Soybean Sudden Death Syndrome Species Diversity Within North and South America Revealed by Multilocus Genotyping

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ABSTRACT

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Sudden death syndrome (SDS) of soybean has become a serious constraint to the production of this crop in North and South America. Phenotypic and multilocus molecular phylogenetic analyses, as well as pathogenicity experiments, have demonstrated that four morphologically and phylogenetically distinct fusaria can induce soybean SDS. Published molecular diagnostic assays for the detection and identification of these pathogens have reported these pathogens as *F. solani*, *F. solani* f. sp. *glycines*, or *F. solani* f. sp. *phaseoli*, primarily because the species limits of these four pathogens were only recently resolved. In light of the recent discovery that soybean SDS and *Phaseolus* and mung bean root rot (BRR) are caused by four and two distinct species, respectively, multilocus DNA sequence analyses were conducted to assess whether any of the published molecular diagnostic assays were species-specific. Comparative DNA sequence analyses of the soybean SDS and BRR pathogens revealed that highly conserved regions of three loci were used in the design of these assays, and therefore none were species-specific based on our current understanding of species limits within the SDS–BRR clade. Prompted by this finding, we developed a high-throughput multilocus genotyping (MLGT) assay which accurately differentiated the soybean SDS and two closely related *Phaseolus* and mung BRR pathogens based on nucleotide polymorphism within the nuclear ribosomal intergenic spacer region rDNA and two anonymous intergenic regions designated locus 51 and 96. The single-well diagnostic assay, employing flow cytometry and a novel fluorescent microsphere array, was validated by independent multilocus molecular phylogenetic analysis of a 65 isolate design panel. The MLGT assay was used to reproducibly type a total of 262 soybean SDS and 9 BRR pathogens. The validated MLGT array provides a unique molecular diagnostic for the accurate identification and molecular surveillance of these economically important plant pathogens.

Additional keywords: Argentina, Brazil, EF-1α, *Glycine max*, *Phaseolus vulgaris*, United States, *Vinga radiata*.

Sudden death syndrome (SDS) of soybean (*Glycine max* (L.) Merr.) is responsible for economically devastating reductions in yields of this crop in North and South America (4). Hallmarks of soybean SDS include foliar necrosis and chlorosis, vascular discoloration of roots and stems, root rot, and death (31). Recent systematic and molecular phylogenetic analyses including morphological studies, multilocus DNA sequence data, and pathogenicity assays have shown that soybean SDS is caused by four closely related soilborne fusaria that are morphologically and phylogenetically distinct species (3,4; present study; *Fusarium virguliforme* O'Donnell & T. Aoki, *F. tucumaniae* T. Aoki, O'Donnell, Yos. Homma & Lattanzi, *F. brasiliense* T. Aoki & O'Donnell, and an undescribed *Fusarium* sp.). These studies have also revealed that bean root rot (hereafter referred to as BRR) of dry edible and snap bean (*Phaseolus vulgaris* L.) and mung bean (*Vigna radiata* (L.) R. Wilczek) is caused by two other distinct species, *F. phaseoli* (Burkh.) T. Aoki & O'Donnell and *F. cuneirostrum* O'Donnell & T. Aoki. Results of the present study, based on sampling of additional isolates and loci, have revealed that the single genetically divergent soybean SDS isolate NRRL 31949 from Brazil previously reported as *F. cuneirostrum* (4) represents an unnamed phylogenetically and morphologically distinct species (T. Aoki, *unpublished data*) herein referred to as *Fusarium* sp. Symptoms of soybean SDS and BRR include deterioration and discoloration of the main and lateral taproots, leaf chlorosis, defoliation, and death (35). All six species form a genealogically exclusive group within the South American clade (i.e., clade 2) of the *F. solani* species complex (FSSC) (20), suggesting that these root rot

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pathogens evolved from a common ancestor prior to the introduction of soybean to the New World. Although the taxonomic treatments of this genus (13,19) used by most North American plant pathologists follow Snyder and Hansen (39) in recognizing *F. solani* (Mart.) Appel & Wollenweber as the only species within the FSSC, molecular phylogenetic analyses indicate that this complex comprises at least 47 phylogenetically distinct species distributed among three strongly supported clades (20,26,49). In addition, the so-called MPI-MPVII mating populations of *Nectria haematococca* Berk. & Br. have been shown to represent biologically and phylogenetically distinct species (18,20). Interesting, approximately two-thirds of all fusarial infection of humans and other animals are caused by at least 20 species which are all nested within FSSC clade 3 (26,49), which also includes the model system organism *F. solani* f. sp. *pisi*, also known as *N. haematococca* MPVI (45).

Because three of the four SDS and the two BRR pathogens were only recently described and given Latin binomials (3,4), these six pathogens have been referred to primarily as *F. solani* f. sp. *glycines* (31), *F. solani* f. sp. *phaseoli* (Burkh.) W. C. Snyder & H. N. Hansen, or *F. solani* (Mart.) Sacc. in most studies published to date. Unfortunately, published molecular diagnostic assays for the specific detection of these pathogens (7,8,14–16,22) were developed either prior to the application of genealogical concordance phylogenetic species recognition (GCPSR) (40) within the SDS–BRR clade (3,4), or else they did not take into account the species diversity reported in the latter two studies (8,15). In so far as species limits were not considered prior to the development of molecular diagnostic assays for these pathogens, it remains to be established whether any of these assays are species specific.

All polymerase chain reaction (PCR)-based assays published to date for the identification of the SDS and BRR pathogens are limited to uniplex amplifications based on the assumption that the etiological agent was a single species. The current gold standard for identification of the six species within the SDS–BRR clade include time-consuming morphological analyses which require taxonomic expertise (3,4; T. Aoki, *unpublished data*) and molecular phylogenetic analyses of DNA sequence data (3,4). Labor-intensive pathogenicity assays have been used to distinguish soybean SDS and BBR pathogens from one another and from other fusarial pathogens but it is now clear that they do not differentiate the six soybean SDS–BBR clade pathogens (31–33). Application of a biological species concept within this clade has been limited to one of the four South American soybean SDS pathogens, *F. tucumaniae* (6), which is the only species within the SDS–BBR clade with a known teleomorph, in spite of a report to the contrary (1).

Recently, multiplexed single-well DNA sequence-based typing schemes have been developed for Fusarium head blight (FHB) species identification and trichothecene chemotype determination (46) and human pathogenic fusaria (25) utilizing multilocus genotyping (MLGT) of single-nucleotide polymorphisms. Both of these MLGT assays employed Luminex technology and flow cytometry, which take advantage of PCR multiplexing and allele-specific primer extension (ASPE) using microsphere arrays which can accommodate up to 100 different probe primers within a single-well assay. Given this background, the primary objectives of the present study were to (i) further evaluate species limits within the SDS–BRR clade using novel multilocus DNA sequence data; (ii) determine whether published molecular diagnostic assays are specific for any of the SDS and BRR pathogens; (iii) develop and validate a high-throughput, allele-specific microsphere array for the simultaneous detection and identification of all four SDS and both BRR pathogens in a single-well assay using flow cytometry; and (iv) use the MLGT assay to develop a baseline of SDS pathogen diversity in the major soybean-growing regions of Argentina and the United States.

MATERIALS AND METHODS

Fungal strains and growth conditions. A total of 262 soybean SDS and nine BRR isolates were genotyped in this study. Isolates were analyzed in the following two groups: a panel of 65 soybean SDS and BRR isolates (Table 1) for the design and validation of the MLGT assay (design panel, DP), and an experimental panel (EP) consisting of 205 soybean SDS isolates and one BRR isolate (Table 2). All of the isolates are stored cryogenically in liquid nitrogen vapor at –175°C in the Agricultural Research Service (NRRL) Culture Collection (NCAUR, Peoria, IL) and are available for distribution to promote a greater understanding of their phenotypic and genotypic diversity (10). Yeast-malt broth cultures were used to obtain mycelium for genomic DNA as described previously (21).

Molecular phylogenetics. Genomic DNA isolation, PCR amplification, and multilocus DNA sequencing were conducted as previously described (6,24). A panel of 65 isolates was chosen to represent the phylogenetic diversity of the soybean SDS and BRR pathogens based on previous phylogenetic analyses (4). We identified species-specific DNA polymorphisms within aligned sequences of the nuclear ribosomal intergenic spacer (IGS) rDNA (2,765 bp alignment) and anonymous locus 51 (1,530 bp alignment) and 96 (1,520 bp alignment) (6). In addition, partial translation elongation factor (*EF-1a*) sequence data were obtained from the same 65-isolate panel (687 bp alignment). Sequencher (version 4.1.2; Gene Codes, Ann Arbor, MI) was used to edit and align DNA sequences, after which positional homology alignments were improved by manual manipulation of the alignment. Phylogenetic reconstructions employed maximum parsimony (MP) in PAUP (42) and maximum likelihood in GARLI (50) as previously described (26). Clade stability was assessed by 1,000 MP pseudoreplicates of the data (Fig. 1).

Assessment of published SDS and BRR species-specific PCR primers and probes. Several PCR primer pairs and probes have been reported for the specific detection and identification of *F. solani* f. sp. *glycines* or *F. solani* f. sp. *phaseoli* (7,8,14–16,22). To assess whether they were specific to one of the four soybean SDS or two BRR pathogens, we PCR amplified and sequenced the three loci employed in the design of the species-specific primers and probes [i.e., nuclear ribosomal internal transcribed spacer region and nuclear large subunit rDNA (22), 5′ portion of the *EF-1a* gene (7,14), and mitochondrial small subunit (mtSSU) ribosomal rDNA (8,14–16)] in two or more isolates of each soybean SDS and BRR pathogen as previously described (20). DNA sequences were edited and aligned with Sequencher version 4.1.2 and then in silico searches were conducted to ascertain whether the published PCR primers or probes could differentiate the soybean SDS and BRR pathogens. DNA sequences have been deposited in GenBank under accession numbers FJ919398 to FJ919561.

Species-specific probe design. MacClade's data editor (17) was used to design all of the species-specific probes. Sequences of the IGS rDNA partition were used to design species-specific probes for two of the four soybean SDS and the two BRR pathogens; aligned sequences of locus 51 and 96 were each used to design species-specific probes for *F. tucumaniae* and *F. virguliforme* (Fig. 2; Table 3). Each species-specific probe primer ends with a 3′ single nucleotide diagnostic for one of the six pathogens, as well as species-specific polymorphisms located internally within the probe primers. In addition, one highly conserved oligonucleotide probe was designed as a positive control for PCR amplification and allele-specific primer extension of each locus for all six pathogens within the SDS–BRR clade. A unique 24-bp tag was added to the 5′ end of each primer (Table 3), which was obtained from the Luminex Corporation website (http://www.luminexcorp.com/).

