Mycoscience (2005) 46:162–183
DOI 10.1007/s10267-005-0235-y

FULL PAPER

Takayuki Aoki · Kerry O'Donnell
María Mercedes Scandiani

Sudden death syndrome of soybean in South America is caused by four species of *Fusarium*: *Fusarium brasiliense* sp. nov., *F. cuneirostrum* sp. nov., *F. tucumaniae*, and *F. virguliforme*

Received: September 14, 2004 / Accepted: January 15, 2005

Abstract Soybean sudden death syndrome (SDS) pathogens and dry bean root-rot pathogens were studied taxonomically, phylogenetically, and pathologically. Detailed phenotypic comparisons of macro- and microscopic features and phylogenetic analyses of multilocus DNA sequence data, including those on the nuclear ribosomal intergenic spacer region and the single copy nuclear gene translation elongation factor 1-a, indicated that they comprised five distinct species of *Fusarium*. Two new species causing soybean SDS in Brazil, *F. brasiliense* and *F. cuneirostrum*, are formally described. *Fusarium cuneirostrum* is responsible for soybean SDS in Brazil and dry bean or mung bean root-rot in the United States, Canada, and Japan. Strains of each species, including *F. cuneirostrum* isolates from dry bean and mung bean and *F. phaseoli* isolates from dry bean, were inoculated on soybean cultivar Pioneer 9492RR to determine their pathogenicity. Although intraspecific variation in pathogenicity was observed, all the species were able to induce typical SDS symptoms on soybean plants in the artificial inoculation tests. Comparisons of the key diagnostic morphological features reveal that all five species can be diagnosed using conidial morphology.

Key words Argentina · Brazil · *Glycine max* · Sudden death syndrome · Pathogenicity · Phylogeny · Taxonomy

T. Aoki (✉)
National Institute of Agrobiological Sciences, Genetic Diversity Department, 2-1-2 Kannondai, Tsukuba, Ibaraki 305-8602, Japan
Tel. +81-29-838-7452; Fax +81-29-838-7408
e-mail: taoki@nias.affrc.go.jp

K. O'Donnell
Microbial Genomics and Bioprocessing Research Unit, National Center for Agricultural Utilization Research, U.S. Department of Agriculture, Agricultural Research Service, Peoria, IL, USA

M.M. Scandiani
Laboratorio Agrícola Río Paraná, San Pedro, Buenos Aires, Argentina

Introduction

Sudden death syndrome (SDS) of soybean (*Glycine max* (L.) Merr.) has been reported from all major growing regions in the United States (Rupe et al. 2001), Canada (Anderson and Tenuta 1998), Argentina (Ploper 1993), Brazil (Nakajima et al. 1993, 1996), Paraguay (Yorinori 1999), Bolivia (Yorinori 2002), and Uruguay (Ploper et al. 2003). Since its discovery in Arkansas in 1972, the etiological agent of this disease has been reported as *F. solani* (Mart.) Sacc. (Roy et al. 1989a; Rupe 1989; Ivancovich et al. 1992; Nakajima et al. 1993, 1996; Abney et al. 1993; Botta et al. 1993; Ploper 1993; Melgar et al. 1994; Hartman et al. 1995; Gray and Achenbach 1996) or its forma specialis, f. sp. *glycines* K.W. Roy (Roy et al. 1997a,b). Recent molecular phylogenetic analyses of DNA sequences, however, indicate the *F. solani* species complex (also known as section *Martiella*) comprises at least 26 phylogenetically distinct species (O'Donnell 2000), including many undescribed species considered as *F. solani* f. sp. *glycines* and *F. solani* f. sp. *phaseoli* (Burkh.) W.C. Snyder & H.N. Hansen. Molecular and morphological analyses of North American isolates of the SDS pathogen indicate they are genetically homogeneous and closely related to a root-rot pathogen of *Phaseolus vulgaris* L., *F. solani* f. sp. *phaseoli* (Achenbach et al. 1996; O'Donnell 2000; Li et al. 2000; Rupe et al. 2001). More recently, Aoki et al. (2003) studied North American and Argentinean isolates of the soybean SDS pathogen, using comparative morphology and genealogical concordance phylogenetic species recognition (GCPSR; Taylor et al. 2000). This study resulted in the discovery of two undescribed species within the *F. solani* species complex capable of inducing soybean SDS, *F. tucumaniae* T. Aoki et al. in Argentina and *F. virguliforme* O'Donnell & T. Aoki in the United States, together with a new combination for a closely related root-rot pathogen of *P. vulgaris*, *F. phaseoli* (Burkh.) T. Aoki & O'Donnell in the United States.

The objective of the present study is to determine what *Fusarium* species are responsible for soybean SDS within South America by extending our studies to include new

isolates from Argentina and Brazil, using morphological and phylogenetic species recognition. Knowledge of the soybean SDS pathogens' genetic diversity is a prerequisite for establishing a robust active molecular surveillance program to monitor any geographic transposition associated with world trade.

Materials and methods

Strains examined

Strains of *Fusarium* species included in the present study originally isolated from soybean (*G. max*) and green or dry bean (*P. vulgaris*) are listed in Table 1. The South American SDS pathogens preserved in Embrapa Soja, Londrina, Brazil and in the Centro de Referencia de Micología (CEREMIC), Fac. de Cs. Bioquímicas y Farmacéuticas, UNR, Rosario, Argentina were studied morphologically and phylogenetically. These isolates were isolated from diseased soybean plants exhibiting typical SDS foliar symptom in farmers' fields in Brazil and Argentina. Four strains of the dry bean root-rot pathogen, *F. solani* f. sp. *phaseoli*, isolated in Japan and the United States (Tanaka and Kitazawa 1956, 1958; O'Donnell and Gray 1995; Schneider et al. 2001) and two strains of the mung bean (*Vigna radiata* (L.) Wilczek = *Phaseolus aureus* Roxb.) root-rot pathogen in Canada (as *F. solani* f. sp. *phaseoli*), were also included because of their close phylogenetic relationship (O'Donnell 2000), together with two additional strains causing soybean SDS in Brazil, NRRL 22743 (=BBA 68441) and 22744 (=BBA 68442) (Hering 1997). Previously studied isolates of *F. tucumaniae* and *F. virguliforme* causing soybean SDS in Argentina and in the United States (Aoki et al. 2003), respectively, were analyzed together with the new isolates. All strains included in this study are stored in liquid nitrogen vapor at –175°C in the Agriculture Research Service Culture Collection (NRRL), National Center for Agricultural Utilization Research, Peoria, Illinois, USA, and at the MAFF Genebank System (Microorganisms Division), National Institute of Agrobiological Sciences, Tsukuba, Ibaraki, Japan.

Examination of morphological characters

Methods for the examination of morphological characters followed Aoki et al. (2003). *Fusarium* strains grown on potato dextrose agar (PDA; Difco, Detroit, MI, USA) and synthetic low nutrient agar (SNA; Nirenberg 1990) in 9-cm plastic Petri dishes were incubated at 20°C in complete darkness, under continuous fluorescent light (Mitsubishi FL40S-W) or under daylight to examine morphological and cultural characteristics. Colony morphology, color, and odor were based on cultures grown on PDA. Colors cited are given according to Kornerup and Wanscher (1978). Average and standard deviation (SD) in the size of conidia were based on the measurement of 50 randomly selected conidia, according to the number of septa and each cultural condition. Descriptive terms for conidia and conidiophore morphology followed Nirenberg and O'Donnell (1998). Dried cultures of the new taxa were deposited as holotypes in the herbarium of the U.S. National Fungus Collection (BPI), USDA/ARS, Beltsville, MD, USA. Two authentic specimens of *Fusarium martii* Appel & Wollenw., BPI 452384 and BPI 452385 (both from *Solanum tuberosum* L., Berlin, Germany, determined by W. Wollenweber, 1909), were also examined to reconfirm and supplement the original description of the species by Appel and Wollenweber (1910).

Mycelial growth rates

To examine mycelial growth rates at various temperatures, agar blocks ~5 × 5 mm were cut from the margins of 2-week-old cultures on SNA and inoculated onto PDA. These cultures were incubated in complete darkness at eight different temperatures at 5°C intervals from 5° to 40°C. Cultures were examined after 1 and 5 days under a dissecting microscope, and colony margins were marked with permanent ink on the reverse side of the Petri dishes. Radial mycelial growth rates were calculated as mean values per day by measuring the difference in colony size in 16 different directions around the colony during the 4-day incubation. Measurements were repeated at least twice and averaged.

Pathogenicity tests on soybean plants

Fifteen *Fusarium* strains examined in the present study were selected for pathogenicity tests, based on their geographical origin and differences in hosts (Tables 1, 2). Two strains of *F. phaseoli*, NRRL 22276 and NRRL 31156 (Aoki et al. 2003), were also examined for pathogenicity to soybean for comparison. Inoculum was prepared by culturing each isolate on PDAS (PDA supplemented with 100 mg/l streptomycin) in 9-cm plastic Petri dishes for 1 week at 25°C in the dark, after which agar plugs (6 mm in diameter) were excised from the growing edge of the colony. Five plugs were added to 125 g sorghum grain that had been autoclaved for 60 min at 121°C in a 500-ml flask on 2 consecutive days, after having been soaked in distilled water overnight, drained, and allowed to cool (Hartman et al. 1997; Li et al. 2000; Rupe et al. 2001; Mueller et al. 2002, 2003). The flask was incubated at 25°C in the dark for 2 weeks and shaken daily. A field soil previously fumigated with methyl bromide (CH_3Br) (Roy et al. 1989b; Melgar et al. 1994) was placed in plastic pots. A layer of infested sorghum seeds (3 g) was distributed in each pot and covered with 2 cm of soil. Two seeds of the susceptible soybean cultivar Pioneer 9492RR were added to each pot and covered with another 2 cm of soil (Scandiani et al. 2002, 2003, 2004a,b). Pots then were placed on a greenhouse bench and grown under natural photoperiod at 25° ± 3°C for 5 weeks. Soil was watered to saturation after planting and maintained at near field capacity throughout the study. In the test each replicate consisted

