Fusarium solani on Soybean Roots: Nomenclature of the Causal Agent of Sudden Death Syndrome and Identity and Relevance of *F. solani* form B

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ABSTRACT

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Two morphological forms of *Fusarium solani* pathogenic on soybean, forms A and B, were further characterized and distinguished from other fungi. Questions regarding their identity were addressed. Soybean plants grown in the field and in a growth chamber were inoculated with *Fusarium solani* form A and *F. solani* f. sp. *phaseoli*. *F. solani* form A incited leaf symptoms typical of sudden death syndrome; *F. solani* f. sp. *phaseoli* did not. It was concluded that the two fungi are different and distinguishable on the basis of host specialization and the types of symptoms they incite on soybean. *F. solani* form A, the causal agent of sudden death syndrome, was designated *F. solani* (Mart.) Sacc. f. sp. *glycines* form. nov. Cultural and morphological characteristics of *F. solani* form B, the cause of seedling disease and root rot of soybean, and *Plectosphaerella cucumerina* were compared to determine the validity of a report indicating that they were conspecific. Results demonstrated that *F. solani* form B and *P. cucumerina* are distinctly different and not conspecific. The relevance of identifying and recognizing the two *F. solani* forms as separate and distinct members of the *F. solani* pathogen complex on soybean is discussed.

Additional keywords: *Fusarium tabacinum*, *Heterodera glycines*, host range

In 1988, Koch's postulates were completed with *Fusarium solani* (Mart.) Sacc. as the causal agent of seedling disease and root rot of older soybean (*Glycine max* (L.) Merr.) plants (19). Later, this same fungus, then called strain B, and an apparently different strain of *F. solani*, then called strain A, were found associated with roots of soybean plants symptomatic for sudden death syndrome (SDS). Through comparative pathogenicity tests, only strain A was found capable of inciting all the symptoms of SDS. Upon completion of Koch's postulates for SDS, a brief report was published in 1988 by Roy et al. (33), followed by comprehensive reports in 1989 by Roy et al. (34-36) and Rupe (40). In the 1989 reports by Roy et al. (34-36), the SDS causal agent was designated a morphological form of *F. solani*, form A. To distinguish the two fungi and ensure that the *F. solani* seedling/root rot pathogen would not be confused with the causal agent of SDS, the seedling/root rot fungus also was designated a morphological form, *F. solani*

form B (FSB). That same year, when Rupe (40) also reported completion of Koch's postulates for SDS, he referred to the causal fungus as a blue-pigmented strain of *F. solani* that is considered to be the same as FSA (41; J. C. Rupe, *personal communication*).

Although cultural and morphological characteristics distinguishing FSA and FSB were described (34-36) and illustrated (34) in 1989, there now exists doubt regarding the identity of these two fungi and confusion regarding the diseases they incite. In one paper (50), a study by Killebrew et al. (19) was cited to document that SDS had yield-reducing potential, although the article by Killebrew et al. dealt solely with the *F. solani* later designated FSB (34-36). In other papers, the study by Killebrew et al. was cited to support observations on variability in the SDS causal agent (51) and to document the production of SDS leaf symptoms on artificially inoculated plants in the greenhouse (52). Recent evidence from rDNA sequence and RFLP analyses (28) of several species within the *F. solani* complex led to the conclusion that *F. solani* f. sp. *phaseoli* (Burkholder) W.C. Snyder & H.N. Hans., the cause of root rot of bean (*Phaseolus vulgaris* L.) (20), was a phylogenetically distinct species comprised of both *F. solani* f. sp. *phaseoli* and FSA. On that basis, and because *F. solani* f. sp. *phaseoli* was described before FSA (48), *F. solani* f. sp. *phaseoli* was declared the causal agent of SDS, which in effect synonymized these two fungi. However, al-

though it was concluded that *F. solani* f. sp. *phaseoli* was the cause of SDS, soybean plants were not inoculated with *F. solani* f. sp. *phaseoli* to determine whether this organism would incite symptoms of SDS (28). Thus, a critical question left unanswered is whether *F. solani* f. sp. *phaseoli* and FSA are similar or dissimilar on the basis of physiological (host) specialization. Clearly, *F. solani* f. sp. *phaseoli* cannot be considered the causal agent of SDS of soybean, or a synonym of FSA, unless it incites all the symptoms characteristic of SDS, as is well documented for FSA (22,23,34-36). The major symptoms of SDS are root rot, crown necrosis, vascular discoloration of roots and stems, interveinal chlorosis and necrosis of leaves, premature defoliation, and flower and pod abortion (23,34,36,37,40,43,44).

The absence of reports of FSB isolation from roots of SDS-symptomatic plants by other researchers is unexpected given that the frequency of isolation of FSB from roots of such plants is usually as high as or higher than that of FSA (23,32,34-36,39). This suggests that FSB is not acknowledged as being separate and distinct from the SDS causal fungus. Recently, *F. solani* isolates from soybean roots that did not produce a blue pigment and produced both macroconidia and microconidia were observed (41). However, although these isolates were similar to FSB (34,35), the authors (41) did not identify them as FSB. In another study, FSB and *Plectosphaerella cucumerina* (Lindf.) W. Gams, a perithecial ascomycete, were found to be conspecific based on results of molecular analyses (28). However, this was not corroborated by a direct comparison of the cultural and morphological characteristics of these two fungi, nor was literature describing (34-36) and illustrating (34) FSB cited in that study. Apparently, this claim of conspecificity was accepted by Achenbach et al. (3), since they included *P. cucumerina* in tests with *F. solani*. Inexplicably, they made no reference to FSB or its association with *P. cucumerina*. In fact, their data conflict with the claim of conspecificity (28). Moreover, the conidial stage of *P. cucumerina* is not a *Fusarium* (31), although it was once thought to be *F. tabacinum* (Van Beymar) W. Gams (4,7,29,56).