Multiplex amplification for MLGT assay. We obtained IGS rDNA and locus 51 and 96 templates for the multiplex primer

extension by co-amplifying portions of these genomic regions using PCR primers NL11 × CNS1, 51-1 × 51-2, and 96-1a × 96-2a, respectively (6). Multiplex PCR amplifications were performed in a total volume of 40-µl following the manufacturer's instructions, and included 1× High Fidelity PCR buffer (Invitrogen Life Technologies, Carlsbad, CA), 1.25 mM $MgSO_4$, 0.2 mM of each deoxynucleoside triphosphate, 10 pmol of each primer, 0.5 U of Platinum *Taq* DNA Polymerase High Fidelity, and

TABLE 1. Design panel of sudden death syndrome and bean root rot fusaria used in the validation of the allele-specific genotyping assay

NRRL[a]	Species	Equivalent no.[b]	Host	Origin	Index of discrimination[c]	Year isolated
22158	*Fusarium cuneirostrum*	ATCC 60860	*Phaseolus vulgaris*	USA, New York	8 (CU-a)	Unknown
22275	*Fusarium cuneirostrum*	Matuo SUF 386 = MAFF 239036	*Phaseolus vulgaris*	Japan, Hokkaido, Sapporo	10 (CU-a)	1955
22276	*Fusarium phaseoli*	van Etten T-162 = MAFF 238544 = CCC 189-05	*Phaseolus vulgaris*	USA	18 (PH-a), 4 (PH-b)	Unknown
22292	*Fusarium virguliforme*	Gray Mont-1	*Glycine max*	USA, Illinois	21 (96-VI-1), 24 (51-VI-1), 44 (51-VI-2)	1991
22411	*Fusarium phaseoli*	ATCC 38466 = CCC 100-03	*Phaseolus vulgaris*	USA, California	10 (PH-a), 4 (PH-b)	Unknown
22678	*Fusarium brasiliense*	FRC S-712 = MAFF 239039	*Glycine max*	USA, California	21 (B-a), 6 (B-b)	1993
22743	*Fusarium brasiliense*	BBA 68441 = MAFF 239041	*Glycine max*	Brazil, Distrito Federal, Brasilia	51 (B-a), 7 (B-b)	1992
22823	*Fusarium virguliforme*	Abney IN-11-60-4	*Glycine max*	USA, Indiana	21 (96-VI-1), 24 (51-VI-1), 41 (51-VI-2)	Unknown
22825	*Fusarium virguliforme*	Abney IN-F2X-11A	*Glycine max*	USA, Indiana	22 (96-VI-1), 22 (51-VI-1), 43 (51-VI-2)	Unknown
31041	*Fusarium virguliforme* (T)	Li #95	*Glycine max*	USA, Illinois	20 (96-VI-1), 21 (51-VI-1), 38 (51-VI-2)	1998
31085	*Fusarium tucumaniae*	Homma MJ-123 = MAFF 238405	*Glycine max*	Argentina, Córdoba, General Roca	9 (TU-a), 13 (TU-c)	2000
31096	*Fusarium tucumaniae* (T)	MAFF 238418	*Glycine max*	Argentina, Tucumán, San Agustín	10 (TU-a), 13 (TU-c)	2001
31100	*Fusarium tucumaniae*	MAFF 238411	*Glycine max*	Argentina, Santa Fe, Las Rosas	10 (TU-a), 13 (TU-c)	2001
31104	*Fusarium cuneirostrum*	MAFF 305607	*Phaseolus vulgaris*	Japan	10 (CU-a)	Unknown
31156	*Fusarium phaseoli*	FRC S-1550 = MAFF 238550 = CCC 190-05	*Phaseolus vulgaris*	USA, Michigan	14 (PH-a), 4 (PH-b)	Unknown
31157	*Fusarium cuneirostrum* (T)	FRC S-1551 = MAFF 239038 = CCC 192-05	*Phaseolus vulgaris*	USA, Michigan	5 (CU-a)	1992
31756	*Fusarium brasiliense*	Yorinori SDS-1 = MAFF 239043 = CCC 124-02	*Glycine max*	Brazil, Distrito Federal, Brasilia	17 (B-a), 5 (B-b)	1992
31757	*Fusarium brasiliense* (T)	Yorinori SDS-5 = MAFF 239050	*Glycine max*	Brazil, Distrito Federal, Brasilia	16 (B-a), 5 (B-b)	1992
31762	*Fusarium brasiliense*	Yorinori 06/98 = MAFF 239051 = CCC 193-05	*Glycine max*	Brazil, Paraná, Campo Mourão	21 (B-a), 6 (B-b)	1998
31776	*Fusarium tucumaniae*	Yorinori 33/00 = MAFF 239054	*Glycine max*	Brazil, Rio Grande do Sul, Tapera	9 (TU-a), 12 (TU-c)	2000
31777	*Fusarium tucumaniae*	Yorinori 34/00 = MAFF 239045	*Glycine max*	Brazil, Rio Grande do Sul, Vila Maria	9 (TU-a), 13 (TU-c)	2000
31778	*Fusarium tucumaniae*	Yorinori 35/00 = MAFF 239046	*Glycine max*	Brazil, Rio Grande do Sul, Sarandi	8 (TU-a), 12 (TU-c)	2000
31779	*Fusarium brasiliense*	Yorinori 36/00 = MAFF 239047= CCC 194-05	*Glycine max*	Brazil, Rio Grande do Sul, Nonai	11 (B-a), 3 (B-b)	2000
31781	*Fusarium tucumaniae*	Yorinori 41/00 = MAFF	*Glycine max*	Argentina, Tucumán	7 (TU-a), 15 (TU-c)	Unknown
31793	*Fusarium tucumaniae*	Yorinori 71/00 = MAFF 239048	*Glycine max*	Brazil, Minas Gerais, Nova Ponte	8 (TU-a), 12 (TU-c)	2001
31949	*Fusarium* sp. (T)	Yorinori 01/00 = MAFF 239052 = CCC 198-05	*Glycine max*	Brazil, Goiás, Cristalina	43 (CR-a), 15 (CR-b)	2000
31950	*Fusarium tucumaniae*	Yorinori 07/00	*Glycine max*	Brazil, Paraná, Ponta Grossa	8 (TU-a), 14 (TU-c)	2000
32392	*Fusarium virguliforme*	NSPCC i101	*Glycine max*	USA, Wisconsin	22 (96-VI-1), 21 (51-VI-1), 40 (51-VI-2)	1998
32458	*Fusarium virguliforme*	Fsg-ISU 1	*Glycine max*	USA, Iowa-Boone	22 (96-VI-1), 21 (51-VI-1), 41 (51-VI-2)	1993
32459	*Fusarium virguliforme*	Fsg-ISU 2	*Glycine max*	USA, Iowa-Cerro	23 (96-VI-1), 22 (51-VI-1), 43 (51-VI-2)	2000
32462	*Fusarium virguliforme*	Fsg-ISU 5	*Glycine max*	USA, Iowa-Chickasaw	22 (96-VI-1), 19 (51-VI-1), 39 (51-VI-2)	2000

(*Continued on next page*)

[a] NRRL = Agriculture Research Service Culture Collection, National Center for Agricultural Utilization Research, USDA-ARS, Peoria, IL.

[b] Equivalent no.: ATCC = American Type Culture Collection, Manassas, VA; BBA = Bundesanstalt für Land und Forstwirtschaft, Berlin; CCC= Culture Collection of CEREMIC (Centro de Referencia de Micología), Fac. de Cs. Bioquímicas y Farmacéuticas, UNR, Rosario, Argentina; FRC = Fusarium Research Center, Department of Plant Pathology, Pennsylvania State University, University Park, PA; Fsg-ISU = Department of Plant Pathology, Iowa State University, Ames, IA; MAFF = NIAS Genbank-Microorganisms Section, National Institute of Agrobiological Sciences (NIAS), 2-1-2 Kannondai, Tsukuba, Ibaraki 305-8602, Japan; NSPCC = National Soybean Pathogen Culture Collection, ARS-USDA, Urbana, IL.

[c] Index of discrimination was determined by first subtracting the average fluorescence intensity of three water negative controls from the value obtained for each DNA sample, and then dividing the minimum fluorescence intensity by the maximum nontarget fluorescence intensity. Species-specific probe primers are indicated in parentheses (Table 3 provides probes used in the assay).

approximately 10 ng of genomic DNA of one of the SDS or BRR pathogens. PCRs employed an initial denaturation of 90 s at 94°C, 40 cycles for 30 s at 94°C, 30 s at 55°C, and 210 s at 68°C, followed by a 4°C soak. Amplicons were purified using Montage PCR_{96} Cleanup filter plates (Millipore, Billerica, MA) and stored at –20°C prior to being used as template for the ASPE reactions.

MLGT assay primer extension, hybridization and detection. Purified IGS rDNA and locus 51 and 96 templates were

TABLE 1. (*Continued from preceeding page*)

NRRL[a]	Species	Equivalent no.[b]	Host	Origin	Index of discrimination[c]	Year isolated
32466	*Fusarium virguliforme*	Fsg-ISU 9	*Glycine max*	USA, Iowa-Clinton	22 (96-VI-1), 20 (51-VI-1), 41 (51-VI-2)	1995
32469	*Fusarium virguliforme*	Fsg-ISU 12	*Glycine max*	USA, Iowa-Greene	23 (96-VI-1), 21 (51-VI-1), 41 (51-VI-2)	1996
32470	*Fusarium virguliforme*	Fsg-ISU 13	*Glycine max*	USA, Iowa-Henry	10 (96-VI-1), 10 (51-VI-1), 22 (51-VI-2)	1994
32472	*Fusarium virguliforme*	Fsg-ISU 15	*Glycine max*	USA, Iowa-Jasper	21 (96-VI-1), 21 (51-VI-1), 39 (51-VI-2)	1994
32473	*Fusarium virguliforme*	Fsg-ISU 16	*Glycine max*	USA, Iowa-Johnson	22 (96-VI-1), 22 (51-VI-1), 40 (51-VI-2)	1994
32474	*Fusarium virguliforme*	Fsg-ISU 17	*Glycine max*	USA, Iowa-Latham	22 (96-VI-1), 19 (51-VI-1), 37 (51-VI-2)	Unknown
32475	*Fusarium virguliforme*	Fsg-ISU 18	*Glycine max*	USA, Missouri-Mont	23 (96-VI-1), 22 (51-VI-1), 39 (51-VI-2)	Prior 1993
32477	*Fusarium virguliforme*	Fsg-ISU 20	*Glycine max*	USA, Missouri-Mont	22 (96-VI-1), 25 (51-VI-1), 50 (51-VI-2)	Prior 1993
32478	*Fusarium virguliforme*	Fsg-ISU 21	*Glycine max*	USA, Iowa-Scott	24 (96-VI-1), 22 (51-VI-1), 42 (51-VI-2)	1993
32481	*Fusarium virguliforme*	Fsg-ISU 24	*Glycine max*	USA, Iowa-Worth	23 (96-VI-1), 20 (51-VI-1), 40 (51-VI-2)	1995
34436	*Fusarium virguliforme*	FRC S-1281L = Gray 269	*Glycine max*	USA, Arkansas	10 (96-VI-1), 10 (51-VI-1), 21 (51-VI-2)	Prior 1993
34437	*Fusarium virguliforme*	FRC S-1286L = Gray L145	*Glycine max*	USA, Arkansas	23 (96-VI-1), 25 (51-VI-1), 42 (51-VI-2)	Prior 1993
34546	*Fusarium tucumaniae*	CCC 125-02	*Glycine max*	Argentina, Buenos Aires, Arrecifes	10 (TU-a), 14 (TU-c)	2000
34547	*Fusarium tucumaniae*	CCC 126-02	*Glycine max*	Argentina, Santa Fe, Las Parejas	14 (TU-a), 16 (TU-c)	2001
34549	*Fusarium tucumaniae*	CCC 129-02	*Glycine max*	Argentina, Buenos Aires, PérezMillán	14 (TU-a), 18 (TU-c)	2000
34550	*Fusarium tucumaniae*	CCC 128-02	*Glycine max*	Argentina, Santa Fe, Pujato	13 (TU-a), 17 (TU-c)	2000
34551	*Fusarium virguliforme*	CCC 101-03	*Glycine max*	Argentina, Buenos Aires, San Pedro	19 (96-VI-1), 23 (51-VI-1), 37 (51-VI-2)	2002
34552	*Fusarium virguliforme*	CCC 102-03	*Glycine max*	Argentina, Santa Fe, Serodino	18 (96-VI-1), 25 (51-VI-1), 38 (51-VI-2)	2002
34553	*Fusarium virguliforme*	CCC 103-03	*Glycine max*	Argentina, Santa Fe, Serodino	16 (96-VI-1), 21 (51-VI-1), 34 (51-VI-2)	2002
34938	*Fusarium brasiliense*	CCC 195-05	*Glycine max*	Brasil, Rio Grande do Sul, Passo Fundo	30 (B-a), 7 (B-b)	2003
34939	*Fusarium tucumaniae*	BRS 137	*Glycine max*	Brasil, Rio Grande do Sul, Passo Fundo	13 (TU-a), 15 (TU-c)	2003
36023	*Fusarium cuneirostrum*	CCC 196-05	*Vigna radiata*	Canada, Ontario, Ridgetown	8 (CU-a)	1996
36605	*Fusarium virguliforme*	CCC 156-05	Soil	Argentina, Buenos Aires, Pergamino	17 (96-VI-1), 23 (51-VI-1), 35 (51-VI-2)	1999
36606	*Fusarium virguliforme*	RupeArg 1.2	Soil	Argentina, Buenos Aires, Pergamino	17 (96-VI-1), 20 (51-VI-1), 32 (51-VI-2)	1999
36607	*Fusarium virguliforme*	CCC 158-05	Soil	Argentina, Buenos Aires, Pergamino	16 (96-VI-1), 20 (51-VI-1), 34 (51-VI-2)	1999
36610	*Fusarium virguliforme*	CCC 159-05	Soil	Argentina, Buenos Aires, Pergamino	18 (96-VI-1), 21 (51-VI-1), 32 (51-VI-2)	1999
36611	*Fusarium virguliforme*	CCC 160-05	Soil	Argentina, Buenos Aires, Pergamino	17 (96-VI-1), 23 (51-VI-1), 35 (51-VI-2)	1999
36877	*Fusarium* sp.	CCC 142-05	*Glycine max*	Argentina, Santa Fe, Zavalla	36 (CR-a), 14 (CR-b)	2004
36878	*Fusarium tucumaniae*	CCC 209-05	*Glycine max*	Argentina, Santa Fe, Zavalla	12 (TU-a), 14 (TU-c)	2004
36880	*Fusarium tucumaniae*	CCC 211-05	*Glycine max*	Argentina, Santa Fe, Los Molinos	13 (TU-a), 18 (TU-c)	2004
36896	*Fusarium virguliforme*	CCC 216-05	*Glycine max*	Argentina, Santa Fe, Los Molinos	17 (96-VI-1), 22 (51-VI-1), 35 (51-VI-2)	2004
36897	*Fusarium virguliforme*	CCC 217-05	*Glycine max*	Argentina, Santa Fe, Los Molinos	17 (96-VI-1), 21 (51-VI-1), 35 (51-VI-2)	2004
36899	*Fusarium virguliforme*	CCC 219-05	*Glycine max*	Argentina, Santa Fe, Los Molinos	17 (96-VI-1), 19 (51-VI-1), 32 (51-VI-2)	2004
36900	*Fusarium virguliforme*	CCC 220-05	*Glycine max*	Argentina, Santa Fe, Los Molinos	14 (96-VI-1), 16 (51-VI-1), 30 (51-VI-2)	2004