Table 1. Strains of *Fusarium* species studied

Species (deposited previously as)	NRRL[a]	Geographic origin
New taxa:		
Fusarium brasiliense sp. nov.		
(*F. solanii*)	22678	USA, California
(*F. martii-phaseoli*)	22743	Brazil, Distrito Federal, Brasilia
(*F. martii-phaseoli*)	22744	Brazil, Distrito Federal, Brasilia
(*F. solani* f. sp. *glycines*)	31756	Brazil, Distrito Federal, Brasilia
(*F. solani* f. sp. *glycines*)	31757[b]	Brazil, Distrito Federal, Brasilia
(*F. solani* f. sp. *glycines*)	31762	Brazil, Paraná, Campo Mourão
(*F. solani* f. sp. *glycines*)	31779	Brazil, Rio Grande do Sul, Nonai
Fusarium cuneirostrum sp. nov.		
(*F. solani* f. sp. *phaseoli*)	22275	Japan, Hokkaido, Sapporo
(*F. solani* f. sp. *phaseoli*)	31104	Japan
(*F. solani* f. sp. *phaseoli*)	31157[b]	USA, Michigan, Presque Isle
(*F. solani* f. sp. *glycines*)	31949	Brazil, Goiás, Cristalina
(*F. solani* f. sp. *phaseoli*)	36023	Canada, Ontario, Ridgetown
(*F. solani* f. sp. *phaseoli*)	36024	Canada, Ontario, Ridgetown
Additional isolates from Argentina and Brazil:		
Fusarium tucumaniae		
(*F. solani* f. sp. *glycines*)	31773	Brazil, Paraná, Ponta Grossa, Embrapa-SPSB
(*F. solani* f. sp. *glycines*)	31776	Brazil, Rio Grande do Sul, Tapera
(*F. solani* f. sp. *glycines*)	31777	Brazil, Rio Grande do Sul, Vila Maria
(*F. solani* f. sp. *glycines*)	31778	Brazil, Rio Grande do Sul, Sarandi
(*F. solani* f. sp. *glycines*)	31793	Brazil, Minas Gerais, Nova Ponte
(*F. solani* f. sp. *glycines*)	31796	Brazil, Paraná, Ponta Grossa, Embrapa-SPSB
(*F. solani* f. sp. *glycines*)	31950	Brazil, Paraná, Ponta Grossa, Embrapa-SPSB
(*F. solani* f. sp. *glycines*)	34546	Argentina, Buenos Aires, Arrecifes
(*F. solani* f. sp. *glycines*)	34547	Argentina, Santa Fe, Las Parejas
(*F. solani* f. sp. *glycines*)	34548	Argentina, Santa Fe, Las Parejas
(*F. solani* f. sp. *glycines*)	34549	Argentina, Buenos Aires, Pérez Millán
(*F. solani* f. sp. *glycines*)	34550	Argentina, Santa Fe, Pujato
Fusarium virguliforme		
(*F. solani* f. sp. *glycines*)	34551	Argentina, Buenos Aires, San Pedro
(*F. solani* f. sp. *glycines*)	34552	Argentina, Santa Fe, Serodino
(*F. solani* f. sp. *glycines*)	34553	Argentina, Santa Fe, Serodino

[a] ATCC, American Type Culture Collection, Fairfax, VA, USA; BBA, Biologische Bundesanstalt für Land- und Forstwirtschaft, Institut für Virologie, Mikrobiologie und biologische Sicherheit, Berlin, Germany; CR, the Centro de Referencia de Micología (CEREMIC), Fac. de Cs. Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Rosario, Argentina; FRC, Fusarium Research Center, Pennsylvania State University, University Park, PA, USA; IFO, Institute for Fermentation, Osaka, Japan; MAFF, MAFF Genebank System, National Institute of Agrobiological Sciences, Tsukuba, Japan; NRRL, The Agriculture Research Service Culture Collection, National Center for Agricultural Utilization Research, USDA/ARS, Peoria, IL, USA; SUF, Shinshu University, Faculty of Textile Science and Technology, Ueda, Nagano, Japan
[b] Ex-holotype strains

of 6 pots with 2 plants. Treatments (isolates) were arranged in a randomized complete block design with four replications. Forty-eight plants were inoculated separately with each fungal isolate. Uninoculated plants served as a negative control. Plants were rated for incidence of SDS-like symptoms on the foliage, plant height, root rot, and for shoot fresh and root dry weight. Disease incidence (DI) of plants was based on the percentage of plants with foliar symptoms typical of SDS (Roy 1997a; Rupe et al. 2001). Symptoms ranged from leaf curling and rugosity, marginal cupping, mottling, chlorotic interveinal spots, interveinal chlorosis and necrosis, to leaf drop and stunting (Roy et al. 1997; Li et al. 1999; Rupe and Hartman 1999). Foliar disease severity (DS) was rated 5 weeks after planting based on a scale of 1 to 5, where 1 = no symptoms; 2 = light symptom development with mottling and mosaic (1%–20% foliage affected); 3 = moderate symptom development with interveinal chlorosis and necrosis (21%–50% foliage affected); 4 = heavy symptom development (51%–80% foliage affected); and 5 = severe symptom development with interveinal chlorosis and necrosis and/or dead plants (81%–100% foliage affected) (Huang and Hartman 1998).

At the end of the experiment, all plants within a replication were rated for basal stem rot, cut off at the soil line, and the fresh shoot weight was determined. Plants were removed from the soil and the roots were washed under running water before the roots were rated for rot using a 1–5 scale where 1 = healthy roots and tap root; 2 = <25% of lateral roots and tap root with necrosis; 3 = 25%–50% of lateral roots and tap root with visible necrosis; 4 = 51%–90% of lateral roots and tap roots with necrosis; and 5 = >90% of root system with necrosis, plants dead (Roy et al. 1989b; Achenbach et al. 1996; Gray and Achenbach 1996; Roy 1997a). Subsequently, all roots were dried for 24 h at 50°C and weighed (Huang and Hartman 1998). Data from this experiment were subjected to analysis of variance (ANOVA). Treatment means were compared by Duncan's test.

Host/substrate	Source[a] (year of isolation)	Equivalent number[a]
Unknown	S.N. Smith, 18W (1993) /P. Nelson	MAFF 239039 = FRC S-712
Glycine max (SDS)	T. Nakajima (1992) /H.I. Nirenberg	MAFF 239041 = BBA 68441
Glycine max (SDS)	T. Nakajima (1992) /H.I. Nirenberg	MAFF 239042 = BBA 68442
Glycine max (SDS)	T. Nakajima (1992) /J.T. Yorinori, SDS-1	MAFF 239043
Glycine max (SDS)	T. Nakajima (1992) /J.T. Yorinori, SDS-5	MAFF 239050
Glycine max (SDS)	J.T. Yorinori, 06/98 (1998)	MAFF 239051
Glycine max (SDS)	J.T. Yorinori, 36/00 (2000)	MAFF 239047
Phaseolus vulgaris (root rot)	I. Tanaka (1955) /T. Matuo, SUF 386	MAFF 239036 = ATCC 42361 = IFO 9974
Phaseolus vulgaris (root rot)	T. Matuo /Tohoku Univ., I-35-1	MAFF 305607
Phaseolus vulgaris (root rot)	Michigan State Univ., FS12 (1992)	MAFF 239038 = FRC S-1551
Glycine max (SDS)	J.R. Menezes (2000) /J.T. Yorinori, 01/00	MAFF 239052
Vigna radiata (root rot)	T.R. Anderson, Mung 1 (1996)	MAFF 239491
Vigna radiata (root rot)	T.R. Anderson, Mung 2 (1996)	MAFF 239492
Glycine max (SDS)	J.T. Yorinori, 15/00 (2000)	MAFF 239044
Glycine max (SDS)	J.T. Yorinori, 33/00 (2000)	MAFF 239054
Glycine max (SDS)	J.T. Yorinori, 34/00 (2000)	MAFF 239045
Glycine max (SDS)	J.T. Yorinori, 35/00 (2000)	MAFF 239046
Glycine max (SDS)	J.T. Yorinori, 71/01 (2001)	MAFF 239048
Glycine max (SDS)	J.T. Yorinori, 86/01 (2001)	MAFF 239049
Glycine max (SDS)	J.T. Yorinori, 07/00 (2000)	MAFF 239053
Glycine max (SDS)	M. Scandiani, 3-2 (2000)	MAFF 239252 = CR 125-2002
Glycine max (SDS)	M. Scandiani, 8-1 (2000)	MAFF 239253 = CR 126-2002
Glycine max (SDS)	M. Scandiani, 8-2 (2000)	MAFF 239254 = CR 127-2002
Glycine max (SDS)	M. Scandiani, WK2 (2001)	MAFF 239255 = CR 129-2002
Glycine max (SDS)	M. Scandiani, Pujato (2001)	MAFF 239256 = CR 128-2002
Glycine max (SDS)	M. Scandiani, LP (2002)	MAFF 239257 = CR 101-2003
Glycine max (SDS)	M. Scandiani, M5 (2002)	MAFF 239258 = CR 102-2003
Glycine max (SDS)	M. Scandiani, M6 (2002)	MAFF 239259 = CR 103-2003

Molecular biology

Mycelium was harvested from yeast-malt broth cultures and DNA isolated following O'Donnell (2000). The entire nuclear ribosomal intergenic spacer (IGS) region was polymerase chain reaction (PCR) amplified with the NL11 (5′-CTGAACGCCTCTAAGTCAG) and CNS1 (5′-GAG ACAAGCATATGACTAC) primer pair (Aoki et al. 2003), using Platinum TAQ DNA polymerase Hi-Fi (Invitrogen Life Technologies, Carlsbad, CA, USA) in an Applied Biosystems (ABI) 9700 thermocycler (Foster City, CA, USA). The following cycling parameters were used for this long PCR: 94°C for 90 s, 40 cycles of 94°C for 30 s, 52°C for 30 s, 68°C for 3 min, followed by 68°C for 10 min and a 4°C soak. Conditions for PCR amplification of the nuclear ribosomal internal transcribed spacer (ITS) region and domains D1 and D2 at the 5′-end of the nuclear large subunit rDNA (28S), using the ITS5 and NL4 primer pair, and a portion of the translation elongation factor (1-α) have been described (White et al. 1990; O'Donnell 2000). All amplicons were purified using Montage PCR_{96} Cleanup Filter Plates (Millipore, Billerica, MA, USA) and then cycle sequenced using ABI BigDye chemistry version 3 in an ABI 9700 thermocycler as follows: 96°C for 15 s, 40 cycles of 96°C for 15 s, 50°C for 10 s, 60°C for 4 min, ending in a 4°C soak. Sequencing primers for the IGS were reported in Aoki et al. (2003), the ITS and 28S rDNA in White et al. (1990) and O'Donnell (1996), respectively, and EF-1a in O'Donnell et al. (1998). Purification of the sequencing reactions was achieved via ethanol precipitation, and they were then run on an Applied Biosystems-Hitachi Prism 3100 or 3730 genetic analyzer (Tokyo, Japan).

