b] Height of mature plants (growth stage R8) from soil line to stem apex. Any two means followed by the same letter are not significantly different ($P = 0.05$), according to Duncan's multiple range test.

TABLE 7. Effect of inoculating continuously irrigated (CI) and periodically irrigated (PI) cultivar Coker 156 soybean with *Fusarium solani* form FS-A either alone or in combination with soybean cyst nematode, *Heterodera glycines* (SCN), on plant yield components in the greenhouse[a]

Inoculation treatment	Pods per plant (no.)			Pod dry weight (g/plant)			Seeds per plant (no.)			Seeds per pod (no.)			Seed size (g/100 seed)			Seed yield (g/plant)		
	CI	PI	Avg.	CI	PI	Avg.	CI	PI	Avg.	CI	PI	Avg.	CI	PI	Avg.	CI	PI	Avg.
FS-A	2.6 c	2.6 c	2.6	1.5	0.8	1.1 bc	9.1 cd	9.9 cde	9.5	1.0	1.3	1.2 a	6.7	9.7	8.2 a	1.0 c	1.1 c	1.1
SCN	5.0 b	1.5 c	3.2	1.0	0.3	0.6 c	20.9 bc	3.6 e	12.2	1.3	0.5	0.9 a	11.4	6.6	9.0 a	2.7 b	0.5 c	1.6
FS-A + SCN	4.7 b	2.3 c	3.5	2.3	0.8	1.6 b	24.6 b	7.9 de	16.2	1.7	0.8	1.2 a	8.7	11.4	10.1 a	2.5 b	0.8 c	1.6
Control	7.6 a	4.6 b	6.1	3.4	1.7	2.6 a	37.0 a	16.9 bcd	26.9	1.6	1.2	1.4 a	11.9	12.3	12.1 a	4.3 a	1.9 bc	3.1
Average	5.0	2.8		2.1 a	0.9 b		22.9	9.5		1.4 a	0.9 b		9.7 a	10.0 a		2.6	1.1	

[a] In the continuous irrigation treatment, soil was kept wet continuously, beginning with the V3 growth stage, by drip irrigation. In the periodic irrigation treatment, plants received sufficient water for optimal growth, but the soil surface was allowed to dry between irrigations. All yield components were measured at plant maturity (growth stage R8). Within a yield component, means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

soybean, it caused SDS-like foliar symptoms (9), but this inoculation involved the application of a highly concentrated bacterial suspension to wounded roots (J. J. Bozzola, *personal communication*). Moreover, production of foliar symptoms has been inconsistent (9; J. J. Bozzola, *personal communication*); the ability of the bacterium to cause root rot as well as other important symptoms characteristic of SDS has not been demonstrated; and a constant association of the bacterium with SDS-symptomatic plants has not been established. The possibility that *L. briosiana* (14) and an unidentified fungus (3) that were found associated with leaves on SDS-symptomatic plants are causally involved in SDS also is remote, because SDS has the general symptomatology and development of a soilborne disease.

The constant association of FS-A with diseased plants, production of SDS symptoms upon inoculation, and reisolation of the fungus from inoculated, symptomatic plants satisfy Koch's postulates. From this and other compatible evidence (16,17), we conclude that FS-A is the primary causal agent of SDS. *H. glycines*, often found in high populations in fields where SDS is present (5,7–9,18), was not indicated to have direct causal involvement in the disease; *H. glycines* caused no foliar symptoms characteristic of SDS, whereas FS-A did cause such symptoms in the absence of the nematode. However, although *H. glycines* is not necessary for infection of soybean by FS-A, our results suggest that its presence on the host may increase the severity of foliar symptoms.

In the 1986 field test, we observed that plants growing near irrigation ditches were more frequently symptomatic than were those growing more distant from ditches. In addition, we found that SDS was more severe in soybean plants irrigated continuously beginning with the V3 growth stage compared with those irrigated continuously beginning with the V8 growth stage (authors, *unpublished*). These and other data (7–9) indicate that high soil moisture, probably early in soybean development, is a critical predisposing factor in SDS development.

With the hypocotyl-inoculation technique, 100% of inoculated seedlings of a susceptible cultivar became infected and were killed by FS-A, indicating that this technique was effective in screening for susceptability to SDS. This technique may be useful for screening soybean cultivars for resistance to FS-A as the apparent causal agent of SDS.

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