TABLE 2. Experimental panel of sudden death syndrome and bean root rot fusaria analyzed by allele-specific genotyping

NRRL[a]	Species	Equivalent no.[b]	Host	Origin	Index of discrimination[c]	Year isolated
22411	*Fusarium phaseoli*	ATCC 38466 = CCC 100-03	*Phaseolus vulgaris*	USA, California	10 (PH-a), 4 (PH-b)	Unknown
22489	*Fusarium virguliforme*	K. Roy FSA Poinset = T-501	*Glycine max*	USA	22 (96-VI-1), 23 (51-VI-1), 43 (51-VI-2)	Unknown
22490	*Fusarium virguliforme*	K. Roy FSA Lu = T-502	*Glycine max*	USA	24 (96-VI-1), 21 (51-VI-1), 42 (51-VI-2)	Unknown
22743	*Fusarium brasiliense*	BBA 68441 = MAFF 239041	*Glycine max*	Brazil, DF, Brasilia	51 (B-a), 7 (B-b)	1992
22744	*Fusarium brasiliense*	BBA 68442 = MAFF 239050	*Glycine max*	Brazil, DF, Brasilia	30 (B-a), 8 (B-b)	1992
31039	*Fusarium virguliforme*	Li #45	*Glycine max*	USA, Illinois	18 (96-VI-1), 17 (51-VI-1), 34 (51-VI-2)	1994
31040	*Fusarium virguliforme*	Li #52	*Glycine max*	USA, Illinois	19 (96-VI-1), 19 (51-VI-1), 37 (51-VI-2)	Unknown
31086	*Fusarium tucumaniae*	Homma MJ-123 = MAFF 238406 = CCC 175-05	*Glycine max*	Argentina, General Roca, Córdoba	6 (TU-a), 11 (TU-C)	2000
31087	*Fusarium tucumaniae*	MAFF 238407 = CCC 176-05	*Glycine max*	Argentina, General Roca, Córdoba	6 (TU-a), 9 (TU-C)	2001
31088	*Fusarium tucumaniae*	MAFF 238408	*Glycine max*	Argentina, General Roca, Córdoba	9 (TU-a), 11 (TU-C)	2001
31089	*Fusarium tucumaniae*	MAFF 238410	*Glycine max*	Argentina, General Roca, Córdoba	6 (TU-a), 9 (TU-C)	2001
31090	*Fusarium tucumaniae*	MAFF 238412	*Glycine max*	Argentina, Cordoba	8 (TU-a)	2001
31091	*Fusarium tucumaniae*	MAFF 238413 = CCC 177-05	*Glycine max*	Argentina, Tucumán, San Agustin	8 (TU-a), 13 (TU-C)	2001
31092	*Fusarium tucumaniae*	MAFF 238414	*Glycine max*	Argentina, Tucumán, San Agustin	9 (TU-a), 16 (TU-C)	2001
31093	*Fusarium tucumaniae*	MAFF 238415	*Glycine max*	Argentina, Tucumán	9 (TU-a)	2001
31094	*Fusarium tucumaniae*	MAFF 238416 = CCC 178-05	*Glycine max*	Argentina, Tucumán, San Agustin	9 (TU-a), 11 (TU-C)	2001
31095	*Fusarium tucumaniae*	MAFF 238417 = CCC 179-05	*Glycine max*	Argentina, Tucumán, San Agustin	9 (TU-a), 12 (TU-C)	2001
31097	*Fusarium tucumaniae*	MAFF 238419 = CCC 180-05	*Glycine max*	Argentina, Tucumán, San Agustin	9 (TU-a), 14 (TU-C)	2001
31098	*Fusarium tucumaniae*	MAFF 238420 = CCC 181-05	*Glycine max*	Argentina, Tucumán, San Agustin	8 (TU-a), 11 (TU-C)	2001
31099	*Fusarium tucumaniae*	MAFF 238409	*Glycine max*	Argentina, General Roca, Córdoba	9 (TU-a), 13 (TU-C)	2001
31773	*Fusarium tucumaniae*	Yorinori 15/11= MAFF 239044	*Glycine max*	Brazil, PR, Ponta Grossa	9 (TU-a), 13 (TU-C)	2000
31796	*Fusarium tucumaniae*	Yorinori 86/01 = MAFF 239049	*Glycine max*	Brazil, PR, Ponta Grossa	7 (TU-a), 8 (TU-C)	1986
32389	*Fusarium virguliforme*	NSPCC i26	*Glycine max*	USA, Illinois	21 (96-VI-1), 22 (51-VI-1), 38 (51-VI-2)	Unknown
32390	*Fusarium virguliforme*	NSPCC i52	*Glycine max*	USA, Illinois	21 (96-VI-1), 19 (51-VI-1), 38 (51-VI-2)	1996
32391	*Fusarium virguliforme*	NSPCC i57	*Glycine max*	USA, Arkansas	21 (96-VI-1), 19 (51-VI-1), 38 (51-VI-2)	1996
32393	*Fusarium virguliforme*	NSPCC i159	*Glycine max*	USA, Illinois	23 (96-VI-1), 21 (51-VI-1), 42 (51-VI-2)	1999
32394	*Fusarium virguliforme*	NSPCC i170	*Glycine max*	USA, Illinois	22 (96-VI-1), 20 (51-VI-1), 39 (51-VI-2)	1999
32395	*Fusarium virguliforme*	NSPCC i171	*Glycine max*	USA, Illinois	20 (96-VI-1), 18 (51-VI-1), 34 (51-VI-2)	1999
32396	*Fusarium virguliforme*	NSPCC i173	*Glycine max*	USA, Illinois	19 (96-VI-1), 19 (51-VI-1), 38 (51-VI-2)	1999
32397	*Fusarium virguliforme*	NSPCC i300	*Glycine max*	USA, Illinois	22 (96-VI-1), 20 (51-VI-1), 41 (51-VI-2)	1991
32398	*Fusarium virguliforme*	NSPCC i501	*Glycine max*	USA, Illinois	22 (96-VI-1), 21 (51-VI-1), 41 (51-VI-2)	2001
32460	*Fusarium virguliforme*	Fsg-ISU 3	*Glycine max*	USA, Iowa-Cerro	21 (96-VI-1), 20 (51-VI-1), 38 (51-VI-2)	2000
32461	*Fusarium virguliforme*	Fsg-ISU 4	*Glycine max*	USA, Iowa-Chickasaw	20 (96-VI-1), 19 (51-VI-1), 37 (51-VI-2)	2000
32464	*Fusarium virguliforme*	Fsg-ISU 7	*Glycine max*	USA, Iowa-Clinton	23 (96-VI-1), 24 (51-VI-1), 44 (51-VI-2)	1995

(*Continued on next page*)

[a] NRRL = Agriculture Research Service Culture Collection, National Center for Agricultural Utilization Research, USDA-ARS, Peoria, IL.

[b] Equivalent no.: ATCC = American Type Culture Collection, Manassas, VA; BBA = Bundesanstalt für Land und Forstwirtschaft, Berlin; CCC= Culture Collection of CEREMIC (Centro de Referencia de Micología), Fac. de Cs. Bioquímicas y Farmacéuticas, UNR, Rosario, Argentina; FRC = Fusarium Research Center, Department of Plant Pathology, Pennsylvania State University, University Park, PA; Fsg-ISU = Department of Plant Pathology, Iowa State University, Ames, IA; MAFF = NIAS Genbank-Microorganisms Section, National Institute of Agrobiological Sciences (NIAS), 2-1-2 Kannondai, Tsukuba, Ibaraki 305-8602, Japan; NSPCC = National Soybean Pathogen Culture Collection, ARS-USDA, Urbana, IL.

[c] Index of discrimination was determined by first subtracting the average fluorescence intensity of three water negative controls from the value obtained for each DNA sample, and then dividing the minimum fluorescence intensity (MFI) by the maximum nontarget fluorescence intensity. Species-specific primer probes are indicated in parentheses (Table 3 provides probes used in the assay).

[d] Sequence data from locus 96 were used to identify NRRL 46137 as *Fusarium tucumaniae*.

TABLE 2. (*Continued from preceeding page*)

