Frequency and Pathogenicity of *Fusarium solani* Recovered from Soybeans with Sudden Death Syndrome

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ABSTRACT

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A blue isolate of *Fusarium solani* was obtained from roots and lower stems of soybeans with sudden death syndrome (SDS) grown in 10 fields in Arkansas, one field in Illinois, and one field in Kentucky. The blue *F. solani* was recovered from plants from all locations and from 86% of all plants sampled. Nineteen percent of isolates from lateral root sections, 23% of isolates from taproot epidermal sections, 12% of isolates from taproot cortical sections, and 6% of isolates from lower stem sections assayed yielded the fungus. One representative isolate was used to inoculate the taproots of flowering 6-wk-old, greenhouse-grown soybeans (cv. Lee 74). Of the 149 plants inoculated, 76.5% developed foliar symptoms similar to SDS. Recovery of the blue *F. solani* from inoculated plants completed Koch's postulates. Eleven single-spore isolates from nine locations were used to inoculate 2-wk-old Lee 74 soybean plants by placing a plug of mycelium next to the stem immediately below the soil line. Disease development was similar to that in older plants. Ten isolates produced symptoms similar to SDS, but the symptoms varied greatly in intensity. These results strongly suggest that a specific type of *F. solani* is involved in SDS.

Sudden death syndrome (SDS) of soybeans (*Glycine max* (L.) Merrill) has been observed periodically in Arkansas since 1971 (H. J. Walters, *personal communication*). Concern about the disease was limited to Arkansas until 1984 and 1985, when SDS was reported in Mississippi, Missouri, Illinois, Tennessee, Kentucky, and Indiana (4,5,11-13). SDS generally occurs in vigorously growing soybeans with high yield potential and can result in total yield loss in severely affected areas.

Initial symptoms of SDS on leaves are interveinal chlorotic spots that may become necrotic or develop into chlorotic streaks. These streaks become necrotic, killing the leaflets, which dehisce and leave only the petiole attached to the plant. Tissue adjacent to the major leaflet veins remains green throughout symptom development. Roots of affected plants are reduced in size and usually discolored, and the vascular tissue is a light brown. Vascular discoloration may extend up the stem several nodes, but the pith remains white. The pattern of symptom development in the field suggests a soilborne etiology (3).

The appearance of foliar symptoms varies by region but is generally associated with cool, wet weather (H. J. Walters, *personal communication*). In regions that grow early-maturing cultivars (maturity groups II, III, and IV), SDS appears at or after flowering. In regions that grow later-maturing cultivars (maturity groups VI, VII, and VIII), SDS may appear several weeks before flowering. In Arkansas, SDS has been observed in the field as early as 5 wk after planting, when plants were in the V-7 to V-9 maturity stage (J. C. Rupe, *unpublished*). These early symptoms on plant leaves varied from a few chlorotic spots to extensive interveinal chlorosis. Reduced seed size and pod abortion caused yield reductions.

Differences in cultivar susceptibility to SDS have been observed (5,8). The soybean cyst nematode, *Heterodera glycines* Ichinohe, is often but not always associated with the disease.

Although many attempts have been made to isolate a causal agent, only two organisms have been reported to incite symptoms similar to SDS in greenhouse tests: *Fusarium solani* (Mart.) Appel & Wollenw. emend. Snyd. & Hans. (7,9,10) and *Xanthomonas* sp. (13). The *F. solani* isolates that produce symptoms similar to SDS grow slowly on potato-dextrose agar (PDA), produce a slimy colony with little aerial mycelium, and produce few if any microconidia. Large numbers of macroconidia are produced in a blue mass, and the agar is stained a dark maroon. Macroconidia measure 30-65 × 6-8 μm and have three to five septa (10). The fungus was identified as *F. solani* by P. E. Nelson and T. A. Toussoun (*personal communication*). In greenhouse tests, soybeans grown in soil amended with rice hulls and oat seeds infested with the fungus developed symptoms similar to SDS (10). The foliar symptoms produced by this isolate of *F. solani* have not been previously associated with *F. solani* on soybeans (2).