Phylogenetic analysis

Sequencher version 4.1.2 (Gene Codes, Ann Arbor, MI, USA) was used to edit DNA sequences and conduct pre-

Table 2. Pathogenicity of selected strains of five *Fusarium* species on soybean cultivar Pioneer 9492RR[a]

Inoculated species of *Fusarium*	NRRL number	DI (%)[b]	DS (1–5)[c]	Plant height (cm)	Basal stem rot (%)	Root-rot (1–5)	Shoot fresh weight (g)	Root dry weight (g)
F. brasiliense	31756	83 abc	3.3 efg	23 mno	33 hijk	3.8 fg	0.7 jklm	0.4 cde
F. brasiliense	31762	5 hi	2.1 l	34 ef	0.5 lm	2.7 no	1.4 de	0.4 cde
F. brasiliense	31779	39 ef	2.4 k	36 e	45 fgh	2.9 lmn	1.4 de	0.4 cde
F. cuneirostrum	31157	61 bcde	3.0 gh	30 ghij	28 jk	3.0 klm	1.1 efg	0.4 cde
F. cuneirostrum	31949	94 a	4.1 bcd	29 hij	89 ab	4.2 cd	0.7 hijklm	0.4 cde
F. cuneirostrum	36023	49 ef	2.5 ijkl	36 de	29 ijk	3.0 klm	1.3 e	0.4 cde
F. cuneirostrum	36024	58 de	3.1 gh	33 efg	33 ghijk	3.4 gh	1.2 ef	0.4 cde
F. tucumaniae	34546	60 cde	3.2 fgh	27 jkl	58 def	3.6 i	1.1 efg	0.4 cde
F. tucumaniae	34547	98 a	4.0 cd	29 ij	79 b	3.8 ef	0.9 fghij	0.5 bc
F. tucumaniae	34548	98 a	4.7 a	24 lmno	96 ab	4.6 a	0.4 lmn	0.3 defg
F. tucumaniae	34549	19 g	2.5 ijkl	41 c	22 k	2.8 mno	1.9 c	0.6 ab
F. tucumaniae	34550	100 a	4.5 a	20 op	91 ab	4.6 a	0.3 mn	0.1 g
F. virguliforme	34551	96 a	3.9 d	27 jkl	60 cdef	3.8 ef	0.8 ghij	0.4 cde
F. virguliforme	34552	98 a	4.5 a	24 klmn	96 ab	4.3 bc	0.6 jklmn	0.3 defg
F. virguliforme	34553	100 a	4.7 a	19 p	100 a	4.5 a	0.3 n	0.2 fg
F. phaseoli	22276	0.5 ij	1.3 n	50 b	0 m	2.2 p	2.3 b	0.5 bc
F. phaseoli	31156	32 fg	2.5 ijkl	31 fghij	49 f	3.1 jkl	1.2 ef	0.4 cde
Control[d]		0 j	1 m	54 a	0 m	1 p	3.5 a	0.7 a

[a] Mean values of 48 plants inoculated separately with each strain
[b] DI, disease incidence; only plants with typical soybean sudden death syndrome (SDS) symptoms were scored
[c] DS, disease severity
[d] Mean values of 48 uninoculated plants used as a negative control
Numbers followed by the same letter are not significantly different ($P = 0.05$) by Duncan's multiple range test

Table 3. Tree statistics

	Number of characters (bp)	SYN	AUT	Number of maximum-parsimony (MP) trees	Tree length	CI	RI
28S rDNA	535	3	7	1	10	1.00	1.00
ITS rDNA	546	9	10	1	19	1.00	1.00
IGS rDNA	2784	145	6	>20000	167	0.93	0.99
EF-1α	695	20	38	6	65	0.95	0.97
Combined data	4560	177	61	156	268	0.92	0.98

SYN, synapomorphies; AUT, autapomorphies; CI, consistency index; RI, rescaled consistency index; ITS, internal transcribed spacer; IGS, intergenic spacer; EF, elongation factor

liminary DNA alignments subsequent to their improvement manually. Sequences generated in this study have been deposited in the GenBank database under accession numbers AY730871–AY730910. Maximum-parsimony phylogenetic analyses were conducted with PAUP* version 4.0b10 (Swofford 2002), using the heuristic search option with 1000 random addition replicates and tree bisection with reconnection branch swapping. Based on results of prior phylogenetic analyses (O'Donnell 2000; Aoki et al. 2003), sequences of *Fusarium* spp. NRRL 22387 and NRRL 22574 were used to root the tree by the outgroup method. Of the 4560 aligned characters in the combined data set, 61 and 177 were autapomorphic and synapomorphic (Table 3), respectively. Clade stability was assessed via 1000 parsimony replications with 10 random addition sequences per replicate and tree bisection with reconnection branch swapping using PAUP*. In addition, a neighbor-joining bootstrap analysis was conducted using the HKY85 distance option, allowing among-site rate variation.

Results

Taxonomy

The present morphological and molecular phylogenetic study of additional South American soybean SDS strains resulted in the discovery of two new species of *Fusarium* and two species new to Brazil or Argentina.

Fusarium brasiliense T. Aoki & O'Donnell, sp. nov.
Figs. 1–21

Coloniae in PDA radium per diem 1.5–2.1 mm ad 20°C obscuritate crescentes, albidae, luteo-albae vel pallide luteae, nonnumquam griseo-virides; in parte sporifera pustulae nonnumquam luteo-albae, dilute flavae, griseo-luteae vel griseo-virides; in luce fluorescente vel naturali pustulis luteo-albis, obscure coeruleis vel viridescentibus; mycelium aerium copiosum, laxe vel dense floccosum, interdum parcum et in coloniis pionnotium simile, album, luteo-album vel pallide luteum compositum; margo integra vel saepe undulate; reversum saepe hyalinum,

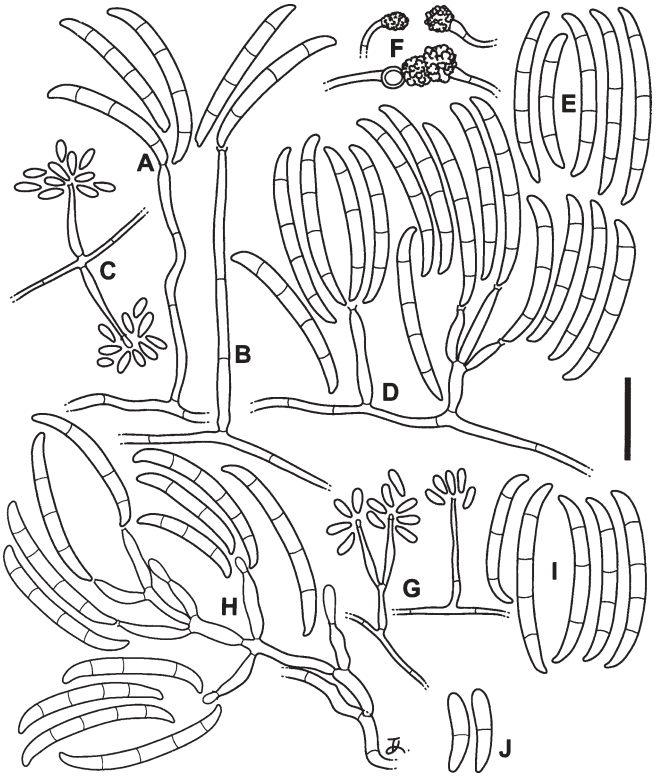
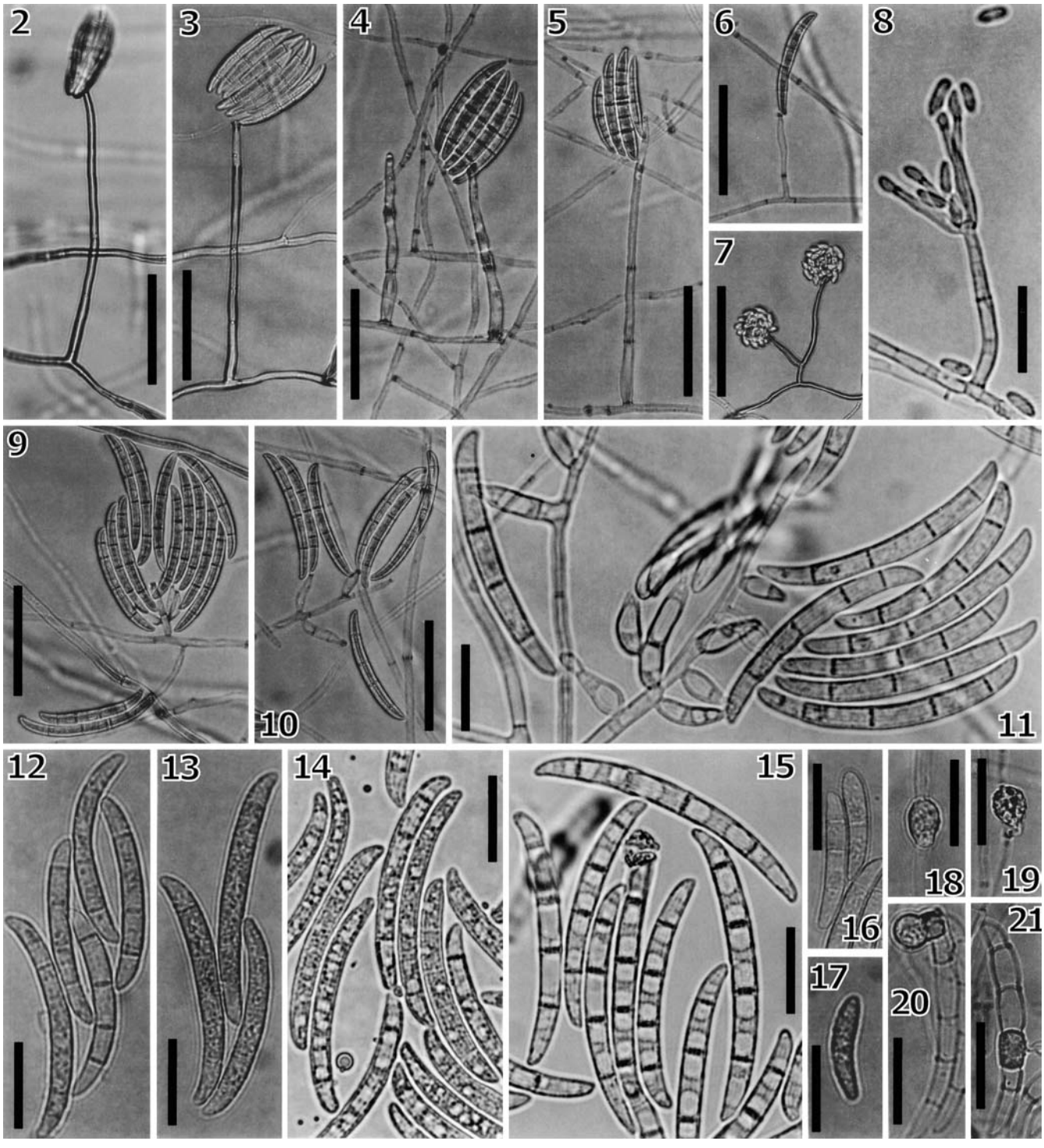