NRRL[a]	Species	Equivalent no.[b]	Host	Origin	Index of discrimination[c]	Year isolated
32465	*Fusarium virguliforme*	Fsg-ISU 8	*Glycine max*	USA, Iowa-Clinton	23 (96-VI-1), 22 (51-VI-1), 42 (51-VI-2)	1995
32467	*Fusarium virguliforme*	Fsg-ISU 10	*Glycine max*	USA, Iowa-Floyd	23 (96-VI-1), 22 (51-VI-1), 42 (51-VI-2)	1994
32468	*Fusarium virguliforme*	Fsg-ISU 11	*Glycine max*	USA, Iowa-Greene	22 (96-VI-1), 20 (51-VI-1), 39 (51-VI-2)	1996
32471	*Fusarium virguliforme*	Fsg-ISU 14	*Glycine max*	USA, Iowa-Jasper	23 (96-VI-1), 25 (51-VI-1), 44 (51-VI-2)	1994
32476	*Fusarium virguliforme*	Fsg-ISU 19	*Glycine max*	USA, Missouri-Mont	22 (96-VI-1), 25 (51-VI-1), 45 (51-VI-2)	Prior 1993
32479	*Fusarium virguliforme*	Fsg-ISU 22	*Glycine max*	USA, Iowa-Scott	22 (96-VI-1), 22 (51-VI-1), 42 (51-VI-2)	1993
32480	*Fusarium virguliforme*	Fsg-ISU 23	*Glycine max*	USA, Iowa-Worth	23 (96-VI-1), 25 (51-VI-1), 49 (51-VI-2)	1995
34438	*Fusarium virguliforme*	FRC S-1288L = Gray 17-1	*Glycine max*	USA, Arkansas	19 (96-VI-1), 19 (51-VI-1), 37 (51-VI-2)	Prior 1993
34439	*Fusarium virguliforme*	FRC S-1292L = Gray 12W-1	*Glycine max*	USA, Illinois	24 (96-VI-1), 27 (51-VI-1), 45 (51-VI-2)	1991
34440	*Fusarium virguliforme*	FRC S-1294L = Gray RW-1	*Glycine max*	USA, Illinois	23 (96-VI-1), 22 (51-VI-1), 42 (51-VI-2)	1991
34441	*Fusarium virguliforme*	FRC S-1295L = Gray Mont-1	*Glycine max*	USA, Illinois	27 (51-VI-1), 56 (51-VI-2)	1991
34442	*Fusarium virguliforme*	FRC S-1296L = Gray 1990-1	*Glycine max*	USA, Illinois	24 (96-VI-1), 30 (51-VI-1), 52 (51-VI-2)	1990
34443	*Fusarium virguliforme*	FRC S-1298L = Gray 11A-1	*Glycine max*	USA, Illinois	24 (96-VI-1), 27 (51-VI-1), 49 (51-VI-2)	1991
34444	*Fusarium virguliforme*	FRC S-1299L = Gray St9055-1	*Glycine max*	USA, Illinois	24 (96-VI-1), 24 (51-VI-1), 46 (51-VI-2)	1990
34547	*Fusarium tucumaniae*	MAFF 239253 = CCC 126-02	*Glycine max*	Argentina, Las Parejas, Santa Fe	14 (TU-a), 16 (TU-C)	2001
34548	*Fusarium tucumaniae*	MAFF 239254 = CCC 127-02	*Glycine max*	Argentina, Las Parejas, Santa Fe	13 (TU-a), 17 (TU-C)	2001
34549	*Fusarium tucumaniae*	MAFF 239255 = CCC 129-02	*Glycine max*	Argentina, Perez Millan, Buenos Aires	14 (TU-a), 18 (TU-C)	2000
34550	*Fusarium tucumaniae*	MAFF 239256 = CCC 128-02	*Glycine max*	Argentina, Pujato, Santa Fe	13 (TU-a), 17 (TU-C)	2000
34551	*Fusarium virguliforme*	MAFF 239257 = CCC 101-03	*Glycine max*	Argentina, San Pedro, Buenos Aires	19 (96-VI-1), 23 (51-VI-1), 37 (51-VI-2)	2002
34552	*Fusarium virguliforme*	MAFF 239258 = CCC 102-03	*Glycine max*	Argentina, Serodino, Santa Fe	18 (96-VI-1), 25 (51-VI-1), 38 (51-VI-2)	2002
34553	*Fusarium virguliforme*	MAFF 239259 = CCC 103-03	*Glycine max*	Argentina, Serodino, Santa Fe	16 (96-VI-1), 21 (51-VI-1), 34 (51-VI-2)	2002
34938	*Fusarium brasiliense*	CD 203	*Glycine max*	Brasil, Passo Fundo, Rio Grande do Sul	30 (B-a), 7 (B-b)	2003
34939	*Fusarium tucumaniae*	BRS 137	*Glycine max*	Brasil, Passo Fundo, Rio Grande do Sul	13 (TU-a), 15 (TU-C)	2003
36023	*Fusarium cuneirostrum*	Mung 1	*Vigna radiata*	Canada, Ontario, Ridgetown	8 (CU-a)	1996
36024	*Fusarium cuneirostrum*	Mung 2	*Vigna radiata*	Canada, Ontario, Ridgetown	12 (CU-a)	1996
36604	*Fusarium virguliforme*	CCC 155-05	Soil	Argentina, Buenos Aires, Pergamino	17 (96-VI-1), 22 (51-VI-1), 37 (51-VI-2)	Unknown
36605	*Fusarium virguliforme*	CCC 156-05	Soil	Argentina, Buenos Aires, Pergamino	17 (96-VI-1), 23 (51-VI-1), 35 (51-VI-2)	Unknown
36606	*Fusarium virguliforme*	CCC 157-05	Soil	Argentina, Buenos Aires, Pergamino	17 (96-VI-1), 20 (51-VI-1), 32 (51-VI-2)	Unknown
36607	*Fusarium virguliforme*	CCC 158-05	Soil	Argentina, Buenos Aires, Pergamino	16 (96-VI-1), 20 (51-VI-1), 34 (51-VI-2)	Unknown
36609	*Fusarium tucumaniae*	CCC 161-05	Soil	Argentina, Buenos Aires, Pergamino	12 (TU-a), 14 (TU-C)	Unknown
36610	*Fusarium virguliforme*	CCC 159-05	Soil	Argentina, Buenos Aires, Pergamino	18 (96-VI-1), 21 (51-VI-1), 32 (51-VI-2)	Unknown
36611	*Fusarium virguliforme*	CCC 160-05	Soil	Argentina, Buenos Aires, Pergamino	17 (96-VI-1), 23 (51-VI-1), 35 (51-VI-2)	Unknown
36612	*Fusarium tucumaniae*	CCC 162-05	Soil	Argentina, Buenos Aires, Pergamino	13 (TU-a), 16 (TU-C)	Unknown
36613	*Fusarium tucumaniae*	CCC 163-05	Soil	Argentina, Buenos Aires, Pergamino	13 (TU-a), 16 (TU-C)	Unknown
36877	*Fusarium* sp.	MAFF 239757 = CCC 142-05	*Glycine max*	Argentina, Zavalla, Santa Fe	36 (CR-a), 14 (CR-b)	2004
36878	*Fusarium tucumaniae*	CCC 209-05	*Glycine max*	Argentina, Zavalla, Santa Fe	12 (TU-a), 14 (TU-C)	2004
36879	*Fusarium tucumaniae*	CCC 210-05	*Glycine max*	Argentina, Zavalla, Santa Fe	13 (TU-a), 16 (TU-C)	2004
36880	*Fusarium tucumaniae*	CCC 211-05	*Glycine max*	Argentina, Los Molinos, Santa Fe	13 (TU-a), 18 (TU-C)	2004
36883	*Fusarium tucumaniae*	CCC 212-05	*Glycine max*	Argentina, Los Molinos, Santa Fe	12 (TU-a), 18 (TU-C)	2004

(*Continued on next page*)

TABLE 2. (*Continued from preceeding page*)

NRRL[a]	Species	Equivalent no.[b]	Host	Origin	Index of discrimination[c]	Year isolated
36886	*Fusarium tucumaniae*	CCC 154-06	*Glycine max*	Argentina, Los Molinos, Santa Fe	16 (TU-C)	2004
36887	*Fusarium tucumaniae*	CCC 213-05	*Glycine max*	Argentina, Los Molinos, Santa Fe	12 (TU-a), 16 (TU-C)	2004
36888	*Fusarium tucumaniae*	CCC 214-05	*Glycine max*	Argentina, Los Molinos, Santa Fe	12 (TU-a), 15 (TU-C)	2004
36895	*Fusarium tucumaniae*	CCC 215-05	*Glycine max*	Argentina, Los Molinos, Santa Fe	14 (TU-a)	2004
36896	*Fusarium virguliforme*	CCC 216-05	*Glycine max*	Argentina, Los Molinos, Santa Fe	17 (96-VI-1), 22 (51-VI-1), 35 (51-VI-2)	2004
36897	*Fusarium virguliforme*	CCC 217-05	*Glycine max*	Argentina, Los Molinos, Santa Fe	17 (96-VI-1), 21 (51-VI-1), 35 (51-VI-2)	2004
36898	*Fusarium virguliforme*	CCC 218-05	*Glycine max*	Argentina, Los Molinos, Santa Fe	9 (96-VI-1), 11 (51-VI-1), 23 (51-VI-2)	2004
36899	*Fusarium virguliforme*	CCC 219-05	*Glycine max*	Argentina, Los Molinos, Santa Fe	17 (96-VI-1), 19 (51-VI-1), 32 (51-VI-2)	2004
36900	*Fusarium virguliforme*	CCC 220-05	*Glycine max*	Argentina, Los Molinos, Santa Fe	14 (96-VI-1), 16 (51-VI-1), 30 (51-VI-2)	2004
37585	*Fusarium virguliforme*	NSPCC i1	*Glycine max*	USA, Arkansas	16 (96-VI-1), 19 (51-VI-1), 33 (51-VI-2)	1991
37586	*Fusarium virguliforme*	NSCPP i12	*Glycine max*	USA, Arkansas	15 (96-VI-1), 16 (51-VI-1), 29 (51-VI-2)	1996
37587	*Fusarium virguliforme*	NSCPP i45	*Glycine max*	USA, Illinois	14 (96-VI-1), 17 (51-VI-1), 29 (51-VI-2)	1994
37588	*Fusarium virguliforme*	NSCPP i98	*Glycine max*	USA, Wisconson	15 (96-VI-1), 17 (51-VI-1), 30 (51-VI-2)	1998
37589	*Fusarium virguliforme*	NSCPP i300	*Glycine max*	USA, Illinois	16 (96-VI-1), 17 (51-VI-1), 32 (51-VI-2)	1991
37590	*Fusarium virguliforme*	NSCPP i502	*Glycine max*	USA, Missouri	16 (96-VI-1), 20 (51-VI-1), 35 (51-VI-2)	2002
37591	*Fusarium virguliforme*	NSCPP i506	*Glycine max*	USA, Missouri	15 (96-VI-1), 16 (51-VI-1), 26 (51-VI-2)	2002
37592	*Fusarium virguliforme*	NSCPP i530	*Glycine max*	USA, Indiana	14 (96-VI-1), 17 (51-VI-1), 28 (51-VI-2)	2003
38411	*Fusarium tucumaniae*	CCC 164-05	*Glycine max*	Argentina, General Roca, Córdoba	13 (TU-a), 13 (TU-c)	2005
38412	*Fusarium tucumaniae*	CCC 165-05	*Glycine max*	Argentina, General Roca, Córdoba	12 (TU-a), 16 (TU-c)	2005
38413	*Fusarium tucumaniae*	CCC 166-05	*Glycine max*	Argentina, General Roca, Córdoba	11 (TU-a), 15 (TU-c)	2005
38414	*Fusarium tucumaniae*	CCC 167-05	*Glycine max*	Argentina, General Roca, Córdoba	12 (TU-a), 13 (TU-c)	2005
38415	*Fusarium tucumaniae*	CCC 168-05	*Glycine max*	Argentina, General Roca, Córdoba	13 (TU-a), 16 (TU-c)	2005
38416	*Fusarium tucumaniae*	CCC 169-05	*Glycine max*	Argentina, General Roca, Córdoba	11 (TU-a), 15 (TU-c)	2005
38417	*Fusarium virguliforme*	CCC 170-05	*Glycine max*	Argentina, Cañada de Gómez, Santa Fe	15 (96-VI-1), 20 (51-VI-1), 35 (51-VI-2)	2005
38418	*Fusarium tucumaniae*	CCC 171-05	*Glycine max*	Argentina, Cañada de Gómez, Santa Fe	12 (TU-a), 17 (TU-c)	2005
38419	*Fusarium tucumaniae*	CCC 173-05	*Glycine max*	Argentina, Cañada de Gómez, Santa Fe	13 (TU-a), 17 (TU-c)	2005
38420	*Fusarium tucumaniae*	CCC 174-05	*Glycine max*	Argentina, Cañada de Gómez, Santa Fe	14 (TU-a), 19 (TU-c)	2005
43326	*Fusarium tucumaniae*	CCC 105-06	*Glycine max*	Argentina, Monte Buey, Córdoba	13 (TU-a), 19 (TU-c)	2006
43327	*Fusarium tucumaniae*	CCC 158-06	*Glycine max*	Argentina, Monte Buey, Córdoba	14 (TU-a), 18 (TU-c)	2006
43328	*Fusarium tucumaniae*	CCC 157-06	*Glycine max*	Argentina, Monte Buey, Córdoba	11 (TU-a), 16 (TU-c)	2006
43329	*Fusarium tucumaniae*	CCC 159-06	*Glycine max*	Argentina, Monte Buey, Córdoba	11 (TU-a), 20 (TU-c)	2006
43331	*Fusarium tucumaniae*	CCC 132-06	*Glycine max*	Argentina, Armstrong, Santa Fe	13 (TU-a), 16 (TU-c)	2006
43332	*Fusarium tucumaniae*	CCC 156-06	*Glycine max*	Argentina, Armstrong, Santa Fe	14 (TU-a), 18 (TU-c)	2006
43333	*Fusarium tucumaniae*	CCC 133-06	*Glycine max*	Argentina, Armstrong, Santa Fe	13 (TU-a), 21 (TU-c)	2006
43334	*Fusarium tucumaniae*	CCC 104-06	*Glycine max*	Argentina, Armstrong, Santa Fe	15 (TU-a), 24 (TU-c)	2006
43335	*Fusarium tucumaniae*	CCC 134-06	*Glycine max*	Argentina, Armstrong, Santa Fe	14 (TU-a), 19 (TU-c)	2006
43336	*Fusarium tucumaniae*	CCC 160-06	*Glycine max*	Argentina, Gral. Roca, Córdoba	13 (TU-a), 17 (TU-c)	2006
43337	*Fusarium tucumaniae*	CCC 167-06	*Glycine max*	Argentina, Gral. Roca, Córdoba	13 (TU-a), 16 (TU-c)	2006
43338	*Fusarium tucumaniae*	CCC 139-06	*Glycine max*	Argentina, Gral. Roca, Córdoba	12 (TU-a), 16 (TU-c)	2006
43339	*Fusarium tucumaniae*	CCC 140-06	*Glycine max*	Argentina, Gral. Roca, Córdoba	12 (TU-a), 15 (TU-c)	2006
43340	*Fusarium tucumaniae*	CCC 141-06	*Glycine max*	Argentina, Gral. Roca, Córdoba	11 (TU-a), 17 (TU-c)	2006
43341	*Fusarium tucumaniae*	CCC 108-06	*Glycine max*	Argentina, Gral. Roca, Córdoba	11 (TU-a), 22 (TU-c)	2006
43342	*Fusarium tucumaniae*	CCC 164-06	*Glycine max*	Argentina, Monte Buey, Córdoba	12 (TU-a), 18 (TU-c)	2006
43343	*Fusarium tucumaniae*	CCC 109-06	*Glycine max*	Argentina, Marcos Juárez, Córdoba	13 (TU-a), 14 (TU-c)	2006
43344	*Fusarium tucumaniae*	CCC 163-06	*Glycine max*	Argentina, Marcos Juárez, Córdoba	12 (TU-a), 18 (TU-c)	2006
43345	*Fusarium tucumaniae*	CCC 142-06	*Glycine max*	Argentina, Marcos Juárez, Córdoba	14 (TU-a), 20 (TU-c)	2006
43346	*Fusarium tucumaniae*	CCC 143-06	*Glycine max*	Argentina, Marcos Juárez, Córdoba	8 (TU-a), 10 (TU-c)	2006
43347	*Fusarium tucumaniae*	CCC 144-06	*Glycine max*	Argentina, Marcos Juárez, Córdoba	12 (TU-a), 17 (TU-c)	2006
43348	*Fusarium tucumaniae*	06-232-1	*Glycine max*	Argentina, Corral de Bustos, Córdoba	12 (TU-a), 17 (TU-c)	2006
43349	*Fusarium tucumaniae*	CCC 138-06	*Glycine max*	Argentina, Corral de Bustos, Córdoba	11 (TU-a), 16 (TU-c)	2006
43350	*Fusarium brasiliense*	CCC 161-06	*Glycine max*	Argentina, Saira, Córdoba	69 (B-a), 8 (B-b)	2006
43351	*Fusarium tucumaniae*	CCC 110-06	*Glycine max*	Argentina, Saira, Córdoba	12 (TU-a), 17 (TU-c)	2006
43352	*Fusarium tucumaniae*	CCC 113-06	*Glycine max*	Argentina, Chañar Ladeado, Santa Fe	12 (TU-a), 18 (TU-c)	2006
43353	*Fusarium tucumaniae*	CCC 107-06	*Glycine max*	Argentina, Chañar Ladeado, Santa Fe	10 (TU-a), 16 (TU-c)	2006
43354	*Fusarium tucumaniae*	CCC 114-06	*Glycine max*	Argentina, Chañar Ladeado, Santa Fe	12 (TU-a), 16 (TU-c)	2006
43355	*Fusarium tucumaniae*	CCC 115-06	*Glycine max*	Argentina, Chañar Ladeado, Santa Fe	9 (TU-a), 12 (TU-c)	2006
43356	*Fusarium tucumaniae*	CCC 116-06	*Glycine max*	Argentina, Chañar Ladeado, Santa Fe	9 (TU-a), 13 (TU-c)	2006
43357	*Fusarium tucumaniae*	CCC 165-06	*Glycine max*	Argentina, Chañar Ladeado, Santa Fe	11 (TU-a), 16 (TU-c)	2006