Questions regarding the identity of FSA and FSB, the diseases they incite, and the relationship among these fungi and other

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strains of *F. solani* on soybean roots and other crops need to be resolved. Unless they are resolved, it is likely that confusion will grow, and with it a confounding of the literature. The main purpose of this report is to address those questions. It is shown that when isolates of FSA and *F. solani* f. sp. *phaseoli* are inoculated onto soybean, only FSA incites the leaf symptoms characteristic of SDS: thus, FSA is distinct from *F. solani* f. sp. *phaseoli*. FSA is further characterized culturally and morphologically, and a forma specialis designation is proposed for it. In addition, the identity of FSB as a form of *F. solani* is reaffirmed on the basis of cultural and morphological characteristics, and the importance of recognizing FSB as a pathogenic member of the *F. solani* complex on soybean is discussed.

An issue concerning the identity of *Nectria haematococca* Berk. & Broome and its connection to the SDS causal agent (1,2,28) is outside the scope of the present study and should be addressed by those directly involved in initially making that connection in 1989 (1).

MATERIALS AND METHODS

Sources and descriptions of *F. solani* and *P. cucumerina* isolates used in this study are listed in Table 1. All isolates were stored at ca. 5°C in slants of potato-dextrose agar prior to use.

Comparative pathogenicity tests with FSA and *F. solani* f. sp. *phaseoli*. Monospore isolates of FSA and *F. solani* f. sp. *phaseoli* were obtained using the method of Booth (4). In preliminary tests, isolates of FSA and *F. solani* f. sp. *phaseoli* were

wound-inoculated on soybean and green bean seedlings, respectively, and the reisolates from necrotic tissue then were used in main tests. Inocula for all tests were prepared by growing the fungi on sterilized sorghum seeds in 500 cm³ flasks. Moist sorghum seeds were autoclaved twice for 20 min, after which mycelial plugs from the isolates were separately and aseptically transferred to and intermixed with cooled seeds. Cultures were incubated under ordinary light conditions in the laboratory and were periodically shaken to obtain uniform distribution of inoculum during 3 weeks of incubation at 25°C.

FSA isolate 91-7 and *F. solani* f. sp. *phaseoli* isolates 13719 and 18006 from the American Type Culture Collection (ATCC) were used in the first two of a total of four pathogenicity tests. In test 1, soybean cv. Asgrow 4715 was planted at the Mississippi State University Plant Science Research Center on 1 May 1992, in each of four replicates (rows). From seven seedlings per replicate, soil was pulled back to expose the taproot, ca. 0.4 g of infested sorghum seeds were uniformly distributed around and in contact with the taproot about 2 cm below the soil line, and the soil was replaced. Equal numbers of control seedlings were treated with sterilized sorghum seeds only. At the time of inoculation, seedlings were in the V4 stage of growth (9). Following inoculation, the soil around test plants was watered to help prevent inoculum desiccation, but irrigation was not available during the growing season. In test 2, concurrent with test 1, Asgrow 4715 seedlings were grown in 10-cm-diameter pots of nonsterile greenhouse

potting soil outdoors in Starkville, Mississippi. The pots were maintained on a concrete surface. Four seedlings in the V2 stage of growth in each of four replicate pots were inoculated with each isolate as described above, and pots were arranged in a randomized complete block design. Plants were watered regularly to maintain high soil moisture, which favors infection (23,34,40). In both tests, the percentage of plants with SDS leaf symptoms was determined. The tests were terminated 7 days after expression of SDS leaf symptoms on the plants that were inoculated with isolates of FSA.

In tests 3 and 4, plants were grown in a controlled-environment growth chamber (Controlled Environment Limited, Winnipeg, Manitoba, Canada) at 25°C and with a 14-h photoperiod. Asgrow 4715 seedlings were grown in 10-cm-diameter plastic cups containing nonsterile greenhouse potting soil. At the V2 stage of growth, four seedlings in each of three replicates were inoculated with FSA isolate IN-F2X-11A or *F. solani* f. sp. *phaseoli* ATCC isolates 18006 and 38466 as described above. The percentage of plants with SDS leaf symptoms was determined during a 5-week incubation period, after which plants were removed from cups and examined for the presence of root necrosis. Symptoms on roots were rated as follows: 0 = no necrosis, 1 = slight necrosis, 2 = moderate necrosis, 3 = extensive necrosis, and 4 = very severe necrosis. The presence of test fungi in symptomatic roots was determined by surface-disinfecting root tissue in 1% sodium hypochlorite (NaOCl) for 1 min and plating tissue on PDA. Test 4 was a repeat of test 3.

Cultural and morphological characteristics of FSA. To supplement the description of FSA beyond that published in 1989 (34-36) and more precisely delineate this fungus, its growth response to temperature and the range in variability of its growth rate, colony characteristics, and macroconidial dimensions were investigated.

Ten isolates from soybean roots and cysts of *Heterodera glycines* Ichinohe, the soybean cyst nematode (SCN) (Table 1), were grown on PDA in darkness at 20, 25, and 30°C. In preliminary tests, relatively little growth occurred at 10, 15, or 30°C compared with 20 and 25°C. Uniform 5-mm-diameter cylinders of mycelium growing on PDA were transferred to the center of 9-cm petri plates of PDA. Four test plates (replicates) each were exposed to the three temperatures for 7 days, after which two measurements of colony diameter were taken at right angles and averaged. For each temperature, the variability in average colony diameter among isolates was estimated with the standard deviation.