Fig. 1. *Fusarium brasiliense* cultured on synthetic low-nutrient agar (SNA) (**A–F**) and potato dextrose agar (PDA) (**G–J**) cultured in the dark (**A** from NRRL 31779; **B–D,F–H,J** from NRRL 31757, ex holotype; and **E,I** from NRRL 22678). **A,B** Septate, falcate to curved-cylindrical conidia with a rounded apex and a foot cell formed on tall, slender aerial conidiophores. **C,G** Aseptate, minute, ovate to short-clavate conidia formed on short aerial conidiophores. **D,E,H,I** Septate, falcate to curved-cylindrical conidia with a rounded apex and a foot cell formed on sporodochial conidiophores. **F** Rough-walled, terminal or intercalary chlamydospores formed in hyphae. **J** Slightly curved, short-clavate 1-septate sporodochial conidia formed in culture on PDA. *Bar* 25 µm

nonnumquam griseo-luteum, griseo-aurantiacum vel brunneo-aurantiacum; exsudatum hyalinum nonnumquam formatum; odor absens vel mucidus. Hyphae in SNA 1–5(–8) µm latae; chlamydosporae in hyphis et conidiis frequenter formantes, plerumque subglobosae, nonnumquam oblongae vel curvatae, terminales vel intercalares, plerumque singulae, nonnumquam catenatae, hyalinae vel dilute flavidae, leves vel rugosae, 7.5–15 × 5–12.5 µm. Sclerotia absentia. Sporulatio plerumque praecox, nonnumquam tarda in stirpe hypharum; in PDA obscuritate saepe subhyalina, in luce viridescens vel caerulescens. Sporodochia copiosa in SNA et in stirpe pionnotium in PDA, plerumque parca in PDA. Conidiophora aeria copiosa in SNA et in PDA, simplicia vel parce ramosa, plerumque ad 195 µm alta, 2–6.5 µm lata, monophialides integras formantia. Phialides aeriae simplices, subulatae vel subcylindricae, saepe collarette conspicuum ferentes. Conidia aeria biformia: (1) curvatim cylindrica vel falcata, 3(–4)-septata, basi clare vel obscure pediformia, a conidiis sporodochialibus saepe indistincta, praecipue in conidiophoris longi formata; (2) minuta, obovata, breviter clavata vel ellipsoidea, 0(–1)-septata, 4–12.5 × 2–3.5 µm, in conidiophoris usque 60 µm longis et 1.5–3 µm latis ad partes paucas coloniae formata. Conidiophora sporodochialia verticillata vel raro simplicia, monophialides ferentia. Phialides simplices, subulatae, ampulliformes vel subcylindricae, nonnumquam collarette conspicuum ferentes. Conidia sporodochialia cylindrica, utrinsecus parallela, medio latissima vel in dimidio superiore leviter dilatata, apice utrinque versus gradatim curvatae, cellula apicali rotundata et nonnumquam ventraliter aduncata, cellula basilari plerumque rotundata vel nonnumquam clare pediformi, (2–)3–4(–6)-septata, in PDA et SNA formata; illa 3-septata 30.5–66 × 4–6.5 µm in SNA et 27.5–59.5 × 4–6 µm in PDA; illa 4-septata 36.5–70.5 × 4.5–6 µm in SNA et 34–63 × 4.5–6 µm in PDA: illa 5-septata 45.5–75 × 4.5–7 µm in SNA. Conidia sporodochialia secundaria praesentia vel absentia, in PDA infrequenter formantia, breviter clavata, leviter curvata, apice rotundata, basi truncata, 0–1-septata, 23–26.5 × 4.5–6.5 µm.

Holotypus: BPI 843352, cultura sicca (NRRL 31757 = MAFF 239050 = J.T. Yorinori SDS-5) e radice *Glycines max* infecta, a T. Nakajima lecta in Brasilia, Distrito Federal, Brasil, 1992, in Herbario BPI, USA deposita.

Colonies on PDA showing radial mycelial growth rates of 1.5–2.1 mm per day at 20°C in the dark. Colony color on PDA white (1A1), yellowish-white (3-4A2) to pale yellow (3A3), sometimes with greyish-green (25B3-4) tint, conidial pustules when present yellowish-white (3-4A2), pale yellow (3-4A3), greyish-yellow (1-2B3-4) to greyish-green (25-30B-D3-5) in the dark and yellowish-white (3-4A2), dull blue (23D-E4-5) to greyish-turquoise (24B-E4-6) under fluorescent light or daylight. Aerial mycelium generally abundant, loose to sometimes dense floccose, white (1A1), yellowish-white (3-4A2) to pale yellow (3A3), sometimes sparse with pionnotal appearance. Colony margin entire or frequently undulate. Reverse pigmentation often absent, sometimes greyish-yellow (4C4-5), greyish-orange (5B4-5) to brownish-orange (5C4-5). Hyaline exudate sometimes present. Odor absent or moldy. Hyphae on SNA 1–5(–8) µm wide. Chlamydospores formed frequently in hyphae and in conidia, mostly subglobose, sometimes oblong or curved, terminal or intercalary, mostly single, sometimes in chains, hyaline to pale-yellow, smooth to rough-walled, 7.5–15 × 5–12.5 µm. Sclerotia absent. Sporulation generally rapid, sometimes slow in mycelial strains on PDA; on PDA often light-colored in darkness, also with a greenish-to-bluish pigmentation under fluorescent light or daylight; sporodochia formed abundantly on SNA and in pionnotal strains on PDA, but generally less frequent on PDA. Aerial conidiophores formed abundantly on SNA and PDA, unbranched or sparsely branched, up to 195 µm long, 2–6.5 µm wide, forming monophialides integrated in the apices. Aerial phialides simple, subulate to subcylindrical, often with a conspicuous collarette at the tip. Aerial conidia of two types; (1) curved cylindrical to falcate, 3(–4)-septate, with a distinct or indistinct foot cell, often morphologically indistinguishable from sporodochial conidia, formed primarily on taller conidiophores; (2) minute, obovate, short-clavate to elliposoidal, 0(–1)-septate, 4–12.5 × 2–3.5 µm in



Figs. 2–21. Morphology of *Fusarium brasiliense* cultured in the dark (**2–12,18–21** cultured on SNA; **13–17** cultured on PDA; **2–7,9,10** aerial view; **8,11–21** mounted in water). **2–6** Falcate aerial conidia formed on slender conidiophores arising from hyphae on the agar surface. **7,8** Minute conidia formed on short aerial conidiophores arising from hyphae on the agar surface. **9–11** Sporodochial conidia and conidiophores formed on the agar surface. **12–15** Falcate sporodochial conidia cultured on SNA (**12**) and PDA (**13–15**); septa are indiscernible in immature conidia on PDA (**13,14**) but become conspicuous as conidia mature (**15**). **16,17** Slightly curved, short-clavate 1-septate sporodochial conidia formed in culture on PDA. **18,19** Chlamydospores in hyphae. **20,21** Chlamydospores in conidia. **2–7,9,10,12,13,16,17** from NRRL 31757; **8,11,14** from NRRL 22744; **15,19,21** from NRRL 22743; and **18,20** from NRRL 31762. *Bars* **2–7,9,10** 50 µm; **8,11–21** 20 µm

total range, 6.4–8.1 × 2.4–2.8 µm on average [ex type: 4.8–7.5 × 2.5–3.5 µm in total range, 6.4 ± 0.78 × 2.8 ± 0.23 µm on average ± SD], formed in a small portion of the colony and on short conidiophores up to 60 µm long, 1.5–3 µm wide. Sporodochial conidiophores branched verticillately, or rarely unbranched, forming apical monophialides. Sporodochial phialides simple, subulate, ampulliform to subcylindric, sometimes with a conspicuous collarette at the tip. Sporodochial conidia cylindrical with dorsal and ventral lines nearly parallel, widest at the midregion or slightly wider in the upper half, gradually curving toward both ends; apex often rounded and sometimes hooked ventrally; base often rounded or sometimes with a distinct foot cell, (2–)3–4(–6)-septate, formed on PDA and on SNA; 3-septate on SNA: 30.5–66 × 4–6.5 µm in total range, 42.1–47.7 × 4.9–5.2 µm on average [ex type: 32.5–51 × 4.5–6 µm in total range, 42.3 ± 3.86 × 5.1 ± 0.34 µm on average ± SD], on PDA: 27.5–59.5 × 4–6 µm in total range, 35.4–50.3 × 4.9–5.1 µm on average [ex type: 28–44.5 × 4–5.5 µm in total range, 37.2 ± 3.23 × 5.0 ± 0.22 µm on average ± SD]; 4-septate on SNA: 36.5–70.5 × 4.5–6 µm in total range, 48.0–59.9 × 5.0–5.4 µm on average [ex type: 45–66 × 4.5–6 µm in total range, 53.2 ± 3.79 × 5.4 ± 0.20 µm on average ± SD], on PDA: 34–63 × 4.5–6 µm in total range, 41.3–55.7 × 5–5.2 µm on average [ex type: 37.5–47.5 × 4.5–5.5 µm in total range, 41.8 ± 2.21 × 5.1 ± 0.16 µm on average ± SD]; 5-septate on SNA: 45.5–75 × 4.5–7 µm in total range, 55.1–62.3 × 5.0–5.4 µm on average [ex type: 45.5–64 × 5–6 µm in total range, 55.3 ± 3.57 × 5.4 ± 0.19 µm on average ± SD]. Sporodochial conidia of the second type present or absent, infrequently formed on PDA, short-clavate, slightly curved, with a rounded apex and a truncate base, 0–1-septate, 23–26.5 × 4.5–6.5 µm.