This paper reports the frequency of isolation of blue *F. solani* from plants with SDS collected from different areas and the results of pathogenicity tests. The preliminary results have been reported (9).

MATERIALS AND METHODS

Isolations from roots and stems. Soybeans with foliar symptoms of SDS were collected from 10 locations in Arkansas, one location in Illinois, and one location in Kentucky. Plants were collected at the R5 to R6 growth stage. Isolation procedures were begun within 3, 4, and 8 days for the plants collected in Arkansas, Illinois, and Kentucky, respectively.

The roots were washed in running tap water and divided into lateral roots, taproot epidermal tissue, taproot cortical tissue, and tissue from the lower 5 cm of stem with vascular discoloration. The epidermis of the lower stems was removed. Plant parts were cut into 1-cm segments and surface-disinfested by dipping in 95% ethanol, soaking in 0.525% sodium hypochlorite for 5 min, and rinsing in sterile water. The segments were placed on 2% water agar amended with 50 mg/L of chloramphenicol and 100 mg/L of streptomycin sulfate. Segments were placed in petri dishes (five segments per dish) and incubated at room temperature.

As fungi grew from the segments, hyphae were transferred to Difco PDA amended with chloramphenicol (50 mg/L), streptomycin sulfate (100 mg/L), and the nonionic polyglycol ether surfactant Tergitol NP-10 (Sigma Chemical Co., St. Louis, MO) (18 drops per liter). The percentage of segments that yielded blue *F. solani* isolates was determined after an incubation of 7-10 days at room temperature. These isolates were identified by the production of a blue, slimy mass of macroconidia, absence of microconidia, appressed mycelium, slow growth on PDA, and maroon discoloration of the agar.

Pathogenicity of the blue *F. solani*. Lee 74 soybeans were grown in steam-pasteurized sandy soil in 500-ml plastic pots, with four seedlings per pot. The plants were watered weekly with a nutrient solution containing nitrogen (All Purpose Soluble Plant Food, 20-20-20, Peters Professional Plant Food, W. R. Grace & Co., Fogelsville, PA). Six weeks after sowing, plants had four fully developed trifoliate leaves and were just beginning to bloom at the time of

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inoculation.

Plants were inoculated by scraping the epidermis of the taproot 0.5 cm below the soil line and pressing a plug (0.5 cm in diameter) of mycelium and macroconidia of the Mar 84 isolate (Table 1) into the wound. The isolates were stored at 4 C on PDA slants under sterile mineral oil until use. Plugs were cut with a sterilized cork borer from 14-day-old cultures growing on PDA at room temperature. The inoculated area was covered with soil, and the plants were returned to the greenhouse. Control plants were wounded in the same manner but not inoculated.

Greenhouse temperatures ranged from 25 to 30 C. The soil was kept moist and allowed to drain through most of each test but was dry for brief periods. The test was conducted seven times, with four to 11 replications per test. In all, 149 plants were inoculated with *F. solani,* and 134 plants served as controls.

Foliar symptoms were assessed three to five times after inoculation on a scale from 0 to 5 based on the percentage of leaf area affected: 0 = 0%; 1 = 1–10%; 2 = 11–30%; 3 = 31–70%; 4 = 41–90%; and 5 = 91–100%. The final evaluation occurred at growth stage R5 to R6 41–55 days after inoculation.

Pathogenicity of isolates. The pathogenicity of 11 single-spore isolates of *F. solani* from nine field locations was compared. All isolates were from soybeans with SDS foliar symptoms. The location, collection date, and cultivar source for each isolate are listed in Table 1. All isolates were grown on PDA by streaking a spore suspension over the surface of the agar and incubating plates at room temperature under fluorescent lights with a 12-hr photoperiod.

Inoculum plugs were cut from the edge of actively growing 10- to 14-day-old cultures with a sterile cork borer 0.5 cm in diameter. Fourteen-day-old soybeans (cv. Lee 74, maturity group VI) were inoculated with a single plug placed next to the stem immediately below the soil surface. The plants were grown in steam-pasteurized sandy soil in 500-ml plastic pots, with four plants per replicate pot and 10 replications per isolate. The plants were placed in the greenhouse with natural light and temperatures of 25–30 C. Soil was kept moist and allowed to drain throughout the experiments. The experiments were conducted between 25 February and 30 April 1988.