Observations on the variability in colony characteristics were based largely on the examination of several hundred colonies of

Table 1. Sources and descriptions of fungi used in this study

Fungus isolate	Isolate no.	Source	Substrate origin	Geographic origin
<i>Fusarium solani</i> f. sp. <i>phaseoli</i>	13719	ATCC ^x	<i>Phaseolus vulgaris</i>	California
<i>F. solani</i> f. sp. <i>phaseoli</i>	18006	ATCC	<i>P. vulgaris</i>	California
<i>F. solani</i> f. sp. <i>phaseoli</i>	38466	ATCC	<i>P. vulgaris</i>	California
<i>F. solani</i> form A	IN-F2X-11A	T. S. Abney ^y	Soybean	Indiana
<i>F. solani</i> form A	91-7	Author	Soybean	Kentucky
<i>F. solani</i> form A	A-15	Author	Soybean	Kentucky
<i>F. solani</i> form A	A-16	Author	Soybean	Kentucky
<i>F. solani</i> form A	A-11	Author	Soybean	Mississippi
<i>F. solani</i> form A	A-12	Author	Soybean	Mississippi
<i>F. solani</i> form A	A-13	Author	Soybean	Arkansas
<i>F. solani</i> form A	A-14	Author	Soybean	Arkansas
<i>F. solani</i> form A	A-17	Author	<i>Heterodera glycines</i> cyst	Arkansas
<i>F. solani</i> form A	94-1	Author	<i>H. glycines</i> cyst	Illinois
<i>F. solani</i> form B	FSB-2	Author	Soybean	Illinois
<i>F. solani</i> form B	FSB-6	Author	Soybean	Kentucky
<i>F. solani</i> form B	F-465	Author	<i>H. glycines</i> cyst	Mississippi
<i>F. solani</i>	46472	ATCC	<i>H. glycines</i> cyst	Florida
<i>F. solani</i>	11712	ATCC	<i>Cucurbita pepo</i>	California
<i>Plectosphaerella cucumerina</i> ^z	IN-11P-12	T. S. Abney	Soybean	Indiana
<i>P. cucumerina</i> ^z	IN-2X-11N	T. S. Abney	Soybean	Indiana
<i>P. cucumerina</i> ^z	IN-11-12	T. S. Abney	Soybean	Indiana

^x American Type Culture Collection.

^y Department of Botany and Plant Pathology, Purdue University, W. Lafayette, Indiana.

^z Determined to be *P. cucumerina* by O'Donnell and Gray (28).

FSA growing on and from surface-disinfested soybean root sections that were cultured on PDA amended with streptomycin B sulfate (100 µg/liter) and aureomycin (2 µg/liter) (PDASA). The roots were obtained from SDS-symptomatic plants during disease surveys in several midwestern and southern states from 1986 to 1994. Publications from that work contain the methodology used for the assay of roots (23,32,34–36,38). Observations from examining the several hundred colonies of FSA were corroborated by examination of monospore cultures and mass transfer subcultures on PDA and PDASA. Cultures containing the root sections were incubated for 2 weeks at ca. 25°C under ordinary light conditions in the laboratory. Monospore and mass transfer cultures were grown for 2 weeks under the same conditions and at ca. 25°C under continuous fluorescent light (38 µE·s⁻¹·m⁻²). For comparison, isolates stored in slant cultures on PDA for 6 months to 1 year were grown under the same conditions.

The 10 isolates of FSA were grown on modified Bilay's medium (4) at 25°C under continuous fluorescent light (38 µE·s⁻¹·m⁻²). After 7 days, macroconidia were mounted in cotton blue-lactophenol, and the dimensions of 50 conidia per isolate were determined microscopically with an ocular micrometer. Variability in mean macroconidial dimensions among isolates was estimated with the standard deviation. In addition, 10 randomly selected soybean roots on which FSA was observed to be sporulating in the field were used to determine the size of macroconidia produced on the host. Subsequent to their collection (23,38), the roots had been stored under

dry condition at 10°C. Macroconidia from each root were mounted in cotton blue-lactophenol, and the dimensions of 25 spores per root were measured as described above.

Confirmation of the identity of FSB. FSB isolates F-465 and FSB-2 were compared with *F. solani* ATCC reference cultures no. 46472 and no. 11712. In contrast to fresh isolates of FSB, which sporulate well on PDA (34,35), the *F. solani* ATCC isolates did not sporulate well on this medium when first received. After selected sporulating sectors from PDA were cultured on modified Bilay's medium in light, the ATCC isolates sporulated profusely. On that basis, modified Bilay's medium was selected for comparison of reproductive structures. Isolates were grown on this medium at 25°C under continuous fluorescent light (38 µE s⁻¹ m⁻²). After 7 days, dimensions of macroconidia (100 per isolate) were determined as described above, and observations on conidial characteristics were made. Isolates were grown on PDA for observations on colony morphology. For chlamydospore production and characteristics, cultures were aged up to 3 weeks and plugs of mycelium were incubated in sterile water.

Comparative cultural and morphological characteristics of FSB and *P. cucumerina*. Characterization of *P. cucumerina* was limited primarily to the conidial stage of this fungus.

Based on reports that the optimal temperatures for growth of *P. cucumerina* (56) and FSB (39) on PDA were 25 and 30°C, respectively, and that *P. cucumerina* did not grow at 35°C (56), growth rates and responses of *P. cucumerina* and FSB to temperature were compared at 25, 30, and 35°C. *P. cucumerina* isolates IN-2X-11, IN-11P-12, and IN-11-12 and FSB isolates F-465, FSB-6, and FSB-2 were grown on PDA in darkness for 7 days at the three temperatures, after which colony diameters were determined as described above. Each treatment was replicated seven times, the experiment was repeated once, and treat-

ments were compared by analysis of variance. Colony characteristics and production of chlamydospores at 25°C also were determined in this experiment. Isolates were grown for 7 days on PDA at 25°C with continuous exposure to fluorescent light (38 µE·s⁻¹·m⁻²) for observations on colony and conidial characteristics and production of chlamydospores. In addition, colonies were aged up to 3 weeks, and plugs of mycelium were placed in sterile water for observations on chlamydospore production. Dimensions of conidia (100 per isolate) were determined as described above.