Holotype: BPI 843352, a dried culture, isolated from a diseased root of soybean plant exhibiting SDS symptoms, collected in Brasilia, Distrito Federal, Brazil, 1992, T. Nakajima, deposited in the herbarium of BPI (US National Fungus Collection, Beltsville, MD), USA.

Ex holotype culture: NRRL 31757 = MAFF 239050 = J.T. Yorinori SDS-5.

Etymology: *brasiliense* (Latin); based on the type locality.

Isolates studied: NRRL 22678 = MAFF 239039, unknown host, California, USA, S.N. Smith, 1993; NRRL 22743 = MAFF 239041, NRRL 22744 = MAFF 239042, NRRL 31756 = MAFF 239043, NRRL 31757 = MAFF 239050, *Glycine max*, Brasilia, Distrito Federal, Brazil, T. Nakajima, 1992; NRRL 31762 = MAFF 239051, *G. max*, Campo Mourão, Paraná, Brazil, J.T. Yorinori, 1998; NRRL 31779 = MAFF 239047, *G. max*, Nonai, Rio Grande do Sul, Brazil, J.T. Yorinori, 2000.

Notes: Important morphological characters observed in *F. brasiliense* include the formation of plural types of conidia on aerial conidiophores, i.e., (1) cylindrical to falcate, septate conidia with a foot cell on tall conidiophores and (2) short-clavate, minute conidia on short conidiophores (Figs. 1A–C,G, 2–8). These characters are common to all the *Fusarium* species that cause soybean SDS and dry bean root-rot, i.e., *F. virguliforme*, *F. tucumaniae*, and *F. phaseoli* as described in Aoki et al. (2003), and *F. brasiliense* and *F. cuneirostrum* spp. nov. described in the present study. Moreover, these characters distinguish these fusaria from all other recognized species within the *F. solani* species complex.

Fusarium brasiliense is differentiated from *F. tucumaniae* by the production of shorter and wider sporodochial conidia. *Fusarium brasiliense* forms distinctive sporodochial conidia that are cylindrical and often rounded at the apical and basal ends (Figs. 1D,E,H,I, 9–15). *Fusarium brasiliense* is most similar morphologically to *F. phaseoli*, because the latter species forms sporodochial conidia with nearly parallel dorsal and ventral lines and a basal foot cell that is distinct but often rounded (Aoki et al. 2003). Sporodochial conidia of *F. phaseoli*, however, have an acuate apical cell, and possess apical and basal parts that are typically curved ventrally and asymmetrically. Although sporodochial conidia of the second type were not observed consistently in all strains of *F. brasiliense*, short-clavate, slightly curved, 0–1-septate sporodochial conidia were occasionally observed on PDA (Figs. 1J, 16, 17) in some strains of this species (NRRL 22678, NRRL 22744, NRRL 31757), suggesting a morphological resemblance of *F. brasiliense* and *F. phaseoli*. Two strains, NRRL 22743 (=BBA 68441) and NRRL 22744 (=BBA 68442), were previously referred to as *F. martiiphaseoli* Burkh. (Hering 1997). This name, however, was based on a misquotation of *F. martii phaseoli* Burkh. (a trinominal citation of *F. martii* Appel & Wollenweber f. *phaseoli* Burkh. ≡ *F. phaseoli*; Burkholder 1919; Snyder and Hansen 1941; Aoki et al. 2003) at the rank of species. Both strains were reidentified as *F. brasiliense* in the present study.

Fusarium brasiliense is also very similar morphologically to *F. martii* (Appel and Wollenweber 1910), a species considered synonymous to *F. solani* by Snyder and Hansen (1941), Booth (1971), Gerlach and Nirenberg (1982), and Nelson et al. (1983). *Fusarium martii* was described as having septate aerial conidia with a foot cell formed on long and slender conidiophores. Sporodochial conidia of *F. martii* are cylindrical, their dorsal and ventral lines are nearly parallel, and they are nearly the same size ranges as in *F. brasiliense* (Appel and Wollenweber 1910). As illustrated by Appel and Wollenweber (1910), apical cells of the sporodochial conidia of *F. martii* are often rounded, as seen in *F. brasiliense*. Sporodochial conidia of *F. martii*, however, were described as having a long protruding papilla at the base. Wollenweber and Reinking (1935) also described and illustrated a protruding papilla at the base of the sporodochial conidia of *F. solani* var. *martii* (Appel & Wollenw.) Wollenw. (=*F. martii*). These features were reconfirmed by microscopic examination of authentic specimens of the species, BPI 452384 and BPI 452385. By way of contrast, sporodochial conidia of *F. brasiliense* are often rounded at both ends and never possess a protruding papilla at the base (Figs. 1D,E,H,I, 9–15). Based on the morphological difference, *F. brasiliense* is considered to be distinct from *F. martii*.

With the exception of NRRL 22678, which was isolated from an unknown host in California, USA, all other strains

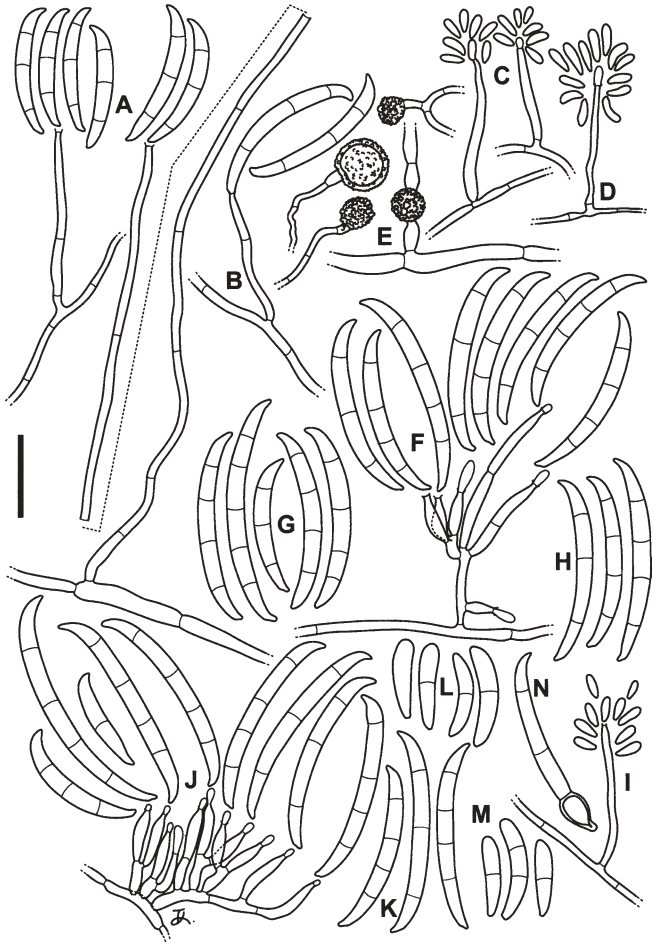


Fig. 22. *Fusarium cuneirostrum* cultured on SNA (**A–H**) and PDA (**I–N**) in the dark (**A,C,E,F,I,J,L,N** from NRRL 31157, ex holotype; **B,D,H** from NRRL 31104; **G,K,M** from NRRL 31949). **A,B** Septate, falcate conidia with a wedge-shaped pointed apex and a foot cell formed on slender aerial conidiophores. **C,D,I** Aseptate, minute, short-clavate conidia formed on short aerial conidiophores. **E** Rough-walled, terminal or intercalary chlamydospores formed in hyphae. **F–H,J,K** Septate, falcate conidia with a wedge-shaped pointed apex and a foot cell formed on sporodochial conidiophores. **L,M** Straight or slightly curved, short-clavate to naviculate, 0–2-septate sporodochial conidia formed in culture on PDA. **N** Chlamydospore in a conidium. *Bar* 25 µm