(*Continued on next page*)

TABLE 2. (*Continued from preceeding page*)

NRRL[a]	Species	Equivalent no.[b]	Host	Origin	Index of discrimination[c]	Year isolated
43358	*Fusarium tucumaniae*	CCC 117-06	*Glycine max*	Argentina, Chañar Ladeado, Santa Fe	14 (TU-a), 16 (TU-c)	2006
43359	*Fusarium tucumaniae*	CCC 118-06	*Glycine max*	Argentina, Chañar Ladeado, Santa Fe	13 (TU-a), 16 (TU-c)	2006
43360	*Fusarium tucumaniae*	CCC 119-06	*Glycine max*	Argentina, Chañar Ladeado, Santa Fe	11 (TU-a), 14 (TU-c)	2006
43361	*Fusarium tucumaniae*	CCC 166-06	*Glycine max*	Argentina, Chañar Ladeado, Santa Fe	11 (TU-a), 15 (TU-c)	2006
43362	*Fusarium tucumaniae*	CCC 106-06	*Glycine max*	Argentina, Victoria, Entre Ríos	11 (TU-a), 17 (TU-c)	2006
43363	*Fusarium tucumaniae*	CCC 135-06	*Glycine max*	Argentina, Victoria, Entre Ríos	11 (TU-a), 16 (TU-c)	2006
43364	*Fusarium tucumaniae*	CCC 136-06	*Glycine max*	Argentina, Victoria, Entre Ríos	11 (TU-a), 18 (TU-c)	2006
43365	*Fusarium tucumaniae*	CCC 137-06	*Glycine max*	Argentina, Victoria, Entre Ríos	11 (TU-a), 18 (TU-c)	2006
43366	*Fusarium tucumaniae*	CCC 207-07	*Glycine max*	Argentina, Victoria, Entre Ríos	10 (TU-a), 14 (TU-c)	2006
43367	*Fusarium tucumaniae*	CCC 129-06	*Glycine max*	Argentina, Leones, Córdoba	12 (TU-a), 13 (TU-c)	2006
43368	*Fusarium tucumaniae*	CCC 130-06	*Glycine max*	Argentina, Leones, Córdoba	13 (TU-a), 16 (TU-c)	2006
43369	*Fusarium tucumaniae*	CCC 112-06	*Glycine max*	Argentina, Leones, Córdoba	14 (TU-a), 16 (TU-c)	2006
43370	*Fusarium tucumaniae*	CCC 131-06	*Glycine max*	Argentina, Leones, Córdoba	14 (TU-a), 22 (TU-c)	2006
43371	*Fusarium tucumaniae*	06-281-1	*Glycine max*	Argentina, Tortugas, Santa Fe	13 (TU-a), 18 (TU-c)	2006
43559	*Fusarium tucumaniae*	CCC 145-06	*Glycine max*	Argentina, Tortugas, Santa Fe	12 (TU-a), 16 (TU-c)	2006
43560	*Fusarium tucumaniae*	CCC 146-06	*Glycine max*	Argentina, Tortugas, Santa Fe	10 (TU-a), 14 (TU-c)	2006
43561	*Fusarium tucumaniae*	CCC 111-06	*Glycine max*	Argentina, Tortugas, Santa Fe	11 (TU-a), 13 (TU-c)	2006
43562	*Fusarium tucumaniae*	CCC 147-06	*Glycine max*	Argentina, Tortugas, Santa Fe	11 (TU-a), 17 (TU-c)	2006
43563	*Fusarium tucumaniae*	CCC 148-06	*Glycine max*	Argentina, Tortugas, Santa Fe	11 (TU-a), 20 (TU-c)	2006
43564	*Fusarium tucumaniae*	CCC 149-06	*Glycine max*	Argentina, Tortugas, Santa Fe	11 (TU-a), 14 (TU-c)	2006
43565	*Fusarium tucumaniae*	CCC 150-06	*Glycine max*	Argentina, Tortugas, Santa Fe	11 (TU-a), 15 (TU-c)	2006
43566	*Fusarium tucumaniae*	CCC 151-06	*Glycine max*	Argentina, Tortugas, Santa Fe	10 (TU-a), 13 (TU-c)	2006
43567	*Fusarium tucumaniae*	CCC 152-06	*Glycine max*	Argentina, Tortugas, Santa Fe	12 (TU-a), 16 (TU-c)	2006
43568	*Fusarium tucumaniae*	CCC 153-06	*Glycine max*	Argentina, Tortugas, Santa Fe	14 (TU-a), 18 (TU-c)	2006
43569	*Fusarium tucumaniae*	CCC 120-06	*Glycine max*	Argentina, Justiniano Posse, Córdoba	13 (TU-a), 20 (TU-c)	2006
43570	*Fusarium tucumaniae*	CCC 121-06	*Glycine max*	Argentina, Justiniano Posse, Córdoba	12 (TU-a), 16 (TU-c)	2006
43571	*Fusarium tucumaniae*	CCC 103-06	*Glycine max*	Argentina, Justiniano Posse, Córdoba	7 (TU-a), 7 (TU-c)	2006
43572	*Fusarium tucumaniae*	CCC 122-06	*Glycine max*	Argentina, Justiniano Posse, Córdoba	9 (TU-a), 13 (TU-c)	2006
43574	*Fusarium tucumaniae*	CCC 124-06	*Glycine max*	Argentina, Justiniano Posse, Córdoba	10 (TU-a), 16 (TU-c)	2006
43575	*Fusarium tucumaniae*	CCC 125-06	*Glycine max*	Argentina, Justiniano Posse, Córdoba	12 (TU-a), 20 (TU-c)	2006
43576	*Fusarium tucumaniae*	CCC 126-06	*Glycine max*	Argentina, Justiniano Posse, Córdoba	11 (TU-a), 17 (TU-c)	2006
43577	*Fusarium tucumaniae*	CCC 127-06	*Glycine max*	Argentina, Justiniano Posse, Córdoba	12 (TU-a), 13 (TU-c)	2006
43578	*Fusarium tucumaniae*	CCC 128-06	*Glycine max*	Argentina, Justiniano Posse, Córdoba	11 (TU-a), 15 (TU-c)	2006
43824	*Fusarium* sp.	CCC 144-07	*Glycine max*	Argentina, Las Cejas, Tucumán	36 (CR-a), 8 (CR-b)	2006
43825	*Fusarium* sp.	CCC 143-07	*Glycine max*	Argentina, Las Cejas, Tucumán	36 (CR-a), 8 (CR-b)	2006
43826	*Fusarium tucumaniae*	CCC 204-07	*Glycine max*	Argentina, Las Cejas, Tucumán	13 (TU-a), 21 (TU-c)	2006
43827	*Fusarium tucumaniae*	CCC 142-07	*Glycine max*	Argentina, Las Cejas, Tucumán	12 (TU-a), 15 (TU-c)	2006
43828	*Fusarium tucumaniae*	CCC 141-07	*Glycine max*	Argentina, Las Cejas, Tucumán	11 (TU-a), 14 (TU-c)	2006
43829	*Fusarium tucumaniae*	CCC 140-07	*Glycine max*	Argentina, Las Cejas, Tucumán	9 (TU-a), 12 (TU-c)	2006
43830	*Fusarium tucumaniae*	CCC 205-07	*Glycine max*	Argentina, Las Cejas, Tucumán	14 (TU-c)	2006
43831	*Fusarium tucumaniae*	CCC 206-07	*Glycine max*	Argentina, Las Cejas, Tucumán	15 (TU-c)	2006
43832	*Fusarium tucumaniae*	CCC 207-07	*Glycine max*	Argentina, Las Cejas, Tucumán	11 (TU-a), 17 (TU-c)	2006
43833	*Fusarium tucumaniae*	CCC139-07	*Glycine max*	Argentina, Las Cejas, Tucumán	18 (TU-c)	2006
43834	*Fusarium tucumaniae*	CCC 144-07	*Glycine max*	Argentina, Las Cejas, Tucumán	15 (TU-c)	2006
43835	*Fusarium tucumaniae*	CCC 208-07	*Glycine max*	Argentina, Las Cejas, Tucumán	15 (TU-c)	2006
43837	*Fusarium tucumaniae*	06-223-2	*Glycine max*	Argentina, Monte Buey, Córdoba	14 (TU-a), 22 (TU-c)	2006
43838	*Fusarium tucumaniae*	CCC 147-07	*Glycine max*	Argentina, Monte Buey, Córdoba	14 (TU-a), 22 (TU-c)	2006
43839	*Fusarium tucumaniae*	06-223-4	*Glycine max*	Argentina, Monte Buey, Córdoba	14 (TU-a), 17 (TU-c)	2006
43840	*Fusarium tucumaniae*	CCC 145-07	*Glycine max*	Argentina, Monte Buey, Córdoba	12 (TU-a), 14 (TU-c)	2006
43842	*Fusarium tucumaniae*	06-225-2	*Glycine max*	Argentina, Camilo Aldao, Córdoba	15 (TU-c)	2006
43843	*Fusarium tucumaniae*	CCC 146-07	*Glycine max*	Argentina, Camilo Aldao, Córdoba	3 (TU-c)	2006
43844	*Fusarium tucumaniae*	06-225-4	*Glycine max*	Argentina, Camilo Aldao, Córdoba	17 (TU-c)	2006
43845	*Fusarium tucumaniae*	06-225-5	*Glycine max*	Argentina, Camilo Aldao, Córdoba	17 (TU-c)	2006
46129	*Fusarium tucumaniae*	CCC 116-07	*Glycine max*	Argentina, Gral.Roca, Córdoba	8 (TU-a), 8 (TU-c)	2007
46130	*Fusarium tucumaniae*	CCC 117-07	*Glycine max*	Argentina, Gral.Roca, Córdoba	13 (TU-a), 20 (TU-c)	2007
46131	*Fusarium tucumaniae*	CCC 118-07	*Glycine max*	Argentina, Gral.Roca, Córdoba	13 (TU-a), 20 (TU-c)	2007
46132	*Fusarium tucumaniae*	CCC 119-07	*Glycine max*	Argentina, Gral.Roca, Córdoba	12 (TU-a), 22 (TU-c)	2007
46133	*Fusarium tucumaniae*	CCC 109-07	*Glycine max*	Argentina, Inriville, Córdoba	15 (TU-a), 25 (TU-c)	2007
46134	*Fusarium tucumaniae*	CCC 110-07	*Glycine max*	Argentina, Inriville, Córdoba	15 (TU-a), 24 (TU-c)	2007
46135	*Fusarium tucumaniae*	CCC 111-07	*Glycine max*	Argentina, Inriville, Córdoba	13 (TU-a), 18 (TU-c)	2007
46136	*Fusarium tucumaniae*	CCC 112-07	*Glycine max*	Argentina, Inriville, Córdoba	8 (TU-a), 10 (TU-c)	2007
46138	*Fusarium tucumaniae*	CCC 114-07	*Glycine max*	Argentina, Leones, Córdoba	13 (TU-a), 18 (TU-c)	2007
46139	*Fusarium tucumaniae*	CCC 115-07	*Glycine max*	Argentina, Leones, Córdoba	7 (TU-c)	2007
46140	*Fusarium tucumaniae*	CCC 166-07	*Glycine max*	Argentina, Casilda, Santa Fe	8 (TU-a), 12 (TU-c)	2007
46141	*Fusarium tucumaniae*	CCC 154-07	*Glycine max*	Argentina, Casilda, Santa Fe	6 (TU-a), 12 (TU-c)	2007
46142	*Fusarium tucumaniae*	CCC 155-07	*Glycine max*	Argentina, Casilda, Santa Fe	12 (TU-c)	2007
46143	*Fusarium tucumaniae*	CCC 164-07	*Glycine max*	Argentina, Pilar, Buenos Aires	8 (TU-a), 13 (TU-c)	2007
46144	*Fusarium tucumaniae*	CCC 163-07	*Glycine max*	Argentina, Pilar, Buenos Aires	8 (TU-a), 12 (TU-c)	2007
46146	*Fusarium tucumaniae*	CCC 153-07	*Glycine max*	Argentina, Pilar, Buenos Aires	8 (TU-a), 12 (TU-c)	2007
46147	*Fusarium tucumaniae*	CCC 157-07	*Glycine max*	Argentina, Pilar, Buenos Aires	7 (TU-a), 10 (TU-c)	2007
46148	*Fusarium tucumaniae*	CCC 138-07	*Glycine max*	Argentina, Tucumán	7 (TU-a), 8 (TU-C)	2007
46149	*Fusarium tucumaniae*	CCC 133-07	*Glycine max*	Argentina, Tucumán	8 (TU-a), 8 (TU-c)	2007