RESULTS

Comparative pathogenicity tests with FSA and *F. solani* f. sp. *phaseoli*. Although mild chlorosis occurred on a few plants, no leaf symptoms typical of SDS occurred on any plants inoculated with isolates of *F. solani* f. sp. *phaseoli* in the field in test 1 or in pots in test 2. Leaf symptoms typical of SDS occurred on 24% of the plants inoculated with FSA in test 1 and on 43% of those inoculated in test 2. In test 1, symptoms did not progress beyond mild interveinal necrosis on those leaves first symptomatic. In test 2, there also was little interveinal necrosis, but newly symptomatic leaves appeared on plants as late as the R5 growth stage. In the growth chamber tests 3 and 4 (Table 2), isolates of *F. solani* f. sp. *phaseoli* did not incite SDS leaf symptoms, but the incidence of FSA-inoculated plants showing SDS leaf symptoms was greater than in tests 1 and 2. Slight root necrosis was incited by *F. solani* f. sp. *phaseoli* no. 38466, and moderate root necrosis was incited by FSA (Table 2). The fungi were reisolated from surface-disinfested, symptomatic roots.

Cultural and morphological characteristics of FSA. FSA isolates grew slowly on PDA (Table 3). After 7 days at 25°C, colony diameters ranged from ca. 23 to 36 mm and averaged 24 mm. Growth at 25°C was greater than at 20 and 30°C. At each temperature, variability in mean growth rate among isolates was relatively low, based on standard deviations (±0.9, 0.9, and 1.2 at 20, 25, and 30°C, respectively).

Colony morphology and pigmentation were the same on PDASA and PDA. Colonies of FSA growing from root sections and those initiated from single spores or mass transfer exhibited similar characteristics. The blue pigmentation that has become associated with the SDS causal agent (34–36,40,41) was invariably produced by fresh cultures of FSA. However, colonies exhibited considerable variation in surface pigmentation due largely to the amount of sporulation. Within 2 to 3 days after infected root tissue was cultured on PDASA, sporodochia formed in situ and on the agar surface. Sporodochia of these wild-type colonies soon became confluent, producing effuse pionnote sporodochia (pseudopion-

Table 2. Comparative ability of *Fusarium solani* form A and *F. solani* f. sp. *phaseoli* to incite symptoms of sudden death syndrome (SDS) after inoculation on soybean cv. Asgrow 4715 in a growth chamber^w

Fungus	Plants with SDS leaf symptoms (%) ^x	Severity of root necrosis ^y
<i>F. solani</i> form A (IN-F2X-11A)	66	2.7*
<i>F. solani</i> f. sp. <i>phaseoli</i> 38466 ^z	0	1.6*
<i>F. solani</i> f. sp. <i>phaseoli</i> 18006 ^z	0	1.2
Control	0	0.7

^w Average of two tests.

^x Symptoms often appear first as chlorotic mottling, sometimes accompanied by upturning of leaf margins, followed by interveinal chlorosis that may progress to interveinal necrosis.

^y Average of two tests. Severity ratings: 0 = no necrosis, 1 = slight necrosis, 2 = moderate necrosis, 3 = extensive necrosis, 4 = very severe necrosis. An asterisk indicates a significant ($P \leq 0.05$) difference from the control as determined by analysis of variance and the LSD test.

^z American Type Culture Collection isolates.

Table 3. Colony diameters (mm) of 10 isolates of *Fusarium solani* form A after growth on potato-dextrose agar in darkness for 7 days at three different temperatures

Isolate	Temperature (°C)		
	20	25	30
IN-F2X-11	17.7	22.8	18.5
91-7	17.9	24.8	18.7
A-15	16.5	25.0	19.0
A-16	18.8	23.8	17.2
A-11	16.8	23.8	17.0
A-12	19.0	25.8	19.7
A-13	16.5	25.0	16.5
A-14	17.8	24.2	18.8
A-17	16.5	24.0	16.8
94-1	17.3	23.6	19.7
Mean	17.5	24.3	18.2
Standard deviations	±0.9	±0.9	±1.2

notes) with slimy masses of blue to blue-green macroconidia (Fig. 1A and B). When viewed in situ microscopically (100×), the conidia appeared reddish blue to dark blue. Isolates with varying degrees of sporula-

tion produced colonies with varying amounts of blue pigmentation. In some profusely sporulating isolates, the blue to blue-green conidial masses occupied nearly the entire colony surface except for an

outer fringe of grayish white, nonsporulating hyphae. The conidial mass radiating outward from the colony center usually was continuous, but small scattered groups of sporodochia sometimes appeared (Fig. 1B). Scattered, small, cream-colored conidial masses sometimes occurred in the older, central part of the colony (Fig. 1A and D). Dark blue (Fig. 1A and B), purple (Fig. 1C and D), and violet (Fig. 1E) spore masses tended to form and become admixed to varying degrees with the blue to blue-green spore masses as colonies aged. The purple or violet spore masses also occurred in wild-type colonies, but much less frequently than blue to blue-green spore masses, and they sometimes occurred as sectors radiating from the center (Fig. 1C and E), particularly in isolates subcultured on PDA for several generations. Transfers from purple or violet spore masses usually produced, at least initially, colonies with blue to blue-green spore masses. Prolonged storage of FSA on PDA slants invariably reduced sporulation and typical surface pigmentation with a concomitant increase in nonsporulating aerial mycelium (Fig. 1F). Transfers from sporulating regions of colonies that had become almost entirely mycelial usually gave rise to sporulating cultures.

