Use of RAPD Markers as a Diagnostic Tool for the Identification of *Fusarium solani* Isolates that Cause Soybean Sudden Death Syndrome

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ABSTRACT

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Fusarium solani is the etiological agent of soybean sudden death syndrome (SDS). Random amplified polymorphic DNA (RAPD) analysis was used to differentiate isolates that cause SDS from those that do not. Greenhouse assays with a sand/cornmeal inoculum method were performed to assess virulence of the fungal isolates and the results were compared with the RAPD pattern analysis. The RAPD results exhibited 100% association with greenhouse virulence assay results. Amplification patterns generated by RAPD reactions were used to generate a phenogram depicting the genetically distinct nature of the cluster defined by the SDS isolates. This cluster was exclusive and distinct from *F. solani* f. sp. *phaseoli* isolates that do not cause SDS. Thus, the RAPD approach proved a sensitive and highly reliable method for quickly identifying fungal pathotypes that cause soybean SDS.

Soybean (*Glycine max* (L.) Merr.) sudden death syndrome (SDS) was first observed in Arkansas and Tennessee in 1971 (13). The disease has now been reported in most states in the lower Midwest. Common symptoms include root rot followed by interveinal necrosis, defoliation, and, eventually, death. Occurrence and severity of the disease appear to be affected by environmental conditions. SDS severity is often greater in soybean crops with high yield potential (12).

In 1989, the soilborne fungus *Fusarium solani* (Mart.) Appel & Wollenweb. emend. W. C. Snyder & H. N. Hans. was identified as the etiological agent of soybean SDS (11,12). Classic morphological techniques are typically used to differentiate the pathogenic isolates that cause SDS from isolates that do not cause the disease (7,11). Although *F. solani* isolates that cause SDS have been used extensively in greenhouse assays to identify SDS-resistant soybean cultivars, little is known of the genotypic diversity of these fungal isolates. Recently, O'Donnell and Gray (9), based on ribosomal internal transcribed spacer and partial 28S rDNA sequencing and restriction mapping, suggested that isolates causing SDS be included in the form species *Fusarium solani* f. sp. *phaseoli*. However, as this group of fungi also includes isolates that do not cause SDS, O'Donnell and Gray state that the isolates causing SDS may represent a distinct population within this form species (9).

Since its development in 1991 (16), random amplified polymorphic DNA (RAPD) analysis has been used to assess genetic variability in a wide variety of organisms, including fungi (2,3,6,15). Because RAPDs are based on the polymerase chain reaction (PCR), very little starting DNA material is necessary in the reaction. Here we report the use of RAPD analysis to quickly and accurately differentiate *F. solani* isolates that cause soybean SDS.

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MATERIALS AND METHODS

Fungal cultures. The species, isolate name, origin, and host plant of the isolates used in this study are shown in Table 1. Each fungal culture was started from a single spore. All isolates were maintained on slants of Bilay's medium at 19°C (4). None of the cultures were serially transferred; rather, the slant culture was used each time inoculum was started.

Genomic DNA isolation. To obtain mycelium for DNA extractions, fungal isolates were grown on Bilay's medium and inoculations were aseptically transferred to 50-ml aliquots of liquid potato dextrose broth (Difco Laboratories, Detroit, MI) in 250-ml Erlenmeyer flasks. Cultures were grown at 28°C with shaking for 1 to 2 weeks. Mycelium was collected by vacuum filtration with a Buchner funnel lined with sterile cheesecloth. DNA extractions were performed according to the method of Nickrent (8).

Primers and PCR amplification conditions. Oligonucleotide primer kits were purchased from Operon Technologies (Alameda, CA). Primers PMO2, PMO4, and RCO8 were synthesized at the DNA Synthesis Facility at the University of Illinois-Urbana. All primers were diluted to 20 ng/µl. Amplification reactions were performed in 25-µl volumes containing 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.1% Triton X-100, 1.2 mM $MgCl_2$, 0.2 mM each dATP, dCTP, dGTP, and dTTP (United States Biochemical, Cleveland, OH), 20 ng of primer, 0.2 µl of *Taq* DNA polymerase (Promega, Madison, WI), and approximately 100 ng of genomic DNA. The samples were overlaid with 30 µl of mineral oil (light white). Amplifications were performed in a Perkin-Elmer Cetus (Norwalk, CT) DNA Thermal Cycler programmed for the following parameters: 94°C for 3 min, followed by 45 cycles of 94°C for 1 min, 36°C for 1 min, 72°C for 2 min, and a final incubation at 72°C for 10 min. Amplification products (4 µl of a 25-µl reaction) were electrophoresed in 0.7% agarose gels with TBE (Tris-borate EDTA) running buffer, stained with ethidium bromide and either scanned into a computer imaging file or photographed (14).