Foliar symptoms were rated weekly as described above. Six weeks after inoculation, stem height from the soil line was measured and the roots were carefully washed. The length of external discoloration was measured, and root mass was rated on a relative scale, with 0 = 0–10%, 1 = 10–30%, 2 = 30–70%, and 3 = 70–100% of the largest root system.

At the time of inoculation, the first trifoliate leaf was expanding. By the end of the experiment, the plants were at V-4 and were beginning to flower. The experiment was conducted twice.

Table 1. Location, date of collection, and source cultivar of isolates of *Fusarium solani* associated with sudden death syndrome of soybean

Isolate	Location	Date	Source cultivar
Mar 84[a]	Marianna, AR	3-11-85	Lee 74
Mar 86	Marianna, AR	9-10-86	Braxton
St. Ch.	St. Charles, AR	9-14-85	Lee 74
Imb	Cross County, AR	10-3-86	DPL 566
Woods	Cross County, AR	10-3-86	DPL 105
Van	Vanndale, AR	8-27-86	Unknown
Crit	Crittenden County, AR	7-24-86	DPL 105
Ill 1	Jackson County, IL	7-24-86	Unknown
Ill 2	Jackson County, IL	7-24-86	Unknown
Ky 1	Graves County, KY	9-15-86	Douglas
Ky 2	Graves County, KY	9-15-86	Douglas

[a]Isolated from soybeans grown in infested soil from Marianna, AR.

Table 2. Percentage of blue *Fusarium solani* (BFS) isolates among all fungi isolated from soybeans with symptoms of sudden death syndrome[a]

Location of collection	Cultivar	Plants with BFS/total plants[b]	Percentage of BFS among isolates from: Lateral roots	Epidermis of taproot	Cortex of taproot	Vascular tissue of lower stem
Marianna, AR	Braxton	6/6	27	13	22	7
Keiser, AR	Stephens	4/4	6	25	31	18
Cross County, AR (Woods farm)	DPL 105	5/5	43	5	25	0
Cross County, AR (Imboden farm)	DPL 566	5/5	38	40	19	0
Stuttgart, AR	Centennial	1/1	22	25	20	0
Arkansas County (Hornbec farm)	Coker 385	2/2	14	11	0	13
Corning, AR	Unknown	1/1	13	60	20	0
TriState Soybean Test	Bragg	3/3	3	27	14	9
Vanndale, AR	Unknown	11/11	41	33	18	6
Crittenden, AR	Lee 74 and DPL 105	5/7	4	14	3	NA[c]
Jackson County, IL	Unknown	12/19	8	13	6	NA[c]
Graves County, KY	Douglas	4/5	18	25	5	0
Total[d]		59/69 = 86%	72/386 = 19%	72/315 = 23%	40/324 = 12%	10/176 = 6%

[a]Samples were collected in 1985 and 1986.
[b]Number of plants with blue *F. solani*/total number of plants sampled.
[c]Data not available.
[d]BFS isolates/total number of isolates from each plant part.

RESULTS

Isolations from roots and stems. The blue *F. solani* was isolated from most parts of the plant root system from all field locations (Table 2). The highest frequency of isolation was generally from the epidermis of the taproot, followed by the lateral roots, the taproot cortex, and the discolored portion of the lower stem. Most of the sampled roots were discolored, but in some, only the secondary lateral roots were discolored. All roots had discolored vascular tissue.