On modified Bilay's medium, macroconidia ranged in size from ca. 34 to 66 µm long by 3.9 to 5.0 µm wide, and across isolates, they averaged 50.5 µm long by 4.6 µm wide (Table 4). Variability in mean length and width of macroconidia among the isolates was relatively low, based on standard deviations (± 2.7 for length and ± 0.2 for width). Dimensions of macroconidia produced on soybean roots in the field ranged from 38 to 48 µm long by 3.8 to 5.0 µm wide and averaged 42.5 µm long by 4.5 µm wide.

Confirmation of the identity of FSB. Colonies of FSB and the ATCC isolates of *F. solani* on PDA were very similar in appearance and, from a practical standpoint, were indistinguishable (Fig. 2). Although colony diameters were not measured, growth rates of the FSB and *F. solani* ATCC isolates were similar, as exemplified in Figure 2. On modified Bilay's medium, there were no fundamental differences among these isolates with respect to conidiogenesis, the morphology of conidiophores, microconidia, and macroconidia, and the morphology and arrangement of chlamydospores within hyphae (4,7,27). There was some variation among isolates in average size of macroconidia, but range in size was comparable (Table 5).

Comparative cultural and morphological characteristics of FSB and *P. cucumerina*. Colony diameters of each of the FSB isolates were significantly different ($P \leq 0.05$) from those of the *P. cucumerina* isolates at 25, 30, and 35°C. At 25°C, the average colony diameter (across three isolates) of *P. cucumerina* was 50%

Table 4. Dimensions of macroconidia of *Fusarium solani* form A isolates after growth of colonies on modified Bilay's medium for 7 days at 25°C under continuous fluorescent light (38 µE·s⁻¹·m⁻²)

Isolate	Size of macroconidia ^z			
	Length (µm)		Width (µm)	
	Mean	Range	Mean	Range
IN-F2X-11A	46.5	38.0 - 55.0	4.5	4.0 - 5.0
91-7	46.1	40.0 - 55.0	4.4	4.0 - 5.0
A-15	53.2	43.8 - 61.3	4.8	3.9 - 4.9
A-16	49.2	41.8 - 59.4	4.7	3.9 - 4.9
A-11	52.6	40.8 - 66.1	4.6	3.9 - 4.9
A-12	49.7	34.1 - 58.4	4.5	3.9 - 4.9
A-13	53.3	40.9 - 59.4	4.5	3.9 - 4.9
A-14	53.3	40.9 - 63.2	4.4	3.9 - 4.9
A-17	51.1	42.8 - 63.2	4.8	3.9 - 4.9
94-1	50.0	40.0 - 60.0	4.5	4.0 - 5.0
Mean across isolates	50.5		4.6	
Standard deviation	± 2.7	± 0.2		

^z Fifty conidia measured per isolate.

Fig. 1. Variability in the surface morphology and pigmentation of approximately 14-day-old colonies of *Fusarium solani* form A isolates on potato-dextrose agar (PDA). Isolates (A) A-15, (B) A-11, (C) A-14, (D) A-16, and (E) A-13 are first- or second-generation subcultures recovered from roots. Note typical blue to blue-green dominant pigmentation (A and B) with admixture of usually later produced dark blue (A and B), purple (C and D), and violet (E) pigmentation, and cream-colored spore masses (arrows; A and D). Isolate (F) A-15 was subcultured on PDA several times and stored in a PDA slant for 8 months prior to being transferred to plates of PDA.

of the average colony diameter (across three isolates) of FSB (56 mm). At 30°C, the average colony diameter of *P. cucumerina* was 43% of the average for FSB (71 mm). At 35°C, a temperature at which FSB grew well (48 mm), *P. cucumerina* did not grow. The disparity in growth rate is exemplified in Figure 3.

On PDA at 25°C, 7-day-old colonies of FSB produced a cream-colored to tan mycelium (Fig. 3). Sporodochia were usually cream-colored to buff, mainly scattered, or less often, in a concentric pattern. With aging, relatively small bluish or greenish sporodochia were produced by some isolates. Three- and four-septate macroconidia predominated in FSB isolates, but macroconidia with more than four septa also were produced. The long microconidiophores with false heads of typically aseptate, but sometimes one-septate, microconidia were perhaps routinely the most easily recognizable and useful character in preliminary identification of FSB. It was common for fresh isolates to produce microconidiophores that exceeded 200 µm in length. Microconidia ranged in size from 11 to 15 µm long by 2 to 4 µm wide. Chlamydospores were produced singly or in pairs in the hyphae and measured 6.9 to 9.4 µm diameter. No sexual fruiting bodies were formed by FSB. Seven-day-old colonies of *P. cucumerina* produced a cream-colored to pinkish mycelium (Fig. 3). Conidiophores of *P. cucumerina* did not aggregate into sporodochia, but were often produced on hyphal ropes in the older part of the colony. Aging colonies grown in darkness were observed to produce radial folds, but this was not observed for colonies in continuous light. The majority of the conidia in *P. cucumerina* were aseptate, some were one-septate, and a few were two-septate. Some of these fell within the size range of FSB microconidia, but they were substantially smaller than FSB macroconidia (Table 6). *P. cucumerina* did not produce chlamydospores. After 8 to 10 days of growth, the fungus began producing perithecia. After an additional 3 to 7 days, brown perithecia typical for this species (4,7) occupied much of the colony of each isolate.