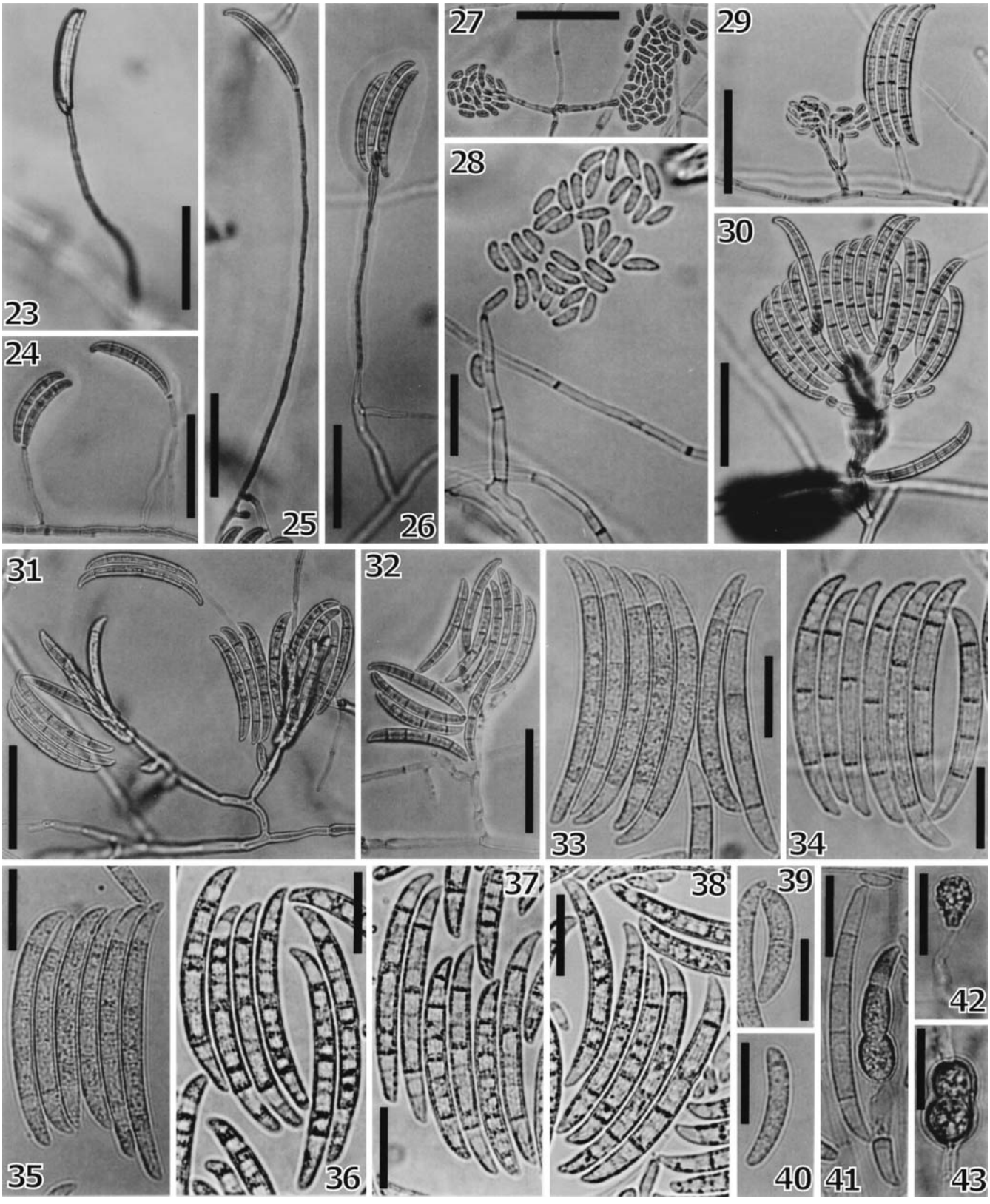
of *F. brasiliense* were isolated from soybean exhibiting SDS foliar symptoms in Brazil (see Table 1).

Fusarium cuneirostrum O'Donnell & T. Aoki, sp. nov.
Figs. 22–43

Coloniae in PDA radium per diem 1.3–1.9 mm ad 20°C obscuritate crescentes, albidae vel luteo-albae, pallide luteae vel aurantio-albae, nonnumquam viridi-griseae; in parte sporifera pustulae luteo-albae, dilute flavae, griseo-luteae vel griseo-virides; in luce fluorescente vel naturali pustulis griseo-aurantiacis, griseo-viridibus vel caerulescentibus; mycelium aerium parcum et in coloniis pionnotium simile, vel nonnumquam copiosum, laxe vel dense floccosum, album vel luteo-album; margo integra vel undulate; reversum saepe hyalinum, nonnumquam griseo-aurantiacum, brunneo-aurantiacum vel luteo-brunneum; odor absens vel nonnumquam mucidus. Hyphae in SNA 1.5–6(–8) µm latae; chlamydosporae in hyphis et conidiis frequenter formantes, plerumque subglobosae, intercalares vel terminales, plerumque singulae, raro catenatae, hyalinae vel dilute flavidae, leves vel rugosae, 6–17.5 × 5–15 µm. Sclerotia absentia. Sporulatio plerumque praecox et copiosa; in PDA saepe subhyalina; in luce nonnumquam viridescens. Sporodochia plerumque copiosa in SNA et PDA, parca in stirpe hypharum. Conidiophora aeria copiosa in SNA, raro in PDA, simplicia vel parce ramosa, plerumque ad 250 µm alta, 2–5 µm lata, monophialides integras formantia. Phialides aeriae simplices, subulatae vel subcylindricae, saepe collarette conspicuum ferentes. Conidia aeria biformia: (1) falcata vel nonnumquam curvatim cylindrica, (2–)3(–4)-septata, basi pediformia, a conidiis falcatis sporodochialibus saepe indistincta, praecipue in conidiophoris majoribus formata; (2) minuta, oblongo-ellipsoidea vel breviter clavata, nonnumquam reniformia, 0(–1)-septata, 5–13 × 1.5–3.5 µm, in conidiophoris usque 74 µm longis et 2–3.5 µm latis ad partes paucas coloniae formata. Conidiophora sporodochialia verticillata vel raro simplicia, monophialides ferentia. Phialides simplices, subulatae, ampulliformes vel subcylindricae, collarette conspicuum ferentes. Conidia sporodochialia biformia: (1) plerumque falcata, nonnumquam cylindrica vel modice curvata, medio latissima vel in dimidio superiore gradatim dilatata, deorsum modice angustata, cellula apicali cuneatim rostrata et cellula basilari clare pediformia, (1–)3–4(–5)-septata, in PDA et SNA formata; illa 3-septata 33–75.5 × 4–6 µm in SNA et 33–68.5 × 4–6.5 µm in PDA; illa 4-septata 45.5–75 × 4.5–6 µm in SNA et 45–81 × 4–6 µm in PDA; illa 5-septata 46.5–93 × 4.5–6 µm in SNA; (2) breviter clavata, nonnumquam ellipsoidea vel naviculiformia, recta vel leviter curvata, apice rotundata et basi truncata, 0–1(–2)-septata, 23–31 × 4.5–6.5 µm, in PDA interdum formata.

Holotypus BPI 843353, cultura sicca (NRRL 31157 = MAFF 239038 = FRC S-1551), e radice *Phaseoli vulgaris* infecti, in Presque Isle, Michigan, USA, lecta in 1992, in Herbario BPI, USA deposita.

Colonies on PDA showing radial mycelial growth rates of 1.3–1.9 mm per day at 20°C in the dark. Colony color on PDA white (1A1) to yellowish-white (4A2), pale yellow (4A3) or orange-white (5A2), sometimes with greenish-grey (25B2) tint, with conidial pustules of yellowish-white (4A2) to pale yellow (4A3) or greyish-yellow (1-2B3-4) to greyish-green (25-30B-D3-4) in the dark and greyish-orange (5B3-5), greyish-green (25B-C3-4) to greyish-turquoise (24B-D3-5) under fluorescent light or daylight. Aerial mycelium sparse with pionnotal colony appearance, or sometimes developed abundantly, then loose to floccose, white (1A1) to yellowish-white (4A2). Colony margin entire to undulate. Reverse pigmentation often absent, sometimes greyish-orange (5B3-6), brownish-orange (5C3-6) to yellowish-brown (5D-E5-6). Exudate not observed. Odor absent or sometimes moldy. Hyphae on SNA 1.5–6(–8) µm wide. Chlamydospores formed abundantly in hyphae and in conidia, mostly subglobose, intercalary, or terminal, mostly single, rarely in chains, hyaline to pale-yellow, smooth to



Figs. 23–43. Morphology of *Fusarium cuneirostrum* cultured in the dark (**23–34,41–43** cultured on SNA; **35–40** cultured on PDA; **23–27,29–32** aerial view; **28,33–43** mounted in water). **23–26** Falcate aerial conidia formed on slender conidiophores arising from hyphae on the agar surface. **27,28** Minute conidia formed on short aerial conidiophores arising from hyphae on the agar surface. **29** Minute conidia formed on a short aerial conidiophore adjacent to a sporodochial conidiophore. **30–32** Sporodochial conidia and conidiophores formed on the agar surface. **33–38** Falcate sporodochial conidia produced on SNA (**33,34**) and PDA (**35–38**); septa are indiscernible in immature conidia on PDA (**35,36**) but become conspicuous as the conidia mature (**37,38**). **39,40** Slightly curved, short-clavate 0–1-septate sporodochial conidia formed in culture on PDA. **41** Chlamydospore in a conidium. **42,43** Chlamydospores in hyphae. **23,25,26,30,31,33,35–37,39,42,43** from NRRL 31157; **24,32,40** from NRRL 31949; **27–29,34,38,41** from NRRL 31104. *Bars* **23–27,29–32** 50 µm; **28,33–43** 20 µm