Pathogenicity of the blue *F. solani*. Greenhouse-grown soybeans inoculated at flowering with the blue *F. solani* exhibited a reddish brown lesion at the soil line within 1 wk. Foliar symptoms began as chlorotic interveinal spots 2 wk after inoculation and progressed to necrotic streaks and defoliation typical of SDS in the field (Fig. 1A). The root systems of the inoculated plants were smaller than those of the controls, and the vascular tissue of the taproot and stem was discolored, but the pith remained white (Fig. 1B). At the end of the tests, 53–91% of the plants had symptoms that resembled SDS. In all seven experiments, 114 of the 149 plants inoculated with *F. solani* (76.5%) had these symptoms. Control plants did not develop the lesion at the soil line, foliar symptoms, or discoloration of the vascular tissue (Fig. 1B). Reisolation of the blue *F. solani* from the inoculated roots completed Koch's postulates.

Pathogenicity of isolates. Inoculations with all 11 isolates of *F. solani* resulted in discolored and reduced root systems, reduced plant height, and (except for isolate KY 1) foliar symptoms (Table 3, Fig. 2). Foliar symptoms were similar to those observed on SDS-affected plants in the field. They included interveinal

chlorotic spots, chlorotic streaks, and necrotic streaks and spots (Fig. 2B–D). The external reddish brown discoloration caused by cortical decay extended up the stem and down into the roots from the point of inoculation (Fig. 2E). This discoloration was observed immediately above the soil line 7 days after inoculation with all isolates, and initial foliar symptoms appeared subsequently with the most pathogenic isolates. Foliar symptoms developed throughout the experiment; expression was greatest 20–43 days after inoculation.

Isolates varied markedly in pathogenicity, as determined by plant size and the intensity of symptoms. Isolate KY 1 was the least pathogenic by all parameters, and Crit, KY 2, Mar 84, and Imb were the most pathogenic (Table 3). Most isolates reduced plant height compared to the control. There were significant correlations ($P < 0.001$) between values for foliar symptoms and plant height ($r = -0.74$), root mass and plant height ($r = 0.71$), and foliar symptoms and root mass ($r = -0.90$). All isolates except KY 1 produced a blue, slimy, appressed growth on PDA with little or no aerial mycelium and a maroon discoloration of the agar. Upon reisolation, isolate KY 1 produced the most aerial mycelium, small amounts of blue pigment, and no discoloration of the agar.

DISCUSSION

The blue *F. solani* was isolated from plants with SDS symptoms in all areas sampled. Although this sample was relatively small, it is significant that the fungus was isolated from diseased plants not only in Arkansas but also in Illinois and Kentucky (Table 2). This fungus has also been isolated from soybeans with SDS in Mississippi and has been shown to produce symptoms similar to SDS in the greenhouse (7).

Like other *F. solani* (2), this fungus produced a cortical root rot and was restricted to the roots and lower stem of diseased plants (Table 3, Fig. 1). The

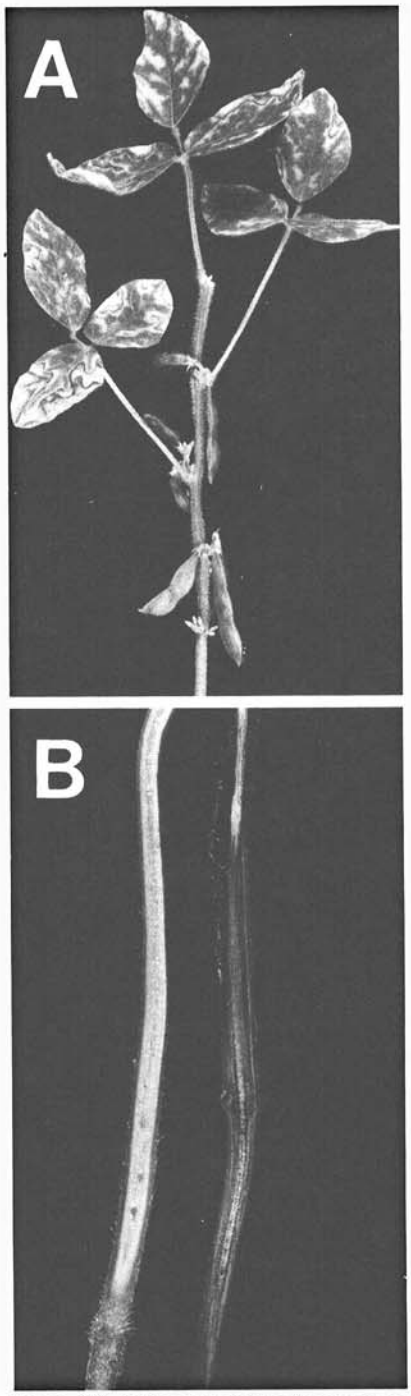

Fig. 1. (A) Foliar symptoms of greenhouse-grown Lee 74 soybean plants inoculated at flowering with mycelial plugs of the blue *Fusarium solani*. **(B)** Vascular tissue of a control plant (left) and an inoculated plant (right).