DISCUSSION

Isolates of *F. solani* f. sp. *phaseoli* did not incite the leaf symptoms characteristic of SDS (23,34,36,37,40), while isolates of FSA did. In other laboratories, isolates of *F. solani* f. sp. *phaseoli* (3) and isolates of *F. solani* from bean (16,17) also did not incite SDS leaf symptoms when inoculated on soybean. Therefore, *F. solani* f. sp. *phaseoli* and FSA are not the same forma specialis, and *F. solani* f. sp. *phaseoli* is not the causal agent of SDS. The present study reaffirms that FSA is the causal agent of SDS, and this is supported by other past and present sources of evidence. Considering that the FSA type of Roy et al. (34)

and the blue-pigmented *F. solani* strain of Rupe (40) are the same fungus (41; J. C. Rupe, *personal communication*), Koch's postulates were completed for SDS with isolates of this fungus at least four times (23,34,40,52). In contrast, Koch's postulates have never been completed for SDS with bona fide cultures of *F. solani* f. sp. *phaseoli*. Although O'Donnell and Gray (28) inoculated soybean with an isolate of *F. solani* ("Mont-1"), reproduced the symptoms of SDS, and reisolated the fun-

gus from diseased tissue, "Mont-1" and isolates of FSA they used in their study were identical based on molecular analyses. Thus, in essence, they completed Koch's postulates with an additional isolate of FSA rather than with *F. solani* f. sp. *phaseoli*, and thereby confirmed the earlier etiological findings (23,34,40,52). In addition, although O'Donnell and Gray emphasized the close genetic relatedness of FSA and *F. solani* f. sp. *phaseoli*, the unique nucleotide base pair substitution they

Fig. 2. Comparison of *Fusarium solani* form B isolate F-465 (left) and *F. solani* American Type Culture Collection isolate no. 46472 (right) colonies and growth rates on potato-dextrose agar.

Fig. 3. Comparison of *Fusarium solani* form B isolate F-465 (left) and *Plectosphaerella cucumerina* isolate IN-2X-11N (right) colonies and growth rates on potato-dextrose agar.

found for FSA may reflect a difference from *F. solani* f. sp. *phaseoli* consistent with pathogenicity results of the present study and with studies by Achenbach et al. (3) and Rupe et al. (42). Although still maintaining that SDS-inciting isolates of *F. solani* represented a subgroup within *F. solani* f. sp. *phaseoli*, Achenbach et al. (3) found such isolates to be distinct from this forma specialis on the basis of random amplified polymorphic DNA (RAPD) analysis. Rupe et al. found from mitochondrial DNA restriction fragment length polymorphism analyses that SDS-inciting isolates of *F. solani* from soybean were genetically distinct from nonsoybean isolates of *F. solani* that did not incite SDS (42), some of which were *F. solani* f. sp. *phaseoli* (J. C. Rupe, *personal communication*).

Host adaptation and the nature of the symptoms a causal agent incites on hosts are essential features of formae speciales (4,15). In the 55 years since the *F. solani* bean root rot pathogen was designated a forma specialis adapted to *Phaseolus* (48), only one report (28) associated this fungus with a disease of soybean. Indeed, soybean has been considered a nonhost of *F. solani* f. sp. *phaseoli* and thus potentially useful as a rotation crop in controlling bean root rot and in reducing *F. solani* f. sp. *phaseoli* soil inoculum (21,45,49). Particularly illuminating in this regard are results of a rotation study conducted in a field with a history of bean root rot: the inoculum density of *F. solani* f. sp. *phaseoli* alternately decreased when soybeans were grown and increased when beans were grown (13),

and furthermore, SDS leaf symptoms were not observed to occur on soybean plants (R. Hall, *personal communication*). Melgar and Roy (22) inoculated 21 plant species with FSA and found that the fungus could infect only nonwounded soybean and mung bean (*Vigna radiata* (L.) R. Wilcz.) and wounded green bean, cowpea (*V. unguiculata* (L.) Walp. subsp. *unguiculata*), and lima bean (*Phaseolus lunatus* L.) plants. Gray (11) also reported that soybean, mung bean, and green bean were hosts of the SDS causal agent. Thus, although FSA and *F. solani* f. sp. *phaseoli* both can infect soybean (Table 2; 22), mung bean (22,46), green bean, cowpea, and lima bean roots (8,14,22), their requirements for pathogenicity are different; wounding of green bean, cowpea, and lima bean roots was required for infection by FSA, indicating that these plant species were less compatible as hosts than soybean and mung bean (22); whereas wounding was not required for infection of these plant species by *F. solani* f. sp. *phaseoli* (45,49,53). More important, however, is the distinction that has been made between FSA and *F. solani* f. sp. *phaseoli* based on the types of symptoms they incite on soybean: *F. solani* f. sp. *phaseoli* incites mild necrosis on soybean roots but does not incite SDS leaf symptoms, while FSA incites greater root necrosis and also typical SDS leaf symptoms (Table 2; 34–36).

Messiaen and Cassini (24) considered *F. solani* to be less specialized in its host adaptation than other fusaria, e.g., *F. oxysporum*, and therefore formae speciales of *F. solani* are determined more by the types

of symptoms they incite than by host specialization per se. For example, *F. solani* f. sp. *phaseoli*, the bean root rot pathogen, and *F. solani* f. sp. *pisi* (F.R. Jones) W.C. Snyder & H.N. Hans., the pea root rot pathogen, each cause mild necrosis on roots of the opposite host but a serious disease only on the designated host (30). Also, *F. solani* f. sp. *eumartii* (C. Carpenter) W.C. Snyder & H.N. Hans. and race 2 of *F. solani* f. sp. *radicicola* (Wr.) W.C. Snyder & H.N. Hans. both infect potato but incite different symptoms (4,24). Thus, a parallel situation appears to exist for FSA and *F. solani* f. sp. *phaseoli*.