Data analysis. RAPD bands generated from all of the isolates listed in Table 1 with eight of the primers were assessed and used to construct a binary (+/–) matrix of 219 characters (+ = band present, – = band absent; matrix available on request). A distance matrix was generated and analyzed by the unweighted pair-group method of analysis (UPGMA) in the Numerical Taxonomy and Multivariate Analysis System (NTSYS-pc, version 1.8) program and by bootstrap analysis in Phylip 3.57 for the PowerMac (developed by J. Felsenstein, Department of Genetics, University of Washington, Seattle).

Inoculum production. Cultures for inoculum were started by removing a small piece of mycelium from the Bilay's slant to a 10-cm plate of 5× Bilay's medium. The cultures were grown at 27°C for 2 weeks. Two agar pieces, 1 × 0.5 cm, were removed from the 2-week-old culture, ground up in 3 ml of sterile water, and

added to a 500-ml flask containing a sterile mix of 70 g of fine sand, 10 g of cornmeal, and 50 ml of distilled water. The cultures were grown at 27°C for 2 weeks. This sand/cornmeal inoculum was then weighed and mixed with a heat-pasteurized clay-loam soil/sand mix (1:1, vol/vol) at a ratio of 20 g of soil/sand mix to 1 g of inoculum.

Virulence assays. Spencer soybean seeds were planted in flats of heat-treated sand and grown for 10 days. Soybean seedlings were removed from the sand flats and placed in individual 4 × 20 cm Cone-Tainers (Stuewe & Sons, Inc., Corvallis, OR). The tubes were pre-filled with a heat-treated soil/sand mix (1:1, vol/vol) to a depth of 12 cm and placed in a tray of water for 1 day before transplanting to allow for uniform moisture. A soybean seedling was placed in the Cone-Tainer on top of the soil and the soil/sand/cornmeal inoculum mix was added to fill the Cone-Tainer. Soybean plants were grown in a growth chamber for 3 weeks at 25°C day, 22°C night temperature with 12-h temperature periods under a day-period light intensity of 250 $\mu E \cdot s^{-1} \cdot m^{-2}$. Uniform soil moisture was maintained in each tube during the course of the experiment by keeping the Cone-Tainers in a tray of water with a constant water level. There were four replications (one plant per replication) per fungal isolate and each experiment was conducted twice. The experimental treatments were arranged in a completely randomized design.

A similar experiment was conducted with Spencer soybean and Top Crop garden bean. The same methodology and experimental design were used. The experiment was conducted twice.

At the end of each experiment, the plants were removed from the Cone-Tainers and the soil washed from the roots. Top and root weights of each plant was determined. The total number of leaflets per plant was counted (average of eight leaflets per plant) and the mean number of leaflets per plant with SDS symptoms (e.g., interveinal necrosis) was determined; leaflet counts were taken before any defoliation occurred. Leaf symptom severity for each plant was determined as a percentage of plant leaflets with SDS symptoms. The severity of root and crown rot on each inoculated plant was assessed according to a rating scale of 1 to 5 as follows: 1 = no root or crown necrosis, root and crown tissue healthy; 2 = 25% of feeder and tap root tissue exhibiting necrosis, no crown necrosis; 3 = 25 to 50% of feeder and tap root tissue exhibiting necrosis; no crown necrosis; 4 = >50% of root and crown tissue necrotic; and 5 = root and crown tissue necrotic, plants dead.

Data were analyzed as a completely random design, using Statistica 4.1 for the Macintosh. Treatment mean differences were compared by least significant difference (LSD).