(*Continued on next page*)

used in the extension reactions for the MLGT assay, together with three positive control probes to confirm the presence of the these three templates in the multiplex ASPE reaction, and 12 species-specific probes. Reactions were performed in a total volume of 20 µl that included 1× PCR buffer, 1.25 mM $MgCl_2$, 5 µM dATP, dGTP, dTTP, 5 µM biotin-dCTP, 0.75 U of Platinum GenoTYPE *Tsp* DNA polymerase (Invitrogen Life Technologies), 25 nM of each probe primer, and 5 µl of purified amplicon (≈0.2 µg). ASPE reactions were run for 120 s at 96°C, followed by 40 cycles of 30 s at 94°C, 60 s at 55°C, 120 s at 74°C, and ending in a 4°C soak. Hybridization and detection followed published protocols (25). Given that the 24-bp tag sequence on the 5′ end of each ASPE product hybridizes to a specific antitag attached to a microsphere with a unique spectral address, it was possible to sort and evaluate the extension products from the different probes individually using a Luminex 100 flow cytometer. Indices of discrimination (ID) for the MLGT identifications were determined by first subtracting the average intensity of three water controls from each value, then dividing the minimum fluorescence intensity (MFI) by the maximum nontarget fluorescence intensity.

RESULTS

Molecular phylogenetics. Nucleotide sequence data were obtained from portions of three loci to investigate species limits within the SDS–BRR clade. Aligned DNA sequences from the *EF-1a* gene (687 bp), anonymous intergenic locus 96 (1,520 bp), and the nuclear ribosomal IGS rDNA (2,765 bp) were analyzed individually and as a combined data set (Fig. 1) (4,972 bp) via MP as implemented in PAUP (42). Four soybean SDS (i.e., *F. tucumaniae*, *F. brasiliense*, *F. virguliforme*, and *Fusarium* sp.) and two BRR (i.e., *F. phaseoli* and *F. cuneirostrum*) pathogens were resolved as genealogically exclusive, phylogenetically distinct species in MP bootstrap analyses of the IGS rDNA and combined data set. Analyses of the partial *EF-1a* gene and locus 96 data partitions resolved *F. brasiliense*, *F. virguliforme*, and *Fusarium* sp. as reciprocally monophyletic. Relationships between the three other SDS–BRR clade species were unresolved in analyses of the latter two loci. Previously, *Fusarium* sp. and *F. cuneirostrum* were treated as conspecific because the monophyly of the former species could not be assessed, given that it was represented by a single isolate from Brazil (4). However, with the inclusion of a second isolate of this *Fusarium* sp. (Fig. 1; NRRL 36877 from Santa Fe, Argentina), the present analyses indicate that it and *F. cuneirostrum* are reciprocally monophyletic sisters. Even though the six species within the SDS–BRR clade are closely related phylogenetically, it is noteworthy that they are all phenotypically distinct (3,4). Concordant results (not shown) were obtained under the maximum likelihood criterion using the general time-reversible model of nucleotide substitution with a proportion of invariant sites and gamma-distributed rate heterogeneity in GARLI (50).

Assessment of published SDS and BRR species-specific primers and probes. Based on our improved understanding of species boundaries within the SDS–BRR clade, we conducted nucleotide sequence comparisons of the three loci used in the design of published SDS and BRR species-specific PCR primers and probes to determine whether they could differentiate the newly delimited SDS and BRR pathogens. Comparative DNA sequence analyses of partial gene sequences of the mtSSU rDNA (8,14–16), *EF-1a* gene (7,14), and ITS + 28S rDNA (22) of the SDS and BRR pathogens revealed that highly conserved regions of these three loci were used in the design of the assays. Therefore none of these assays are species-specific based on our current understanding of species limits within the SDS–BRR clade.

Design and validation of the allele-specific genotyping assay. Given the inability of published molecular diagnostics to differentiate the fusaria associated with SDS and BRR, multilocus DNA sequence data from the 65-isolate design panel (DP) (Fig. 1) was used to design probes for MLGT analysis of the SDS and BRR fusaria (Table 3). This panel included 12 species-specific probes and three positive control probes to confirm the successful amplification of the IGS rDNA and locus 51 and 96 amplicons in the ASPE reaction. Probe performance data for the DP are provided in Table 1. ID values for the 12 MLGT probes ranged from 3 to 44 which means that MFI values for isolates with a negative genotype were less than one-third the MFI values for strains with a positive genotype. Sequences in the primer annealing sites independently confirmed that the MLGT assay should accurately identify all 65 isolates in the DP (Table 1).

MLGT of experimental panel. The validated microsphere array successfully identified 205 soybean SDS isolates in the EP.

TABLE 2. (*Continued from preceeding page*)

NRRL[a]	Species	Equivalent no.[b]	Host	Origin	Index of discrimination[c]	Year isolated
46150	*Fusarium tucumaniae*	CCC 158-07	*Glycine max*	Argentina, Tucumán	7 (TU-a), 12 (TU-c)	2007
46151	*Fusarium tucumaniae*	CCC 152-07	*Glycine max*	Argentina, La Cocha, Tucumán	8 (TU-a), 11 (TU-c)	2007
46152	*Fusarium tucumaniae*	CCC 159-07	*Glycine max*	Argentina, La Cocha, Tucumán	8 (TU-a), 8 (TU-C)	2007
46154	*Fusarium tucumaniae*	CCC 137-07	*Glycine max*	Argentina, La Cocha, Tucumán	6 (TU-a), 6 (TU-C)	2007
46155	*Fusarium tucumaniae*	CCC 132-07	*Glycine max*	Argentina, La Cocha, Tucumán	7 (TU-a), 12 (TU-c)	2007
46157	*Fusarium tucumaniae*	07-620-2	*Glycine max*	Argentina, La Virginia Burruyacú, Tucumán	7 (TU-a), 11 (TU-c)	2007
46158	*Fusarium tucumaniae*	CCC 149-07	*Glycine max*	Argentina, La Virginia Burruyacú, Tucumán	5 (TU-c)	2007
46160	*Fusarium tucumaniae*	CCC 136-07	*Glycine max*	Argentina, Leones, Córdoba	12 (TU-c)	2007
46161	*Fusarium tucumaniae*	CCC 149-07	*Glycine max*	Argentina, Marcos Juarez, Córdoba	9 (TU-a), 13 (TU-c)	2007
46162	*Fusarium tucumaniae*	CCC 136-07	*Glycine max*	Argentina, Marcos Juarez, Córdoba	9 (TU-a), 12 (TU-c)	2007
46163	*Fusarium tucumaniae*	CCC 172-07I	*Glycine max*	Argentina, Marcos Juarez, Córdoba	8 (TU-a), 12 (TU-c)	2007
46165	*Fusarium tucumaniae*	CCC 151-07	*Glycine max*	Argentina, Marcos Juárez, Córdoba	6 (TU-a), 7 (TU-C)	2007
46166	*Fusarium tucumaniae*	CCC 162-07	*Glycine max*	Argentina, Inriville, Córdoba	7 (TU-a), 9 (TU-C)	2007
46167	*Fusarium tucumaniae*	CCC 165-07	*Glycine max*	Argentina, Inriville, Córdoba	8 (TU-a), 9 (TU-C)	2007
46168	*Fusarium tucumaniae*	CCC 173-07	*Glycine max*	Argentina, Inriville, Córdoba	7 (TU-c)	2007
46169	*Fusarium tucumaniae*	CCC 161-07	*Glycine max*	Argentina, Tucumán	9 (TU-a), 9 (TU-c)	2007
46170	*Fusarium* sp.	CCC 169-07	*Glycine max*	Argentina, Las Lajitas, Salta	39 (CR-a), 10 (CR-b)	2007
46171	*Fusarium* sp.	CCC 168-07	*Glycine max*	Argentina, Las Lajitas, Salta	32 (CR-a), 7 (CR-b)	2007
46172	*Fusarium* sp.	07-664-3	*Glycine max*	Argentina, Las Lajitas, Salta	22 (CR-a), 5 (CR-b)	2007
46173	*Fusarium* sp.	CCC 174-07	*Glycine max*	Argentina, Las Lajitas, Salta	34 (CR-a), 10 (CR-b)	2007
46174	*Fusarium* sp.	CCC 175-07	*Glycine max*	Argentina, Las Lajitas, Salta	37 (CR-a), 9 (CR-b)	2007
46175	*Fusarium* sp.	CCC 176-07	*Glycine max*	Argentina, Las Lajitas, Salta	37 (CR-a), 9 (CR-b)	2007
46177	*Fusarium tucumaniae*	CCC 178-07	*Glycine max*	Argentina, Entre Ríos	8 (TU-c)	2007
46178	*Fusarium tucumaniae*	CCC 177-07	*Glycine max*	Argentina, Entre Ríos	14 (TU-c)	2007
46179	*Fusarium tucumaniae*	CCC 171-07	*Glycine max*	Argentina, Entre Ríos	8 (TU-c)	2007

Probe performance data for the EP are provided in Table 2. ID values for the species probes ranged from 3 to 56, meaning the MFI values for isolates with a positive genotype were at least 3 times greater than the MFI values for strains with a negative genotype. Combined analysis of the DP and EP ($N = 271$) provide an initial estimate of soybean SDS species diversity in Argentina and the United States. Results of the present study revealed that *F. tucumaniae* is the predominant SDS pathogen in five of the six Argentine provinces surveyed, comprising 87.2% of the SDS isolates recovered. The Argentinean province of Salta is the only exception in that the undescribed *Fusarium* sp., formerly thought to be conspecific with the BRR pathogen *F. cuneirostrum* (4), accounted for all six SDS isolates recovered in this province (Fig. 3). The present survey revealed that three other SDS pathogens are present in Argentina, but in low frequencies (*F. virguliforme* 7.5%, *Fusarium* sp. 4.8%, and *F. brasiliense* 0.5%). In addition, limited sampling within five Brazilian districts revealed that three of the four soybean SDS pathogens are present in Brazil (Fig. 3). By way of contrast, surveys of eight major soybean-growing states suggests that a highly clonal population of *F. virguliforme* appears to be responsible for all soybean SDS within the United States (3,29,43), with the exception of a single isolate of *F. brasiliense* from California (Tables 1 and 2).