The ability of *F. solani* f. sp. *phaseoli* to incite limited necrosis on soybean roots is not unusual, since FSB (18,19,34), the *F. solani* associated with Essex syndrome (12), and other *F. solani* strains from soybean (3,10,55) can also cause necrosis on soybean roots. None of these fungi, however, incite SDS leaf symptoms. An ability to incite necrosis on soybean roots is not decisive in assigning causality for SDS because leaf symptoms can be incited independently of root symptoms, as shown by Roy et al. (34). They wound-inoculated FSA into soybean hypocotyls, and with no involvement of root necrosis, leaf symptoms typical for SDS were produced. Such results suggest the involvement of a phytotoxin in leaf symptom expression, and this is substantiated by more direct evidence. Jin et al. (16) identified a polypeptide from culture filtrates of SDS-inciting isolates of *F. solani* from soybean that caused some of the leaf symptoms of SDS. Isolates of *F. solani* that did not incite SDS leaf symptoms on soybean also failed to produce the phytotoxic polypeptide; most of the nonsoybean isolates were from leguminous plant species, including two from bean (16,17). In contrast, phytotoxins are not involved in the etiology of bean root rot, caused by *F. solani* f. sp. *phaseoli* (5,20). The results of Jin et al. (16,17) are compatible with those in the present study and strongly suggest that the unique ability of FSA to incite SDS leaf symptoms is conferred, at least in part, by the phytotoxic polypeptide.

In addition to the above distinctions between FSA and *F. solani* f. sp. *phaseoli*, the host range of FSA (22) differs from the host ranges of other formae speciales of *F. solani* (4,20,48). Thus, cumulative evidence indicates that the unique ability of FSA to incite SDS, particularly the leaf symptoms, and its relatively limited host range, warrant its designation as a forma specialis (15). Therefore, FSA is designated *F. solani* (Mart.) Sacc. f. sp. *glycines* form. nov. Representative cultures of the fungus from soybean roots and SCN cysts have been deposited with the ATCC. Isolates representative of *F. solani* f. sp. *glycines* are described in this and other studies (34–36). In addition to their capacity to incite SDS symptoms in soybean, *F. solani*

Table 5. Comparative dimensions of macroconidia of *Fusarium solani* form B isolates and isolates of *F. solani* from the American Type Culture Collection after growth of colonies on modified Bilay's medium for 7 days at 25°C under continuous fluorescent light (38 $\mu\text{E}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$)

Fungus	Isolate no.	Size of conidia ^y			
		Length (μm)		Width (μm)	
		Mean	Range	Mean	Range
<i>F. solani</i> form B	F-465	33.8	24-46	4.4	4-5
<i>F. solani</i> form B	FSB-2	34.6	29-43	4.4	4-5
<i>F. solani</i>	46472 ^z	36.1	29-46	4.7	4-6
<i>F. solani</i>	11712 ^z	31.6	24-39	4.3	4-5

^y One hundred conidia measured per isolate.

^z American Type Culture Collection isolate numbers.

Table 6. Comparative dimensions of *Fusarium solani* form B macroconidia and *Plectosphaerella cucumerina* conidia after growth of colonies on potato-dextrose agar for 7 days at 25°C under continuous fluorescent light (38 $\mu\text{E}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$)

Fungus	Isolate no.	Size of conidia ^z			
		Length (μm)		Width (μm)	
		Mean	Range	Mean	Range
<i>F. solani</i> form B	F-465	37.8	27-51	4.8	4-6
<i>F. solani</i> form B	FSB-6	34.5	26-50	4.7	4-6
<i>F. solani</i> form B	FSB-2	38.6	32-48	4.7	4-6
<i>P. cucumerina</i>	IN-11-12	8.0	7-9	2.7	2-3
<i>P. cucumerina</i>	IN-11P-12	7.4	6-11	2.4	2-3
<i>P. cucumerina</i>	IN-2X-11	6.7	6-8	2.4	2-3

^z One hundred conidia measured per isolate.

f. sp. *glycines* isolates are further distinguished from other forms of *F. solani* that occur on soybean roots (12,34,38) by several morphological features. *F. solani* f. sp. *glycines* grows more slowly (Table 3) and has longer macroconidia (Table 4) than FSB (34-36), the Essex syndrome *F. solani* (12), and *F. solani* f. sp. *pisi* (20,54). Moreover, *F. solani* f. sp. *glycines* rarely, if ever, produces microconidia, in contrast to FSB (34-36), the Essex syndrome *F. solani* (12), and *F. solani* f. sp. *pisi* (20,30).

Evidence from this study clearly shows that FSB and *P. cucumerina* are not conspecific. Apparently, authentic cultures representative of FSB (Figs. 2 and 3; 18,19,34,35) were not used in an earlier study that concluded FSB and *P. cucumerina* were conspecific (28). Colonies of FSB are easily distinguishable from those of *P. cucumerina*, and FSB grows faster and at temperatures beyond what *P. cucumerina* will tolerate. Other characteristics of FSB that distinguish it from *P. cucumerina* are the production of sporodochia, lack of hyphal ropes, production of both macroconidia and microconidia, production of conidia that are larger and have more septa, production of chlamydospores, and absence of a sexual stage.

There are other distinctions, ecological and pathological in nature, between the two fungi. *F. solani*, including FSB, is one of the most common fungi isolated from surface-disinfested SCN cysts (6,26,32, 38,39). Although *P. cucumerina* has been isolated from cysts (6), it is not a common isolate. Carris et al. (6) isolated *P. cucumerina* from three, and *F. solani* from about 800 of 5,000 assayed cysts. *P. cucumerina* causes no known disease on soybean, its pathogenicity to plants is seldom demonstrated, and it is a relatively weak pathogen on its plant hosts (4,7,29,56). Pathogenicity of FSB on soybean is well documented (18,19,34).