Table 1. Origins of fungal isolates used in this study

Species	Isolate	Origin	Host	Source
Septoria glycines	Septoria	Illinois	*Glycine max*	G. Hartman
Plectosphaerella cucumerina	22819(NRRL)[a]	Indiana	*Glycine max*	K. O'Donnell
	22821(NRRL)	Indiana	*Glycine max*	K. O'Donnell
	22822(NRRL)	Indiana	*Glycine max*	K. O'Donnell
Nectria haematococca MPVI	22820(NRRL)	Indiana	*Glycine max*	K. O'Donnell
Fusarium solani f. sp. *pisi*	T8	. . .	*Pisum sativum*	H. Van Etten
Fusarium solani	D1	Wisconsin	*Medicago sativum*	C. Grau
	D5	Wisconsin	*Medicago sativum*	C. Grau
	S1177	Australia	*Lupinus augusti-folium*	P. Nelson
	91-10-1	North Dakota	*Glycine max*	B. Nelson
	91-11-2	North Dakota	*Glycine max*	B. Nelson
Fusarium solani f. sp. *phaseoli*	Mont-1	Illinois	*Glycine max*	P. Stevens
	RW1	Illinois	*Glycine max*	P. Stevens
	VR309	Illinois	*Glycine max*	P. Stevens
	St1-1	Illinois	*Glycine max*	L. Gray
	St90-1	Illinois	*Glycine max*	P. Stevens
	17-1	Arkansas	*Glycine max*	J. Rupe
	269	Arkansas	*Glycine max*	J. Rupe
	Mo1792	Missouri	*Glycine max*	T.D. Wyllie
	FSA1	Mississippi	*Glycine max*	K. Roy
	KS	Kansas	*Glycine max*	J. Rupe
	22823(NRRL)	Indiana	*Glycine max*	K. O'Donnell
	22825(NRRL)	Indiana	*Glycine max*	K. O'Donnell
	B1-1	Illinois	*Glycine max*	L. Gray
	B2-3	Illinois	*Glycine max*	L. Gray
	B3-5	Illinois	*Glycine max*	L. Gray
	B4-10	Illinois	*Glycine max*	L. Gray
	B7-9	Illinois	*Glycine max*	L. Gray
	B9-3	Illinois	*Glycine max*	L. Gray
	B10-10	Illinois	*Glycine max*	L. Gray
	I1-2	Illinois	*Glycine max*	L. Gray
	I2-5	Illinois	*Glycine max*	L. Gray
	I2-11	Illinois	*Glycine max*	L. Gray
	I3-8	Illinois	*Glycine max*	L. Gray
	I3-15	Illinois	*Glycine max*	L. Gray
	I4-7	Illinois	*Glycine max*	L. Gray
	I4-18	Illinois	*Glycine max*	L. Gray
	18006(ATCC)	California	*Phaseolus vulgaris*	ATCC[b]
	38135(ATCC)	Washington	*Phaseolus vulgaris*	ATCC
	42361(ATCC)	. . .	*Phaseolus vulgaris*	ATCC

[a] NRRL numbers correspond to cultures from the USDA/ARS NCAUR, Peoria, IL, and were originally designated by T. S. Abney (1) and cross-referenced in O'Donnell and Gray (9).
[b] ATCC isolates are from the American Type Culture Collection, Rockville, MD.

RESULTS AND DISCUSSION

RAPD patterns generated from 45 different primers were initially screened to check for those that differentiated SDS and non-SDS fungal isolates. Optimization of the amplification was performed by varying the buffer components and the concentration of genomic DNA and primers. As with most RAPD reactions, the critical parameter proved to be the concentration of $MgCl_2$. Addition of gelatin to the buffer did not significantly alter the results. The PCR results were consistent from run to run and were repeatable in different labs with different *Taq* DNA polymerase preparations (e.g., Promega, Perkin-Elmer, and Gibco BRL).

Of the 45 primers screened, eight primers produced polymorphisms useful for classification of the fungal isolates and were selected for further analysis (Table 2). Each of the selected primers resulted in different RAPD patterns. Representative banding patterns from two different primers are shown in Figure 1. The number of bands produced from amplification of each of the genomic DNAs ranged from one to 12 with observed molecular weights from approximately 220 to 3,800 base pairs.

RAPD marker patterns were assessed with the set of eight primers on DNA from 40 fungal isolates, of which 35 were *F. solani*. Each of the 22 SDS-causing iso-

Table 2. Primers used for random amplified polymorphic DNA analysis in this study[a]

Code	Sequence (5′ to 3′)
PMO2[b]	CGATGCCAGA
PMO4	GACTCCCTAC
RCO8	GGATGTCGAA
OPA-02[c]	TGCCGAGCTG
OPA-04	AATCGGGCTG
OPA-13	CAGCACCCAC
OPF-10	GGAAGCTTGG
OPF-13	GGCTGCAGAA

[a] Only those primers diagnostic for sudden death syndrome isolates are listed.
[b] PM and RC primers are referenced in Crowhurst et al. (5).
[c] OP primers were purchased from Operon Technologies (Alameda, CA).

lates (as determined from virulence assays, see below) gave identical RAPD patterns with the same primer from the selected set. These patterns were exclusive to the SDS isolates. No non-SDS isolate examined produced the same RAPD pattern as an SDS isolate, including three *F. solani* f. sp. *phaseoli* isolates: ATCC accession numbers 18006, 38135, and 42361.