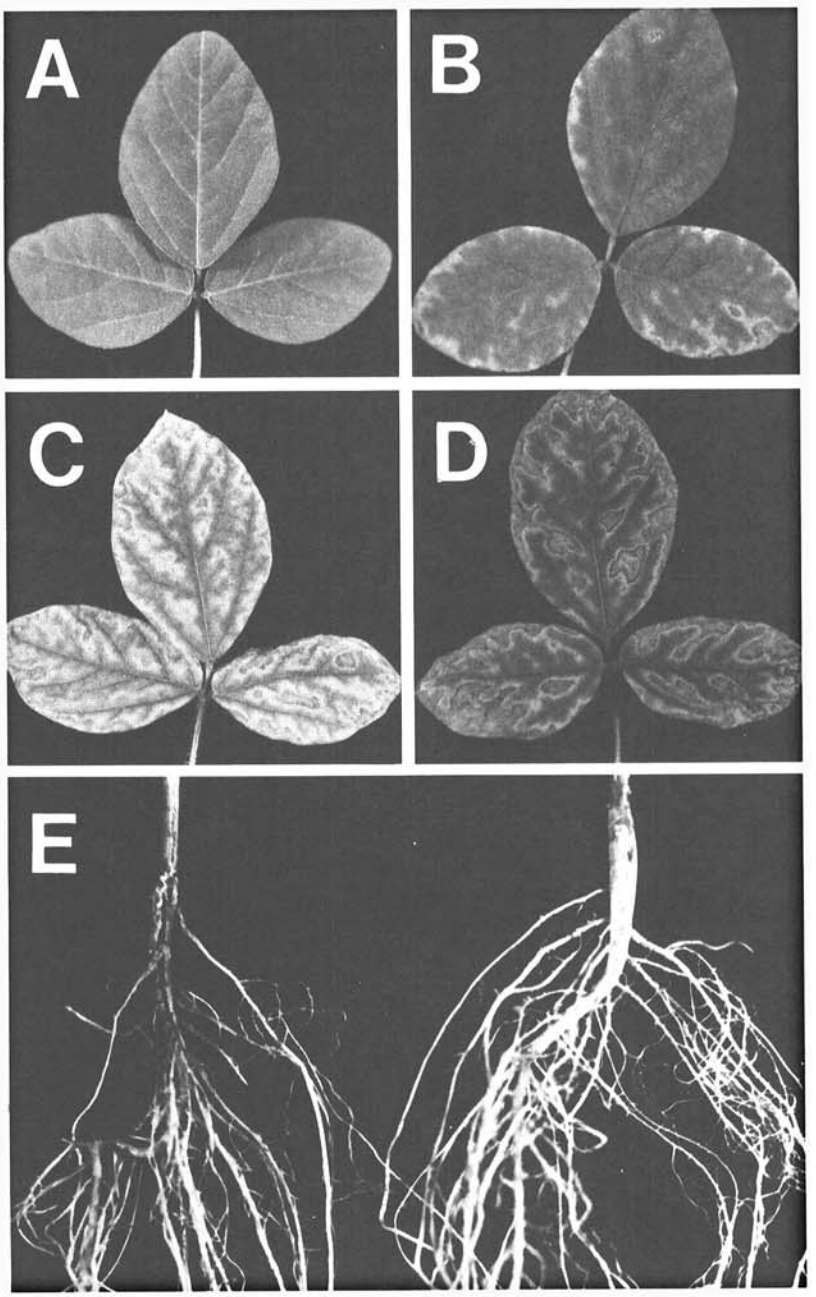

Fig. 2. (A) Leaf from uninoculated Lee 74 soybean seedling. **(B–D)** Leaves from Lee 74 soybean plants 6 wk after inoculation with mycelial plugs of the blue *Fusarium solani*. **(E)** Roots from an inoculated plant (left) and a control plant (right).