DISCUSSION

The present study extends our knowledge of species boundaries within the SDS–BRR clade by the discovery of a fourth SDS species, *Fusarium* sp., which is currently only known from the District of Goiás in Brazil (NRRL 31949) and the northern provinces of Salta, Tucumán, and Santa Fe in Argentina (Tables 1 and 2). Previously, this soybean SDS species was tentatively considered a divergent isolate of the BRR pathogen *F. cuneirostrum* because it was represented by a single isolate (NRRL 31949), and because it formed a clade with the latter species (4). However, with the discovery of additional isolates of this species from Argentina, multilocus bootstrap analyses of the three individual and combined gene partitions demonstrated that *F. cuneirostrum* and *Fusarium* sp. represent genealogically exclusive, reciprocally monophyletic sister taxa. Even though isolates of *F. cuneirostrum* from the United States, Canada, and Japan share the same 3-locus haplotype (Fig. 1), electrophoretic karyotypes of this species from Japan (NRRL 22276 and 31104) are polymorphic (41), suggesting that genome organization within this species is variable. Moreover, isolates of *F. brasiliense* (NRRL 22678 and 22743) and *F. cuneirostrum* (NRRL 22158) appear to possess the same unique mtSSU rDNA RFLP haplotype (32), indicating that this locus is too conserved to differentiate these species.

Application of the forma specialis naming system for the SDS and BRR pathogens (i.e., f. sp. *glycines* and f. sp. *phaseoli*), and for other phytopathogens within the FSSC (20,26,49) and *F. oxysporum* species complex (23), obscures the genetic diversity, phylogenetic relationships, geographic distribution, and host range of these pathogens. Based on the discovery of four SDS and two BRR pathogens (3,4; present study), in the absence of detailed phenotypic or molecular systematic data, it is impossible to conclude with certainty what soybean SDS pathogens were studied when they are reported as *F. solani* f. sp. *glycines*, *F. solani* f. sp. *phaseoli*, or *F. solani*. Furthermore, even though the six SDS and BRR species form a genealogically exclusive subclade within the putatively South American clade 2 of the FSSC (20), the molecular phylogenetic results indicate that evolution of host specificity is homoplastic in that these two formae speciales exhibit a paraphyletic set of relationships. The available data suggests that pathogenicity to soybean may represent the plesiomorphic condition, given that the SDS pathogen *F. virguliforme* appears to represent the most basal divergence within the SDS–BRR clade (4). Because soybean was introduced to South and North America

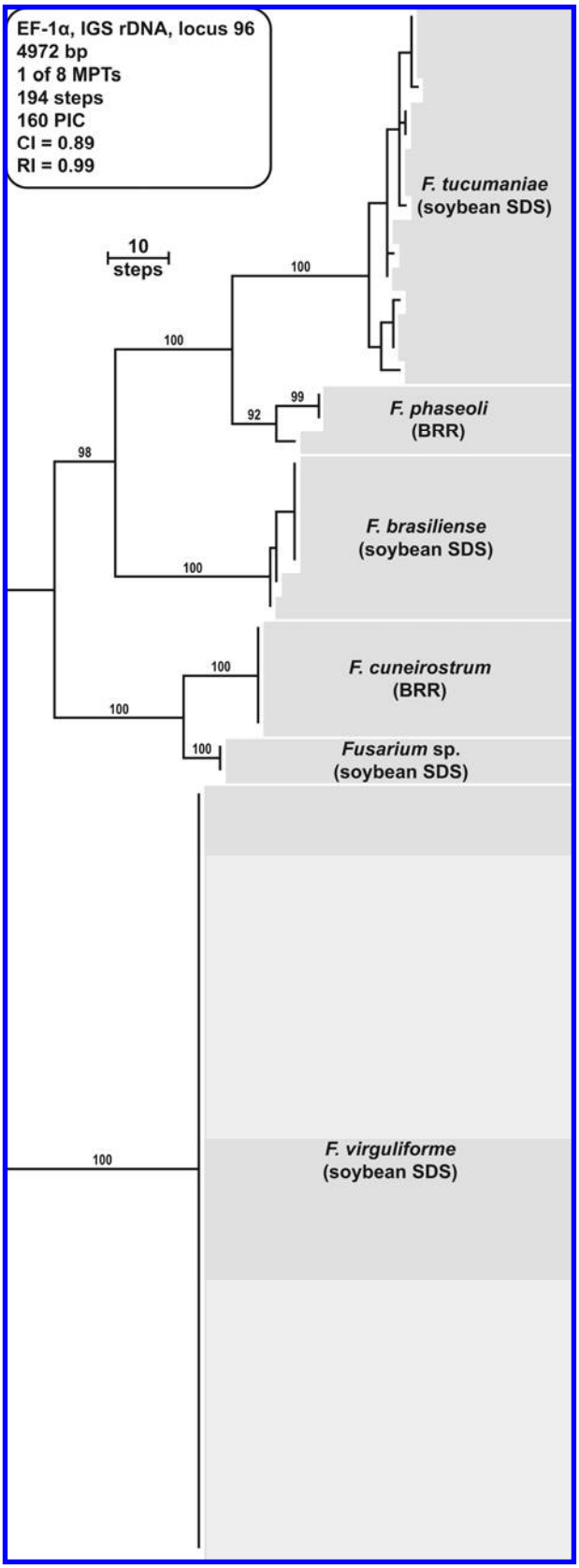

Fig. 1. One of eight most-parsimonious trees inferred from DNA sequence data from portions of three loci (*EF-1a*, intergenic spacer region [IGS] rDNA, and locus 96) from 58 soybean sudden death syndrome (SDS) and 8 bean root rot (BRR) pathogens. Sequences of *Fusarium virguliforme* were used to root the phylogeny based on more inclusive analyses (3,4). Numbers above nodes represent maximum parsimony (MP) bootstrap support >90% from 1,000 pseudoreplicates of the data. Note that the soybean SDS and BRR pathogens exhibit a paraphyletic set of relationships. PIC, parsimony informative character; CI, consistency index; RI, retention index.

approximately one and two centuries ago, respectively, and the available data strongly support a South or Mesoamerican origin of the SDS–BRR pathogens, we theorize that the SDS fusaria jumped from some unknown host(s) to soybean after the relatively recent introduction of this Asian species to the New World (38). Given the very close and paraphyletic relationship of the SDS and BRR fusaria, it seems plausible that one or more of these species may have evolved in one or more of the three centers of diversity of wild *Phaseolus* species in Mexico, Ecuador, or Argentina (29,37). It should be possible to test whether one or more of these regions are the source of origin of these pathogens by analyzing haplotype and nucleotide diversity of isolates collected from wild *Phaseolus* populations in these regions (9). Such an investigation may also improve our understanding of their population structure

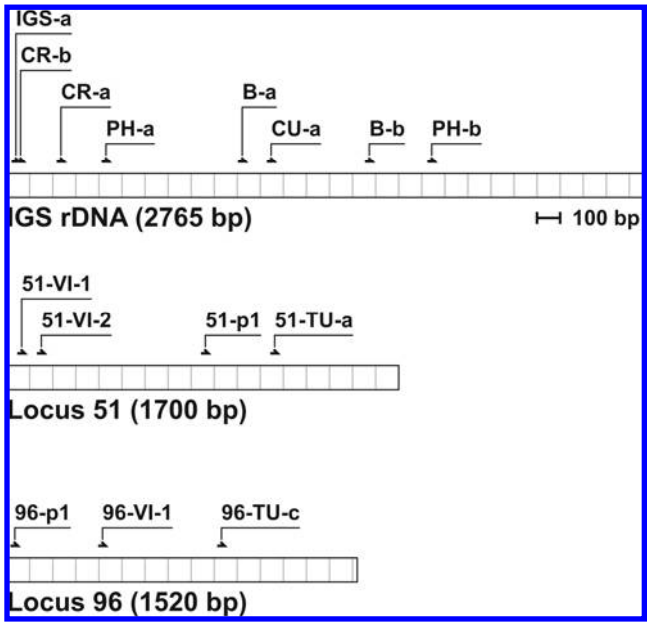

Fig. 2. Location of 12 species-specific and three positive control forward primers used in the soybean sudden death syndrome and bean root rot multiplex genotyping assay. Note that eight probe primers prime on the intergenic spacer region rDNA, four on locus 51, and three on locus 96.

and reproductive mode. Of the six SDS–BRR species discovered to date, only *F. tucumaniae* has been shown to possess high levels of genetic variability, suggesting sexual recombination in the field (3,4). A hypothesized sexual cycle was subsequently confirmed in this species employing heterothallic crosses in the laboratory (6). By comparison, three of the six SDS–BRR species may be strictly clonal on soybean or *Phaseolus* and mung bean, based on multilocus phylogenetics and analyses of other molecular markers (2,6,16,32).

As discussed previously (6), we strongly advocate using only the fusarial anamorph name, even for species with a known sexual state such as *F. tucumaniae*, to avoid confusion associated with duplicity of names for a single species and because several teleomorph names have recently been used for members of the FSSC (i.e., *Hypomyces*, *Nectria*, *Neocosmospora*, and *Haematonectria*) (20,30). Molecular phylogenetic analyses have demonstrated that *Nectria sensu lato* is a paraphyletic grade, and that this generic name should be restricted to members of the monophyletic *N. cinnabarina* species group (36). It is worth noting that the name *Haematonectria* has been applied to a paraphyletic group of fusaria within which *Neocosmospora* forms a distinct lineage (20,26,30), providing two unsatisfactory options: recognizing a paraphyletic taxon that is also a later synonym of *Neocosmospora* according to the botanical code's principle of priority, or broadening the concept of *Neocosmospora*, the earliest available legitimate generic name, far beyond its original circumscription (20). Fusaria in the SDS–BRR clade are unique within the FSSC in that all of the species except the SDS pathogen *Fusarium* sp. have been formally described with Latin binomials (3,4), and the latter species is currently being formally described (T. Aoki, *unpublished data*). Unfortunately, even two-thirds of a century after Snyder and Hansen (39) circumscribed the overly broadly defined species *F. solani* (13,19), most of the species within the FSSC are still reported in the phytopathological and clinical microbiology literature under this name. Several independent multilocus molecular phylogenetic analyses, however, have clearly demonstrated that the FSSC comprises at least 47 phylogenetically distinct species distributed among three strongly supported clades (20,26, 40,49). Although morphological apomorphies have yet to be identified for most of the species within the FSSC, a robust multilocus species and haplotype nomenclature has been proposed for all of the members of clade 3, which comprises most of the eco-

TABLE 3. Allele-specific and positive control probe primers used in the genotyping assay