The eight RAPD primers were utilized in a complete analysis of all 40 fungal isolates. All isolates were scored for either the presence (+) or absence (–) of any given RAPD marker produced by a specific primer. Bands of the same mobility (molecular weight) were scored as identical. There was no correction for band intensity and all visual bands were scored. The resulting binary matrix was used as the input file for a computer program that computes distances from qualitative data by a simple matching coefficient (SIM QUAL in NTSYS-pc 1.8). The distance matrix was then used in a UPGMA to produce a phenogram (Fig. 2). The use of alternative coefficients in computing the distance matrix (e.g., Jaccard coefficient) did not significantly alter the resulting phenogram. To verify the major clusters depicted in the phenogram, the binary matrix was subjected to bootstrap analysis (SeqBoot in Phylip 3.57) from which the following sets (clusters) were represented in 100 out of 100 resulting phenograms: (i) NRRL 22819, 22821, and 22822; (ii) 91-10-1 and 91-11-2; (iii) T8 and D5; and (iv) all 22 SDS-causing isolates (results not shown).

The relationships depicted in the phenogram (Fig. 2) indicate that most of the *F. solani* strains that were isolated from soybean were differentiated into two distinct groups, the first represented by isolates B1-1, B2-3, and B3-5 and the second group represented by Mont-1, RW1, and 20 other isolates.

To ascertain the significance of this dichotomous relationship among *F. solani* isolates isolated from soybean plants, virulence assays were conducted in a growth chamber environment for *F. solani* isolates on the SDS-susceptible soybean cv. Spencer (Tables 3 and 4). All *F. solani* isolates, including those isolated from hosts other than soybean, were capable of inducing root and/or crown rot in the virulence assays, although to varying degrees. For example, *F. solani* f. sp. *pisi* isolate T8 resulted in a 95% decrease in crown weight and a 99% decrease in root weight compared with the uninfested control. However, this isolate and many others were unable to induce the characteristic leaf symptoms of SDS, notably, interveinal necrosis. Thus, although all isolates caused root rot on the SDS-susceptible soybean cv. Spencer, not all were able to cause SDS leaf symptoms.

An additional complicating factor was the observation that not all isolates previously described in the literature as causing SDS (e.g., FSA1, NRRL 22823, and NRRL 22825; 7,9) were able to cause foliar symptoms indicative of this disease. The reason for this may be twofold. First, our virulence assays were performed with a sand/cornmeal soil-infestation inoculum whereas FSA1 and the two NRRL isolates were tested with a modified toothpick inoculation method (7,9). Second, we have observed that *F. solani* isolates capable of causing SDS that are cultured repeatedly on high carbohydrate media (such as potato dextrose agar [PDA]) occasionally lose not only the characteristic blue pigmentation but also the ability to cause the foliar symptoms of SDS (results not shown). Isolates FSA1, NRRL 22823, and NRRL 22825 were maintained on PDA prior to our receiving them. These three isolates failed to produce leaf symptoms but did produce high mean root rot ratings (≥3.00) compared with other isolates isolated from soybean that did not cause SDS symptoms