rough-walled, 6–17.5 × 5–15 μm. Sclerotia absent. Sporulation generally rapid and abundant; on PDA often light-colored, sometimes with a greenish pigmentation under fluorescent light or daylight; sporodochia normally formed abundantly on SNA and PDA, but sparsely in mycelial strains. Aerial conidiophores formed abundantly on SNA, infrequently on PDA, unbranched or sparsely branched, up to 250 μm long, 2–5 μm wide, forming monophialides integrated in the apices. Aerial phialides simple, subulate to subcylindrical, often with a conspicuous collarette at the tip. Aerial conidia of two types; (1) falcate to sometimes curved cylindrical, (2–)3(–4)-septate, with a foot cell, morphologically indistinguishable from falcate sporodochial conidia, formed primarily on taller conidiophores; (2) minute, oblong-ellipsoidal to short clavate, occasionally reniform, 0(–1)-septate, 5–13 × 1.5–3.5 μm in total range, 6.2–9.8 × 2.4–2.8 μm on average [ex type: 5–9.5 × 1.5–3 μm in total range, 6.6 ± 0.96 × 2.4 ± 0.28 μm on average ± SD], formed in a small portion of the colony and on short conidiophores up to 74 μm long, 2–3.5 μm wide. Sporodochial conidiophores branched verticillately, or rarely unbranched, forming apical monophialides. Sporodochial phialides simple, subulate, ampulliform to subcylindrical, with a conspicuous collarette at the tip. Sporodochial conidia of two types; (1) mostly falcate, sometimes cylindrical and gradually curved, widest at the midregion or widening gradually upward, with a rostrate apical cell like a wedge-shaped beak, gradually narrowing toward the base with a distinct, slightly protruding basal foot cell, (1–)3–4(–5)-septate, formed on PDA and SNA; 3-septate on SNA: 33–75.5 × 4–6 μm in total range, 45.7–54.8 × 4.9–5.3 μm on average [ex type: 42–57 × 4.5–6 μm in total range, 50.7 ± 3.30 × 5.2 ± 0.28 μm on average ± SD], on PDA: 33–68.5 × 4–6.5 μm in total range, 45.3–54.5 × 4.7–5.5 μm on average [ex type: 45–62 × 4.5–6 μm in total range, 54.5 ± 3.28 × 5.4 ± 0.27 μm on average ± SD]; 4-septate on SNA: 45.5–75 × 4.5–6 μm in total range, 53–63.5 × 5.1–5.3 μm on average [ex type: 50–67.5 × 5–6 μm in total range, 56.7 ± 3.99 × 5.3 ± 0.23 μm on average ± SD], on PDA: 45–81 × 4–6 μm in total range, 54.6–63.4 × 4.9–5.3 μm on average [ex type: 50.5–68.5 × 5–6 μm in total range, 57.4 ± 4.46 × 5.2 ± 0.23 μm on average ± SD]; 5-septate on SNA: 46.5–93 × 4.5–6 μm in total range, 59.7–69.3 × 5.1–5.4 μm on average [ex type: 50.5–67.5 × 5–6 μm in total range, 59.7 ± 3.90 × 5.4 ± 0.22 μm on average ± SD]; (2) short-clavate to sometimes ellipsoidal or naviculate, straight or slightly curved, with a rounded apex and a truncate base, 0–1(–2)-septate, 23–31 × 4.5–6.5 μm, sometimes formed on PDA.

Holotype: BPI 843353, a dried culture, isolated from a diseased root of dry bean exhibiting root-rot symptoms, collected in Presque Isle, Michigan USA, 1992, deposited in the herbarium of BPI, USA.

Ex holotype culture: NRRL 31157 = MAFF 239038 = FRC S-1551 = Michigan State Univ. FS12.

Etymology: *cunei-* (Latin, wedge-shaped) + *rostrum* (Latin, beak); based on the apical cell morphology of sporodochial conidia.

Isolates studied. NRRL 22275 = MAFF 239036, *Phaseolus vulgaris*, Sapporo, Hokkaido, Japan, I. Tanaka, 1955; NRRL 31104 = MAFF 305607, *P. vulgaris*, Japan, T. Matuo, (collection date unknown); NRRL 31157 = MAFF 239038, *P. vulgaris*, Presque Isle, Michigan, USA, 1992; NRRL 31949 = MAFF 239052, *Glycine max*, Cristalina, Goiás, Brazil, J.T. Yorinori, 2000; NRRL 36023 = MAFF 239491 = Mung 1, NRRL 36024 = MAFF 239942 = Mung 2, *Vigna radiata*, Ridgetown, Ontario, Canada, T.R. Anderson, 1996.

Notes: The rostrate apical cell of the sporodochial conidia distinguishes *F. cuneirostrum* from all other species. Because the apical cells of the conidia of *F. cuneirostrum* often taper and hook abruptly at the upper third or quarter of their length (Figs. 22F–H,J,K, 29–38), they look like a wedge-shaped beak. Together with a distinct basal foot cell, falcate conidia of *F. cuneirostrum* often have a pointed appearance at both ends. *Fusarium cuneirostrum* and *F. tucumaniae* are morphologically similar in that both produce sporodochial conidia with a pointed apical cell and a distinct basal foot cell (Aoki et al. 2003). However, sporodochial conidia in *F. tucumaniae* are longer and narrower than those of *F. cuneirostrum*, and the curvature of conidia in *F. tucumaniae* is less pronounced.

Fusarium cuneirostrum is also morphologically similar to *F. phaseoli* in that both species produce sporodochial conidia with an acuate apical cell and a distinct basal foot cell (Aoki et al. 2003). The second type of sporodochial conidia produced by *F. cuneirostrum* are short-clavate to ellipsoidal and they are also similar to those of *F. phaseoli*. All but one of the strains of *F. cuneirostrum* was isolated from *P. vulgaris*, the original host of *F. phaseoli*. The apical cell of the first type of sporodochial conidia of *F. cuneirostrum*, however, is typically more pointed than *F. phaseoli* and it frequently possesses a pediform and slightly protruding basal foot cell (Figs. 22F–H,J,K, 33–38). Apical and basal parts of the sporodochial conidia in *F. cuneirostrum* are less significantly curved compared with those of *F. phaseoli*. In general, *F. cuneirostrum* produces longer sporodochial conidia of the first type than *F. phaseoli*.

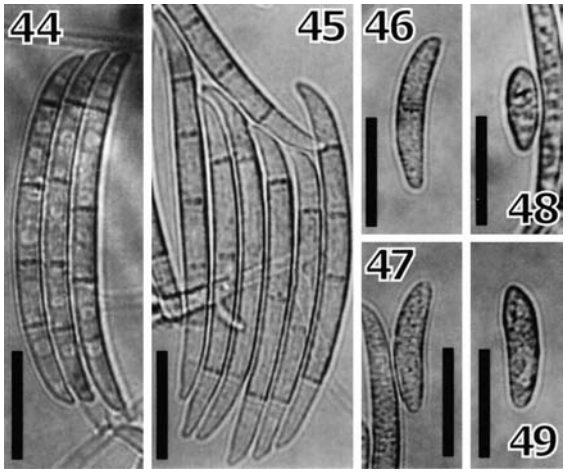
Fusarium cuneirostrum has three different hosts: dry bean, mung bean and soybean. Three strains of *F. cuneirostrum* were isolated from dry bean plants exhibiting root-rot symptoms in Japan and in the United States, two strains of the species were isolated from mung bean plants exhibiting root-rot symptoms in Canada, and one strain, NRRL 31949, was isolated from soybean SDS in Brazil (see Table 1). The single strain of *F. cuneirostrum*, NRRL 31949 causing soybean SDS in Brazil has a slight morphological difference from other strains of this species. Sporodochial conidia of strain NRRL 31949 on SNA and PDA are mostly typical for *F. cuneirostrum*, i.e., falcate, gradually curved, with a rostrate apical cell and a distinct basal foot cell (Figs. 22G,K, 32), but occasionally conidia are produced with rounded apical and basal ends (Fig. 32), similar to the conidial morphology of *F. brasiliense*.

The following two species, *F. tucumaniae* based on Brazilian isolates and *F. virguliforme* based on the Argentinean isolates, are described here as additional distribution records outside of the countries where they were originally

reported (i.e., Argentina and the United States, respectively; Aoki et al. 2003).

Fusarium tucumaniae T. Aoki, O'Donnell, Yos. Homma & Lattanzi, Mycologia 95: 664, 2003.

Colonies on PDA showing radial mycelial growth rates of 1.3–1.6 mm per day at 20°C in the dark. Colony color on PDA white (1A1) to yellowish-white (3-4A2), sometimes with greenish-grey (25-26B2-3) to greyish-green (25-26D6) tint, conidial pustules yellowish-white (2-4A2), pale yellow (2-4A3), light yellow (4A4-5), or greyish-green (28-30B3) in the dark and yellowish-white (2-4A2), greenish-white (28-30A2), greyish-green (27-30B-C3-5, 25-30D4-5, 27-28E4-7) to dark green (25-26F6-8) under fluorescent or daylight. Aerial mycelium generally sparse with a pionnotal colony appearance, sometimes developed abundantly, then loose to dense floccose, white (1A1) to yellowish-white (1A2). Colony margin entire to often undulate. Reverse pigmentation often absent, sometimes greyish-yellow (4C4-6) to yellowish-brown (5D-E4-6) to dark brown (5F6-7). Exudate not observed. Odor absent or sometimes putrid and moldy. Hyphae on SNA 1–5(–14) µm wide. Chlamydospores formed frequently in hyphae and in conidia, mostly subglobose, often terminal, occasionally intercalary, single, rarely in chains, hyaline to pale, yellowish-gray or pale-yellow, smooth to rough-walled, sometimes verruculose, 8–20 × 10–20 µm. Sclerotia absent. Sporulation generally rapid and abundant; on PDA often light-colored in dark, with a greenish-to-bluish pigmentation under fluorescent light or daylight; sporodochia often formed abundantly on SNA and PDA, but sparsely in mycelial strains on PDA. Aerial conidiophores formed abundantly on SNA, sometimes on PDA, generally unbranched, or branched sparsely, up to 230 µm long, 2.5–5 µm wide, forming monophialides integrated in the apices. Aerial phialides simple, subulate to subcylindrical. Aerial conidia of two types; (1) curved cylindrical to falcate, (2–)3(–5)-septate, with foot, morphologically indistinguishable from falcate sporodochial conidia, formed primarily on taller conidiophores; (2) minute, oval, 0(–1)-septate, 4.5–13 × 2–3.5 µm in total range, 6.7–7.7 × 2.4–2.7 µm on average, formed in a small portion of the colony and on short conidiophores of up to 50 µm long, 2–3 µm wide. Sporodochial conidiophores branched verticillately, or rarely unbranched, forming apical monophialides. Sporodochial phialides simple, subulate, ampulliform to subcylindrical, often with a conspicuous collarette at the tip. Sporodochial conidia generally cylindrical and gently curved, sometimes falcate, with nearly parallel dorsal and ventral lines, with an acuate apical cell and a distinct basal foot cell, (2–)3–4(–6)-septate (Figs. 44, 45); 3-septate on SNA: 38–72.5 × 4–6.5 µm in total range, 52.2–63.3 × 4.7–4.9 µm on average, on PDA: 34.5–71 × 4–5.5 µm in total range, 55.0–58.3 × 4.7–4.9 µm on average; 4-septate on SNA: 50.5–81 × 4–5.5 µm in total range, 62.5–67.3 × 4.8–5.0 µm on average, on PDA: 51–77.5 × 4–5.5 µm in total range, 58.0–61.4 × 4.7–5.0 µm on average; 5-septate on SNA: 57.5–88.5 × 3.5–6 µm in total range, 65.3–74.1 × 4.8–5.1 µm on average. Second type of sporodochial conidia present or absent, short-clavate to ellipsoidal or naviculate, straight or slightly curved, with a rounded apex and a truncate base, 0–1-septate, 20–27(–51.5) × 2.5–6 µm, occasionally formed in some strains on PDA (Figs. 46–49).