Probe for[a]	Locus[b]	Species specific	Probe	Tag designation[c]	Position[d]	Tag + probe sequence[e]
F. brasiliense	IGS rDNA	Yes	B-a	12	1,079	TACACTTTCTTTCTTTCTTTCTTTACTTGGTGGTTTTGCCCGCTA
F. brasiliense	IGS rDNA	Yes	B-b	85	1,572	ATACTACATCATAATCAAACATCAAGGGTAGGTGAGAAAATCCGT
F. cuneirostrum	IGS rDNA	Yes	CU-a	23	1,152	TTCAATCATTCAAATCTCAACTTTGTATAGGGTAAGCTCCATTAG
F. phaseoli	IGS rDNA	Yes	PH-a	18	432	TCAAAATCTCAAATACTCAAATCAAGGGTAGGCTGGCTGGACTTA
F. phaseoli	IGS rDNA	Yes	PH-b	28	1,855	CTACAAACAAACAAACATTATCAATAGGGTAGGTGAAAATTTTGA
Fusarium sp.	IGS rDNA	Yes	CR-a	30	79	TTACCTTTATACCTTTCTTTTTACGATTTCCCCAGCCAAGTGACT
Fusarium sp.	IGS rDNA	Yes	CR-b	29	241	AATCTTACTACAAATCCTTTCTTTCTTCCACGGCGCTCGAAGCGT
F. tucumaniae	Locus 51	Yes	51-TU-a	54	1,160	CTTTTTCAATCACTTTCAATTCATCTGCATCTCTTCCTCTTCGGG
F. tucumaniae	Locus 96	Yes	96-TU-c	63	936	CTACTTCATATACTTTATACTACATTCTGCACCGAGACGTTTCGA
F. virguliforme	Locus 51	Yes	51-VI-1	96	70	ATACTAACTCAACTAACTTTAAACGCCAAGTCCGAGGTGACTATT
F. virguliforme	Locus 51	Yes	51-VI-2	77	153	CAATTAACTACATACAATACATACCAAATCATCAAACGATAACGT
F. virguliforme	Locus 96	Yes	96-VI-1	97	418	AATCTCATAATCTACATACACTATCAGACGGGCCTGACAAAGACA
Positive control	Locus 51	NA	51-p1	88	864	TTACTTCACTTTCTATTTACAATCCGAGAGTTTGGCACGTTGAGC
Positive control	Locus 96	NA	96-p1	1	40	CTTTAATCTCAATCAATACAAATCAGCACTCAGTAGACAATCGAC
Positive control	IGS rDNA	NA	IGS-a	59	59	TCATCAATCAATCTTTTCACTTTTAGCGTCTTAGCAGGCGATTC

[a] Species or positive control for polymerase chain reaction (PCR) amplification of locus 51, locus 96, or the intergenic spacer (IGS) rDNA.
[b] Two anonymous loci, designated 51 and 96, and the IGS rDNA were targeted in the allele-specific assay.
[c] A unique number between 1 and 100 was assigned at the Luminex Corporation website for each tag sequence.
[d] 3′-Most nucleotide position of probe primer in the sequence alignment of the respective locus targeted.
[e] The unique tag sequence (underlined) attached to the 5′ end of each probe sequence was assigned at the Luminex Corporation website. Each tag is designed to hybridize to the antitag attached to only 1 of the 15 different fluorescent microspheres used in the assay. The 3′-most nucleotide of each probe sequence is specific for one of the four sudden death syndrome pathogens, or was used as a positive control for the PCR amplification of the loci targeted in the allele-specific assay.

nomically important phytopathogens and all of the medically important species within the FSSC (26,49).

Results of the present study, and our parallel studies on the genetic diversity and mycotoxin potential of the FHB pathogens (24,27,28,46,48), highlight the importance of rigorously identifying species limits, based on reciprocal monophyly at multiple loci (i.e., GCPSR [43]), rather than relying on a homoplasious character such as host specificity to delimit taxa (44). Through comparative DNA sequence analyses, we have determined that published PCR primer pairs and probes for the detection and identification of the SDS and BRR pathogens prime on highly conserved regions of the three loci used in the design of these molecular diagnostic assays (ITS – 28S rDNA [22]; mtSSU rDNA [8,14–16]; and *EF-1a* [7,14]). Given the inability of the published molecular assays to differentiate the fusaria associated with SDS and BRR, incorporation of these diagnostics in pathogen surveillance programs could have serious negative consequences for crop biosecurity because the center of origin of these six pathogens is theorized to be outside the United States. As such, the available DNA-based pathogen detection systems are unable to distinguish, for example, between foreign soybean SDS pathogens that already have entered the United States (i.e., *F. virguliforme* and *F. brasiliense*) and the most important soybean SDS pathogen in South America (i.e., *F. tucumaniae* [3,4,33; present study]). The latter species, unlike the highly clonal population of *F. virguliforme* on soybean in North and South America, possesses a sexual cycle and concomitant high levels of genetic diversity on soybean in Argentina and Brazil. Because resistance to soybean SDS is quantitative, a sexually recombining pathogen such as *F. tucumaniae*, in theory, is much more likely to

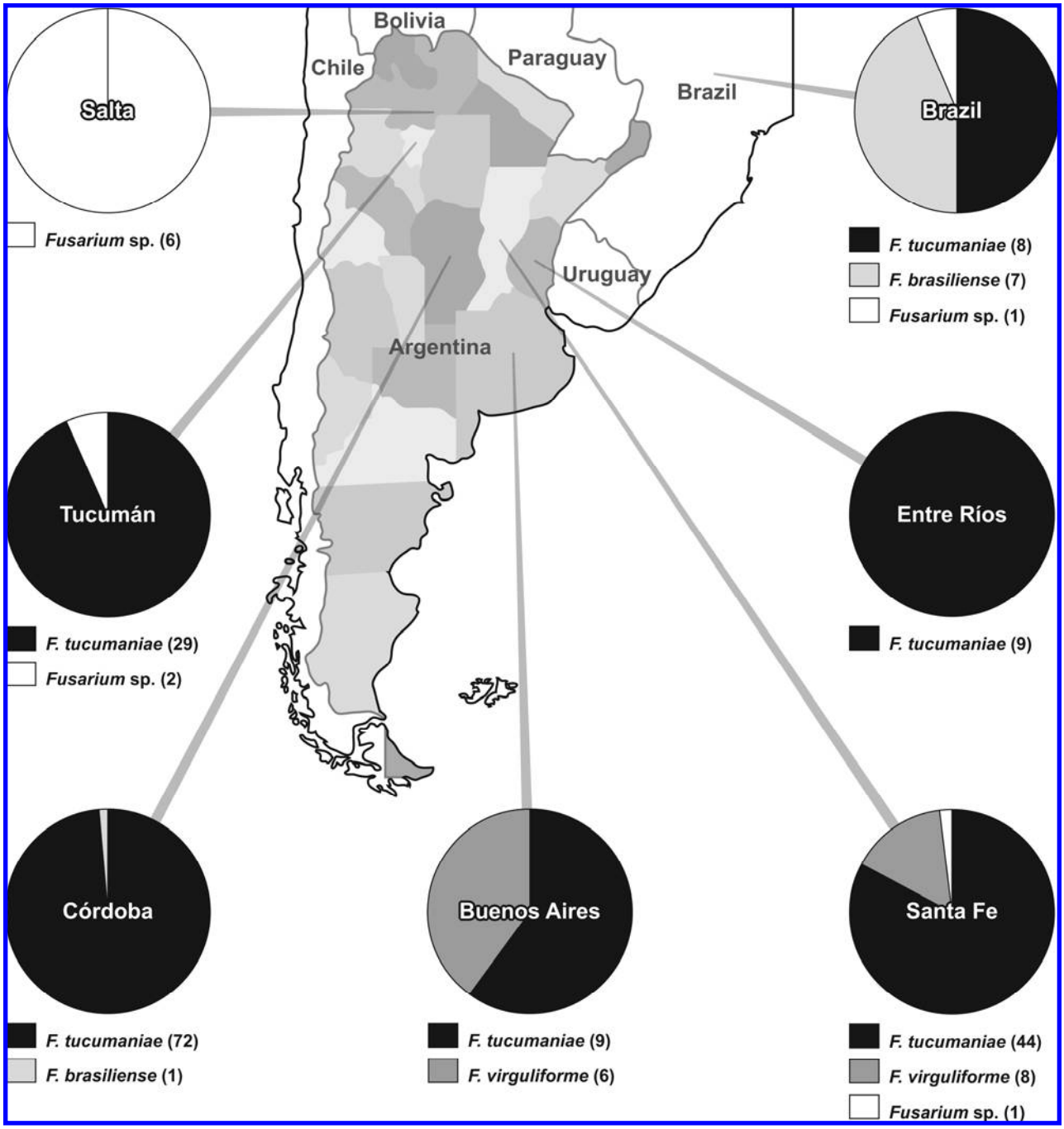

Fig. 3. Soybean sudden death syndrome species frequencies in six Argentinean provinces and preliminary sampling of five Brazilian districts based on multilocus genotyping analyses. Note that one isolate from Córdoba (NRRL 46137) was identified as *Fusarium tucumaniae* based on DNA sequence analysis of locus 96. Pie charts show the species composition in each area.

overcome host resistance, which may help explain why it is the dominant SDS pathogen in South America. Moreover, given the close relationship of the SDS and BRR pathogens and the fact they colonize some of the same hosts, the potential for interspecific hybrids with novel pathogenicity traits poses an additional threat to the production of soybean and dry edible bean. These findings highlight the need to not only expand our arsenal of soybean SDS species-specific diagnostics, but to also develop diagnostics for distinguishing the mating types idiomorphs so as to minimize the significant threat poised by a sexually recombining SDS pathogen to soybean production within the United States and elsewhere. Given that only 15 probe primers were used in the MLGT assay, and up to 100 can be accommodated in the single-well microsphere array, it should be relatively easy to expand the current assay to accommodate newly discovered genetic variation within known and novel soybean SDS and BRR pathogens, and even include probes for nonfungal pathogens such as the soybean cyst nematode (*Heterodera glycines*) if needed. Because the probe primers were designed based on known species-specific DNA sequence variation, updating the assay as novel diversity is discovered can be done easily as evidenced by our continuous updating of the MLGT array for FHB species and trichothecene toxin potential determination (27,46,48). Though beyond the scope of the present study, methods are available which should make it possible to analyze complex DNA samples from field samples using the MLGT assay (11,12), thereby obviating the time-consuming step of culturing the notoriously slow growing SDS and BRR fusaria. Even though the Luminex system is ideally suited for distinguishing multiple pathogens in a single-well assay, the multilocus sequence data could be used to design a species-specific primer pair for detection of any one of the soybean SDS and BRR fusaria now that their species limits have been clearly resolved. Although DNA sequence data from the IGS rDNA can be used to accurately identify all of the SDS–BRR fusaria, sequencing is much more time-consuming, labor-intensive, and more expensive than using the high-throughput MLGT assay which can identify 96 samples in 1 to 2 h.

Results of the present study add to the growing repertoire of DNA arrays for the detection and identification of agriculturally (11,12,46) and medically important fusaria (25). We designed and validated the MLGT assay for differentiating the six SDS and BRR pathogens only after species limits were delimited using GCPSR (43). Independent identifications of the 65-isolate design panel using a 3-locus MLST scheme and the MLGT assay both yielded species identifications with 100% accuracy. However, isolate NRRL 46137 from Córdoba, Argentina in the EP, identified as *F. tucumaniae*, based on sequence analysis of locus 96, was not subjected to the MLGT analysis due to repeated failed attempts to generate a multiplex PCR. We attribute this result to poor quality DNA. It is worth noting that none of the MLGT species probes gave false positives.

Our MLGT analyses provide a preliminary estimate of soybean SDS species diversity in the United States (N = 64), Argentina (N = 187) and Brazil (N = 16), revealing that *F. tucumaniae* and *F. virguliforme* are the dominant SDS pathogens in South and North America, respectively. Although several surveys suggest that virtually all soybean SDS is caused by *F. virguliforme* within the United States (2,16,32,47), this pathogen was found in only two of the six provinces we surveyed in Argentina, and then only in very low frequencies compared to *F. tucumaniae*. In addition, *F. virguliforme* has not been detected in Brazil based on very limited sampling (5; present study). These results punctuate the need for additional pathogen surveys to identify the center of origin(s) of soybean SDS in South or Mesoamerica. In summary, the MLGT assay provides agricultural scientists with a unique molecular diagnostic for surveillance and identification of the soybean SDS and BRR pathogens (34). With the development and validation of this assay, it is now possible to monitor changes in soybean SDS and BRR species composition in South and North America over time. Furthermore, this assay should assist plant inspection and quarantine officials in their efforts to prevent these economically destructive pathogens from being spread into additional non-indigenous areas.

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