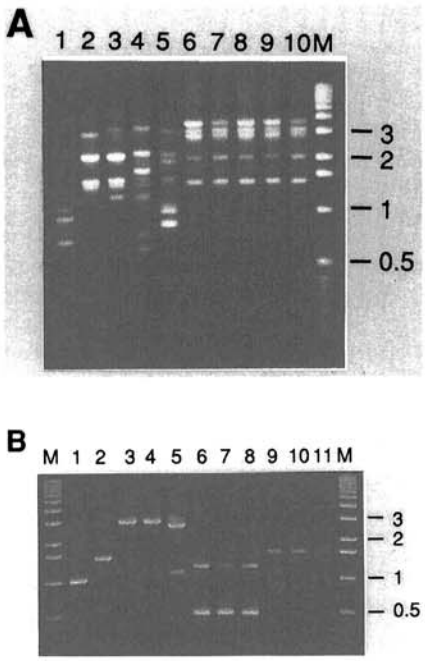

Fig. 1. Random amplified polymorphic DNA pattern obtained by amplification of DNA from fungal isolates. **(A)** Amplification with primer PMO2. Lane 1: *Septoria;* lane 2: T8; lane 3: D1; lane 4: S1177; lane 5: 91-10-1; lane 6: Mont-1; lane 7: St90-1; lane 8: 269; lane 9: Mo1792; lane 10: FSA1; lane M: 1-kb marker. **(B)** Amplification with primer PMO4. Lane1: *Septoria;* lane 2: T8; lane 3: D1; lane 4: 91-11-2; lane 5: B1-1; lane 6: Mont-1; lane 7: 269; lane 8: Mo1792; lane 9: ATCC 18006; lane 10: ATCC 38135; lane 11: ATCC 42361; lane M: 1-kb marker. The fragment size in kilobase pairs is indicated on the right.

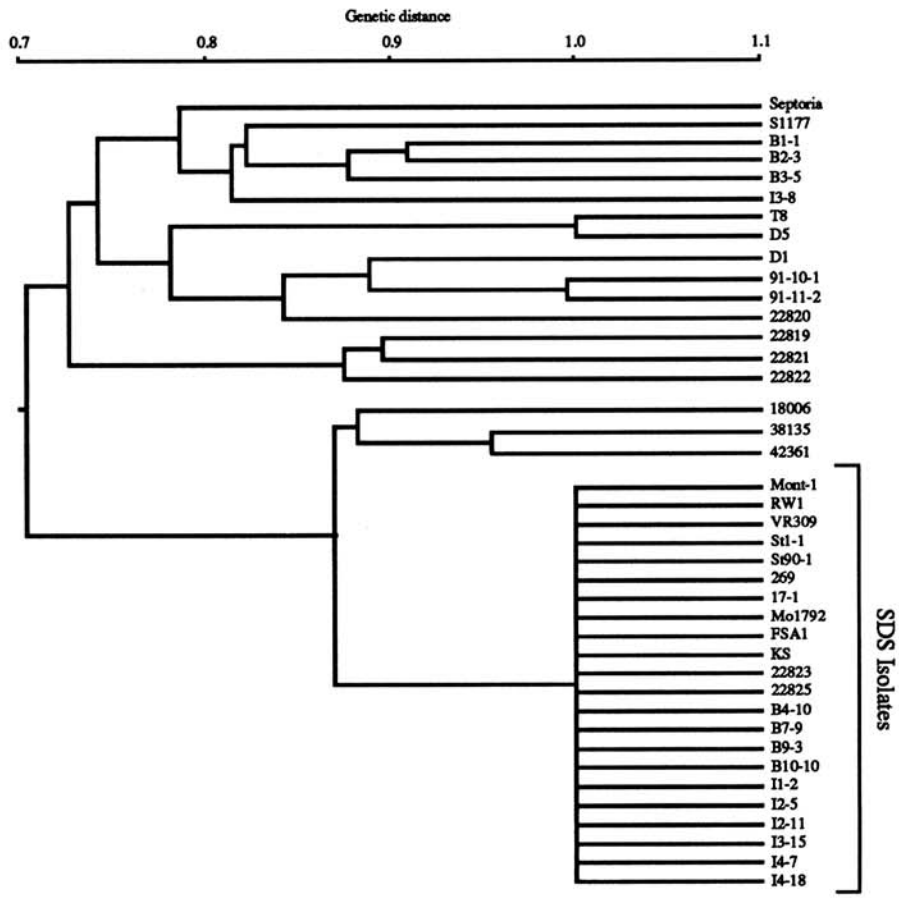

Fig. 2. Phenogram produced from unweighted pair-group method of analysis showing the relationship of the *Fusarium solani* isolates that cause soybean sudden death syndrome to other fungal isolates. Genetic distances were computed from data generated by eight random amplified polymorphic DNA primers.

(e.g., B1-1, B2-3, B3-5, and I3-8). This may be a distinguishing factor between less virulent SDS-causing isolates (i.e., those that do not cause foliar symptoms) and isolates that do not cause SDS.