Table 3. Symptoms and plant growth response of Lee 74 soybeans 6 wk after inoculation with 11 isolates of the blue *Fusarium solani* recovered from soybeans with symptoms of sudden death syndrome collected from nine locations[x]

Isolate	Root mass[y]	Length of discolored lesions on roots and lower stem (cm)	Foliar symptoms[z]	Plant height (cm)
Mar 84	1.3 e	5.6 cd	3.1 e	15.6 e
Mar 86	1.7 cd	5.5 cd	1.7 cd	17.6 bcd
St. Ch.	2.3 b	3.0 b	0.3 a	19.0 ab
Imb	1.6 cd	5.3 cd	1.7 bcd	17.1 cd
Woods	1.8 cd	5.1 b	1.1 bc	18.6 abc
Van	1.6 de	5.2 cd	2.1 d	17.0 d
Crit	0.9 f	4.9 c	3.7 e	14.2 f
Ill 1	1.7 cd	4.9 c	1.8 c	18.1 abcd
Ill 2	1.9 c	5.8 d	1.0 d	18.2 abcd
Ky 1	2.5 b	2.5 b	0.0 a	19.0 ab
Ky 2	1.6 de	5.0 c	2.0 d	17.9 abcd
Control	2.6 a	0.1 a	0.0 a	19.4 a

[x] Plants were inoculated by placing an infested agar block next to the stem. Data are means of four seedlings in each of 10 replicate pots. Numbers followed by the same letter are not significantly different ($P = 0.05$) by Duncan's multiple range test.

[y] Root mass relative to largest uninoculated root system: 0 = 0–10%, 1 = 10–30%, 2 = 30–70%, 3 = 70–100%.

[z] Based on a visual rating of leaf area affected: 0 = 0%, 1 = 1–10%, 2 = 11–30%, 3 = 31–70%, 4 = 71–90%, 5 = 91–100%.

low frequency of recovery of the fungus from some plant parts could be the result of competition from secondary invaders or may reflect the limitations of the isolation method used because of the slow growth of this fungus. This study was restricted to fungi infecting the roots of plants with SDS. The levels and types of bacteria associated with these roots, such as *Xanthomonas* sp., were not determined.

Inoculations of greenhouse-grown soybeans with the blue *F. solani* resulted in symptoms typical of SDS in the field. The inoculated plants developed chlorotic interveinal spots that progressed to necrotic streaks and defoliation. Root systems were reduced, the vascular tissue was discolored, and the pith remained white. The fungus was reisolated from the inoculated plants, completing Koch's postulates. Although previous studies of the pathogenicity of *F. solani* on soybeans have reported reduced root systems, plant height, and yield, none have mentioned the striking foliar symptoms reported here (2). These foliar symptoms and the lack of microconidia suggest that the blue *F. solani* is a unique strain of this species.

As observed in the field, the physiologic age of the plant did not appear to affect disease development. In both 2-wk-old plants and plants at flowering, inoculation produced a similar pattern of symptom development typical of SDS, including vascular discoloration (Figs. 1 and 2). The midseason development of symptoms generally observed in the field may be the result of lower inoculum levels than those used in this study.

All isolates except KY 1 were nearly identical morphologically and produced symptoms similar to SDS in inoculation experiments (Table 3). After reisolation from inoculated plants, isolate KY 1 did not stain the agar or produce macroconidia in a blue, slimy mass as did the other isolates. This suggests that morphological characteristics may distinguish *F. solani* isolates that cause SDS symptoms from those that do not. Except for isolate KY 1, all isolates produced symptoms similar to SDS with varying degrees of severity (Table 3).