Results of the comparison of FSB with ATCC isolates of *F. solani* confirm that FSB is *F. solani*. The ATCC isolates were virtually indistinguishable from FSB, and they are representative of FSB isolates originating from soybean root tissue or SCN cysts (19,32,34,39). Cultures of FSB from soybean roots and SCN cysts, and cultures of *P. cucumerina* from soybean roots, have been deposited with the ATCC.

It is possible that a *Cephalosporiopsis* sp. isolated from soybean reproductive structures (25), which has some similarities with the conidial stage of *P. cucumerina* (4,7), is actually the latter species. Pascoe et al. (29) initially identified *P. cucumerina* as *Cephalosporiopsis*, and *C. imperfecta* F. & V. Moreau is a synonym of the anamorph (7).

FSB is one of several strains of *F. solani* that occur on soybean roots (10,12,18,19, 34,55). This situation is typical for soil-borne fusaria, and it parallels the situation in bean, where saprophytic and plant-

pathogenic clones of *F. solani* coexist (47). FSB is not defined as a narrowly circumscribed morphological entity (34,35). This fungus was never formally described in a taxonomic sense, and the word "form" implies no taxonomic significance. To have defined it as such would have disregarded the other strains of *F. solani* on soybean that also are not well delineated morphologically (10,12,55). Rather, FSB represents a strain that is morphologically and pathogenically distinct from *F. solani* f. sp. *glycines* and is comprised of pathogenic *F. solani* isolates that cause seedling disease and root rot of older soybean plants (18,19,34). FSB should not be equated with strains of *F. solani* that occur on aerial parts of soybean because no comparison has been made of their morphological characteristics and pathogenic capabilities. Strains from aerial parts of soybean may differ from FSB, based on previous observations (25).

It is clear from careful reading of the literature and from the results presented in this study that *F. solani* f. sp. *glycines* and FSB are morphologically distinct fungi that incite separate and distinct soybean diseases. Further, more subtle distinctions were recently reported. In contrast to *F. solani* f. sp. *glycines*, FSB occurs in soybean roots and SCN cysts regardless of the presence of SDS (39), and it does not produce conspicuous blue masses of macroconidia on diseased roots in the field (24,38). Because the complex of *F. solani* strains on soybean roots includes, in addition to *F. solani* f. sp. *glycines* and FSB, the *F. solani* involved in the etiology of Essex syndrome (12), *F. solani* f. sp. *pisi* (38), and possibly other strains (10,12,55), much work remains in characterizing the entire complex and distinguishing members of the complex from each other and particularly from *F. solani* f. sp. *glycines* and FSB. Meanwhile, it is not sufficient to refer to isolates of *F. solani* from soybean roots merely as *F. solani* without at least briefly describing their salient morphological features. Failure to do so likely will lead to their confusion with *F. solani* f. sp. *glycines* or FSB, or to other complications. The cultural history, pathogenic history, and source of an *F. solani* isolate from soybean cannot be disregarded. For example, Achenbach et al. (3) provided little such information with respect to isolates of FSA, particularly FSA-1. They incorrectly cited Melgar et al. (23) as "Melgar and Roy" to document cultural conditions under which FSA-1 was maintained and the method by which it had previously been tested for pathogenicity, while omitting the correct article (22). FSA-1 was not used by Melgar et al. (23); the FSA isolate they used was inoculated by infestation of soil in field microplots, and SDS leaf symptoms were incited. Furthermore, Achenbach et al. (3) listed K. Roy as the source of FSA-1 for their study, which implies

that Roy maintained FSA-1 in culture immediately prior to its use in their study. In fact, Roy recovered FSA-1 in 1989 from soybean plants exhibiting SDS foliar symptoms, gave a culture of this isolate to another researcher (not a coauthor of Achenbach et al.) in 1990, and thereafter had no knowledge of its history. Conclusions by Achenbach et al. (3) that the isolates of FSA they tested no longer "produce" SDS foliar symptoms and that this might be explainable on the basis of repeated subculturing of isolates or dissimilar methods of inoculations in their study compared with the study by "Melgar and Roy" must be evaluated in light of the aforementioned facts.

Confusion regarding the identity and characteristics of pathogenic forms of *F. solani* from soybean confounds the literature and impedes progress in understanding and managing the soybean diseases caused by *F. solani*. This is particularly important with respect to SDS, a disease already complicated enough by the interactive involvements of soybean, *F. solani* f. sp. *glycines*, and SCN (23,32,34,36,39,40,44).

ADDENDUM

Four papers published in 1996 or 1997 in the journal Crop Science referred to the SDS causal agent as *F. solani* type A (Njiti et al., 36:1165-1170) or *F. solani* f. sp. *phaseoli* type A (Chang et al., 36:1684-1688; Hnetkovsky et al., 36:393-400; and Njiti et al., 37:132-138). The ill-chosen and certain-to-be-confusing abbreviation "FSA" was used by Hnetkovsky et al. for *F. solani* f. sp. *phaseoli* type A. An article published in 1996 by Gray and Achenbach in the journal Plant Disease (80:1197-1199) ironically referred to the SDS causal agent as represented by "specific isolates" of *F. solani* rather than *F. solani* f. sp. *phaseoli*, as proposed by Gray only a year earlier in O'Donnell and Gray (28). Moreover, Gray and Achenbach cited J. Rupe as the source of isolate FSA-1, and in contrast to the report by Achenbach et al. (3), which was also published in 1996, this isolate incited SDS leaf symptoms on all soybean plants inoculated by Gray and Achenbach in each of four trials. It was noted that neither the Crop Science papers nor the Gray and Achenbach paper cited the work of O'Donnell and Gray which originally applied the name *F. solani* f. sp. *phaseoli* to the SDS causal agent (28).

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