When the results from virulence assays were compared with the results of molecular analysis, a good association in the ability of the RAPD approach to identify those fungal isolates capable of causing soybean SDS was found. This association held true for each of the eight RAPD primers used. In order to test the accuracy of our results, we performed double-blind tests in which *F. solani* isolates were chosen at random by a lab technician and labeled with only a code number. These double-blind tests included all of the *Fusarium* isolates listed in Table 1 and an additional 86 *F. solani* isolates from central and southern Illinois (not included in the list). Coded fungal isolates were then subjected to both RAPD analysis and virulence assays (as described above) and independently assigned as either SDS or non-SDS isolates according to the results. The results between the RAPD analysis and virulence assays were compared and, again, 100% association was achieved. All isolates that were (or had previously been described as being) capable of causing soybean SDS resulted in identical, discrete RAPD patterns. Furthermore, no isolate incapable of producing SDS ever produced a RAPD pattern identical to the SDS-causing isolates. Thus, it was possible to predict with 100% accuracy which *F. solani* isolates would cause SDS in the greenhouse by performing a single RAPD amplification that included at least one known SDS isolate, such as Mont-1, in the RAPD analysis as a positive control.

This study demonstrates the applicability of the RAPD approach in differentiating *F. solani* isolates that cause soybean SDS from those that do not. This method is fast, requiring only 2 days of laboratory work once the fungal cultures have produced sufficient mycelia to perform genomic DNA extractions. Because both PCR kits and RAPD primers are commercially available, the method is also convenient and can be performed in any laboratory with access to a thermal cycler. Most importantly, the accuracy of the results have been borne out through the high degree of association (100%) between the RAPD analyses and virulence assays conducted in the greenhouse environment. Thus, this paper describes a molecular diagnostic approach for the identification of SDS-causing fungal isolates that obviates the need for time-consuming greenhouse tests.

The RAPD analysis was useful not only for identifying SDS-causing *F. solani* isolates, but also for determining the genetic similarity of these isolates. The cluster defined by RAPD analysis that includes only those isolates that cause SDS shows 100% similarity among these isolates if one assumes that co-migrating bands are homologous. (The SDS isolates would still exhibit a very high level of genetic similarity even if some percentage of the co-migrating RAPD bands were proven to be heterologous.) This low level of genetic diversity is not unusual, as researchers have previously reported such findings through RAPD analysis of other *Fusarium* species such as *F. graminearum* (10).

Recently, O'Donnell and Gray (9) classified *F. solani* isolates that cause SDS as *F. solani* f. sp. *phaseoli,* based on nucleotide sequence analysis of the ribosomal intergenic spacer region and flanking rDNA sequences. However, this conclusion was qualified by the observation that the SDS-causing isolates could represent a population within *F. solani* f. sp. *phaseoli.* We performed sequence analysis of the D2 region of the 28S rDNA in several select SDS isolates and the three *F. solani* f. sp. *phaseoli* isolates (ATCC accession numbers 18006, 38135, and 42361), which resulted in identical nucleotide sequences as predicted by O'Donnell and Gray (9; results not shown). Moreover, our RAPD analyses were able to unambiguously distinguish the SDS-causing isolates from the *F. solani* f. sp. *phaseoli* isolates. None of the primers in the set generated exactly the

Table 3. Plant growth and disease severity for soybean cv. Spencer plants grown in Cone-Tainers infested with different *Fusarium solani* f. sp. *phaseoli* isolates[a]

Isolate	Top weight (g)	Root weight (g)	Root rot rating[b]	Leaf symptoms (%)[c]
Experiment 1				
Mont-1	0.58	0.33	4.75	100
FSA1	1.50	2.15	4.00	0[d]
22823	1.75	2.28	4.00	0[d]
22825	1.85	3.13	3.00	0[d]
KS	1.25	0.75	4.00	75
St90-1	0.75	0.55	4.00	94
St1-1	1.08	1.10	4.00	75
RW1	1.23	0.70	4.75	83
VR309	1.05	0.95	4.00	66
Mo1792	1.60	1.25	4.00	38
269	1.23	1.10	4.00	72
17-1	0.98	0.85	4.25	88
22819	2.48	3.63	2.50	0
22820	2.55	3.75	2.00	0
22821	1.80	2.90	2.00	0
22822	2.23	3.80	2.00	0
S1177	1.70	2.20	2.50	0
T8	0.13	0.03	5.00	0
91-11-2	2.50	3.38	2.50	0
91-10-1	2.25	3.33	2.75	0
D1	2.10	2.93	2.00	0
D5	0.10	0.02	5.00	0
Control[e]	2.77	4.42	1.00	0
LSD (0.05)	0.43	0.72	0.40	3
Experiment 2				
Mont-1[f]	0.25	0.13	5.00	100
RW1[f]	0.80	1.03	4.00	90
B4-10	1.03	0.80	4.25	85
B7-9	0.58	0.33	4.75	100
B9-3	0.93	0.55	4.50	85
B10-10	1.58	1.60	4.00	33
I1-2	0.55	0.33	4.75	100
I2-5	0.65	0.23	5.00	94
I2-11	0.75	0.48	4.75	100
I3-15	0.45	0.13	5.00	100
I4-7	0.48	0.15	5.00	100
I4-18	0.58	0.18	5.00	93
B1-1	2.18	2.95	2.50	0
B2-3	2.48	3.80	2.00	0
B3-5	1.78	2.60	2.75	0
I3-8	2.03	2.98	2.25	0
Control[e]	2.75	4.08	1.0	0
LSD (0.05)	0.52	0.71	0.50	15