SDS shares certain characteristics with other diseases caused by *F. solani*. Root rots of *Phaseolus vulgaris* and *Pisum sativum* caused by *F. solani* develop under conditions that reduce root growth, such as anaerobic soil, drought, and hardpans (1,6). SDS has been associated with wet, cool weather at or after flowering, which favors saturated, anaerobic soil conditions. Cool weather may also stress the plant or favor the fungus. Development of SDS, however, has also been observed to increase under drought conditions in Arkansas (J. C. Rupe, *unpublished*). The soybean cyst nematode, often found associated with SDS, may represent another root stress factor that favors disease. In general, SDS has been observed in well-managed soybean fields with high yield potential.

The results presented here strongly suggest that a specific type of *F. solani* is involved in SDS. This fungus has been isolated from plants with SDS symptoms in Arkansas, Kentucky, and Illinois, demonstrating its wide distribution and consistent association with the disease. The fungus has also been isolated in Mississippi and shown to cause symptoms similar to SDS (7). The inoculation tests demonstrated that this fungus can, alone, produce symptoms consistent with those of SDS. Whether the blue *F. solani* is the sole causal agent of SDS or part of a complex with other pathogens such as *Xanthomonas* sp. or *H. glycines* remains to be determined.

Added in galley: A paper on the same subject by K. W. Roy, G. W. Lawrence, H. H. Hodges, K. S. McLean, and J. F. Killebrew appeared in *Phytopathology*, volume 79, number 2.

LITERATURE CITED

1. Burke, D. W., and Miller, D. E. 1983. Control of Fusarium root rot with resistant beans and cultural management. Plant Dis. 67:1312-1317.
2. Cheng, Y.-H. 1977. Pathogenicity of *Neocosmospora vasinfecta* and *Fusarium* spp. on soybean and their survival in the soil. M.S. thesis. University of Florida, Gainesville. 102 pp.
3. Hirrel, M. C. 1983. Sudden death syndrome of soybean—A disease of unknown etiology. (Abstr.) Phytopathology 73:501-502.
4. Hirrel, M. C. 1985. Sudden death syndrome: Assessment of cause and severity. (Abstr.) Page 78 in: Proc. Annu. Meet. South. Soybean Dis. Workers, 12th.
5. Hirrel, M. C. 1986. Disease severity and yield loss comparison of soybean maturity groups affected in sudden death syndrome. (Abstr.) Page 61 in: Proc. Annu. Meet. South. Soybean Dis. Workers, 13th.
6. Miller, D. E., and Burke, D. W. 1985. Effects of soil physical factors on resistance in beans to Fusarium root rot. Plant Dis. 69:324-327.
7. Roy, K. W., Lawrence, G. W., Hodges, H. H., McLean, K. S., and Killebrew, J. F. 1988. Etiology of sudden death syndrome of soybean. (Abstr.) Page 30 in: Proc. Annu. Meet. South. Soybean Dis. Workers, 15th.
8. Rupe, J. C. 1986. Soybean cultivar response to sudden death syndrome. Arkansas Farm Res. 36:7.
9. Rupe, J. C. 1987. Occurrence and pathogenicity of *Fusarium solani* recovered from soybean with sudden death syndrome. (Abstr.) Phytopathology 77:1689.
10. Rupe, J. C., and Weidemann, G. J. 1986. Pathogenicity of a *Fusarium* sp. isolated from soybean plants with sudden death syndrome. (Abstr.) Phytopathology 76:1080.
11. Sciumbato, G. L., and Keeling, B. L. 1985. Sudden death syndrome (SDS) of soybeans in Mississippi in 1984. (Abstr.) Page 64 in: Proc. Annu. Meet. South. Soybean Dis. Workers, 12th.
12. von Qualen, R. H., Abney, T. S., and Huber, D. M. 1986. Effect of tillage and crop rotation on premature dying of soybeans. (Abstr.) Phytopathology 76:1093.
13. Yopp, J., Krishnamani, M. R. S., Bozzola, J., Richardson, J., Myers, O., and Klubek, B. 1986. Presumptive role of a pathovar of *Xanthomonas* in sudden death syndrome of soybean. Microbios Lett. 32:75-79.