[a] Plants were grown for 3 weeks in Cone-Tainers in soil mix infested at a rate of 5%.

[b] Based on the following scale: 1 = no root or crown necrosis, root and crown tissue healthy; 2 = 25% of feeder and tap root tissue exhibiting necrosis, no crown necrosis; 3 = 25 to 50% of feeder and tap root tissue exhibiting necrosis, no crown necrosis; 4 = >50% of root and crown tissue necrotic; 5 = root and crown tissue necrotic, plants dead.

[c] Mean number of leaflets exhibiting sudden death syndrome (SDS) symptoms/total number of leaflets per plant × 100.

[d] *F. solani* isolates that no longer produce SDS foliar symptoms.

[e] Spencer soybean plants grown in uninfested soil.

[f] Mont-1 and RW1 were included as positive SDS controls.

Table 4. Plant growth and disease severity for soybean cv. Spencer and Top Crop garden bean plants grown in Cone-Tainers infested with different *Fusarium solani* f. sp. *phaseoli* isolates[a]

	Cultivars					
	Root weight (g)		Root rot rating[b]		Leaf symptoms (%)[c]	
Isolate	Spencer	Top Crop	Spencer	Top Crop	Spencer	Top Crop
18006	4.0	4.0	2.5	2.0	0	0
38135	3.7	3.2	3.0	3.0	0	0
42361	3.6	4.6	2.0	2.0	0	0
Mont-1[d]	0.6	2.0	5.0	4.0	100	0
Control[e]	4.8	5.5	1.0	1.0	0	0
LSD (0.01)	1.3		0.3			

[a] Plants were grown for 3 weeks in Cone-Tainers in soil mix infested at a rate of 5%.
[b] Based on the following scale: 1 = no root or crown necrosis, root and crown tissue healthy; 2 = 25% of feeder and tap root tissue exhibiting necrosis, no crown necrosis; 3 = 25 to 50% of feeder and tap root tissue exhibiting necrosis; no crown necrosis; 4 = >50% of root and crown tissue necrotic; 5 = root and crown tissue necrotic, plants dead.
[c] Mean number of leaflets exhibiting sudden death syndrom (SDS) symptoms/total number of leaflets per plant × 100.
[d] Mont-1 was included as a positive SDS control.
[e] Plants grown in uninfested soil.

same RAPD pattern in these two groups and, although some RAPD bands were present in both the SDS isolates and the three ATCC isolates, certain primers, such as PMO4 and RCO8, resulted in very different patterns (see Fig. 1B). Greenhouse assays confirmed that the ATCC isolates designated as *F. solani* f. sp. *phaseoli* in Table 1 were unable to cause SDS symptoms on soybean (Table 4).

In light of the current results, the classification of SDS isolates as *F. solani* f. sp. *phaseoli* does not adequately indicate the genetic and pathogenic distinctions among this group. The random and genome-wide nature of the RAPD approach is better able to indicate overall genetic dissimilarity than sequence analysis of a single region of the genome (such as the ribosomal operon). Given that the fungal isolates capable of causing soybean SDS exhibit 100% genetic similarity in the RAPD analyses reported herein and that this cluster forms an exclusive group distinct from *F. solani* f. sp. *phaseoli* isolates unable to cause SDS (Fig. 2), those fungal isolates that cause soybean SDS form a biologically meaningful subgroup within *F. solani* f. sp. *phaseoli* and may ultimately represent a separate form species.

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