Figs. 44–49. Sporodochial conidia formed by Brazilian isolates of *Fusarium tucumaniae* cultured in the dark (mounted in water). **44,45** Falcate sporodochial conidia observed on SNA (**44**) and PDA (**45**); septa are often indiscernible in immature conidia on PDA but become conspicuous as the conidia mature (**45**). **46–49** Slightly curved, short-clavate to naviculate, 0–1-septate sporodochial conidia. **44** from NRRL 31793; **46,48,49** from NRRL 31773; **45,47** from NRRL 31796. *Bars* 20 µm

Isolates studied: NRRL 31773 = MAFF 239044, NRRL 31950 = MAFF 239053, *Glycine max*, Ponta Grossa, Paraná, Brazil, J.T. Yorinori, 2000; NRRL 31776 = MAFF 239054, *G. max*, Tapera, Rio Grande do Sul, Brazil, J.T. Yorinori, 2000; NRRL 31777 = MAFF 239045, *G. max*, Vila Maria, Rio Grande do Sul, Brazil, J.T. Yorinori, 2000; NRRL 31778 = MAFF 239046, *G. max*, Sarandi, Rio Grande do Sul, Brazil, J.T. Yorinori, 2000; NRRL 31793 = MAFF 239048, *G. max*, Nova Ponte, Minas Gerais, Brazil, J.T. Yorinori, 2001; NRRL 31796 = MAFF 239049, *G. max*, Ponta Grossa, Paraná, Brazil, J.T. Yorinori, 2001.

Notes: The foregoing description of *F. tucumaniae* is based entirely on Brazilian isolates (see Table 1). An additional morphological character was observed among the Brazilian isolates of *F. tucumaniae*. Brazilian isolates NRRL 31773, 31778, 31793, and 31796 formed a second type of sporodochial conidia, which are short-clavate to ellipsoidal, 0–1-septate, straight or slightly curved, with a rounded apex and a truncate base on PDA (Figs. 46–49). This conidial morphology was similar to that of *F. phaseoli*, *F. brasiliense* and *F. cuneirostrum*. The second type of sporodochial conidia was not observed in the other Brazilian isolates and in the Argentinean isolates of *F. tucumaniae* examined. Except for the production of the second type of sporodochial conidia, all other morphological and cultural features of the Brazilian isolates of *F. tucumaniae* matched the original description of the species, which was based entirely on the Argentinean isolates (Aoki et al. 2003). The first type of sporodochial conidia of the Brazilian isolates of *F. tucumaniae* (Figs. 44, 45) is septate, cylindrical, and gently curved or sometimes falcate, with an acuate apical cell

and a distinct basal foot cell. On average, conidial sizes of the Brazilian isolates exceeded 50 µm in length and were mostly 4.5–5 µm wide, corresponding to the values obtained from the Argentinean isolates of *F. tucumaniae*. In addition, all morphological and cultural features of the five new Argentinean isolates (see Table 1) agreed well with the original description of *F. tucumaniae* (Aoki et al. 2003).

The long and slender sporodochial conidia of *F. tucumaniae* differentiate it from other *Fusarium* species causing soybean SDS and dry bean root-rot. Even though some strains may have ability to produce the second type of sporodochial conidia, as observed in some of the Brazilian isolates, the size ranges of the first type of sporodochial conidia clearly distinguish this species from all other related fusaria. This species has only been isolated from soybean plants exhibiting SDS foliar symptoms in Argentina and Brazil (see Table 1; Aoki et al. 2003).

Fusarium virguliforme O'Donnell & T. Aoki, Mycologia 95: 667, 2003.

Colonies on PDA showing radial mycelial growth rates of 1.4–1.6 mm per day at 20°C in the dark. Colony color on PDA white (1A1) to yellowish-white (2-4A2) or pale yellow (4A3), sometimes with bluish-grey (22-23B2-3) tint, conidial pustules pale yellow (4A3) to light yellow (4A4-5) in the dark and pale yellow (4A3), greyish-yellow (3-4B3-4), greenish-white (26-29A2), pale green (26-29A3), greyish-turquoise (24B-D3-6), pastel green (28A4), greyish-green (25-28B-C3-5, 25-27D-E4-7), dark green (25-27F5-8) to dark turquoise (24F7-8) under fluorescent light or daylight. Aerial mycelium sparse with a pionnotal colony appearance, sometimes developed abundantly, then loose to dense floccose, white (1A1), yellowish-white (2-4A2), sometimes greenish-grey (25-27B2). Colony margin entire to often undulate. Reverse pigmentation often absent, sometimes greyish-yellow (4B-C4-5) to brownish-orange (5C3-4), or olive-brown (4D-E5) to yellowish-brown (5D-F5-6). Exudate not observed. Odor absent or sometimes putrid or moldy. Hyphae on SNA 1.5–6(–9) µm wide. Chlamydospores formed abundantly in hyphae and in conidia, mostly subglobose, intercalary or terminal, single, rarely in chains, hyaline to pale or pale-yellow, smooth to rough-walled, 8–15 × 5.5–12 µm. Sclerotia absent. Sporulation generally rapid and abundant; on PDA often light-colored in dark, with a greenish-to-bluish pigmentation under fluorescent light or daylight; sporodochia formed abundantly on SNA and PDA. Aerial conidiophores formed abundantly on SNA, sometimes on PDA, unbranched or branched sparsely, up to 250 µm long, 3–5 µm wide, forming monophialides integrated in the apices. Aerial phialides simple, subulate to subcylindrical, often with a conspicuous collarette at the tip. Aerial conidia of two types; (1) curved cylindrical to falcate, (2–)3(–4)-septate, with a foot cell, morphologically indistinguishable from falcate sporodochial conidia, formed mainly on taller conidiophores; (2) minute, oblong-ellipsoidal to short-clavate, 0(–1)-septate, 4–11.5 × 1.5–3.5 µm in total range, 6.8–7.6 × 2.5–2.6 µm on average, formed in a small portion of the colony and on short conidiophores up to 56 µm long, 2.5–3 µm wide. Sporodochial conidiophores branched verticillately, or rarely unbranched, forming apical monophialides. Sporodochial phialides simple, subulate, ampulliform to subcylindrical, with a conspicuous collarette at the tip. Sporodochial conidia of two types; (1) typically falcate, dorsiventral, most frequently widest at the midregion of their length, often tapering and curving equally toward both ends, with the apex and foot cell typically similarly pointed and often indistinguishable, (2–)3–4(–5)-septate, formed on PDA and on SNA; apical and basal halves often morphologically symmetrical; 3-septate on SNA: 38–62 × 4.5–6 µm in total range, 50.2–53.5 × 5.1–5.2 µm on average, on PDA: 36–61 × 4.5–6 µm in total range, 45.7–49.0 × 5.2 µm on average; 4-septate on SNA: 45–65 × 5–6 µm in total range, 53.8–57.5 × 5.3 µm on average, on PDA: 46–65 × 4.5–6 µm in total range, 54.6–55.7 × 5.0–5.3 µm on average; 5-septate on SNA: 45.8–85.5 × 5–6.5 µm in total range, 62.1–63.5 × 5.2–5.4 µm on average; (2) comma-shaped to sometimes short-clavate, with a swollen apex often rounded and with a tapering and curving base, formed only on PDA often in the dark, 0–1(–2)-septate, 15.5–30.5 × 4.5–8 µm in total range, 22.0–23.2 × 5.8–6.3 µm on average.

Isolates studied: NRRL 34551 = MAFF 239257, *Glycine max*, San Pedro, Buenos Aires, Argentina, M. Scandiani, 2002; NRRL 34552 = MAFF 239258, *G. max*, Serodino, Santa Fe, Argentina, M. Scandiani, 2002; NRRL 34553 = MAFF 239259, *G. max*, Serodino, Santa Fe, Argentina, M. Scandiani, 2002.

Notes: The foregoing description of *F. virguliforme* is based strictly on Argentinean isolates (see Table 1). Morphological and cultural features of the three Argentinean isolates matched the original description of *F. virguliforme* reported from the United States (Aoki et al. 2003). *Fusarium virguliforme* is distinguished from other species within the *F. solani* complex by the production of comma-shaped sporodochial conidia on PDA, together with the apically and basally symmetrical sporodochial conidia formed on SNA and PDA. Septate, falcate aerial conidia with a foot cell on tall slender conidiophores and minute, oblong-ellipsoidal to short-clavate conidia formed on short aerial conidiophores on SNA provide additional diagnostic features of all the species that induce soybean SDS within the *F. solani* complex.

Morphological comparison of four *Fusarium* species causing soybean SDS in South America

A morphological character common to the four *Fusarium* species (*F. brasiliense*, *F. cuneirostrum*, *F. tucumaniae*, and *F. virguliforme*) causing soybean SDS in Argentina and Brazil is the formation of plural types of conidia, especially on aerial conidiophores. Two types of aerial conidia were produced by all four species: (A-1) falcate, multiseptate aerial conidia with a foot cell formed mainly on tall and slender conidiophores observed on SNA; and (A-2) minute, short-clavate to ellipsoidal aerial conidia formed on short conidiophores in a small portion of the colony on SNA and

PDA. Two types of sporodochial conidia were also found among the species: (S-1) falcate, multiseptate conidia with a foot cell formed on SNA and PDA; and (S-2a) short-clavate to ellipsoidal or naviculate conidia or (S-2b) comma-shaped conidia on PDA. Considering the second type of sporodochial conidia, naviculate conidia (S-2a) were formed in *F. brasiliense*, *F. cuneirostrum*, and *F. tucumaniae* and comma-shaped conidia (S-2b) were formed in *F. virguliforme*. In the present study, the S-2a conidial type observed in *F. tucumaniae* was also found in some Brazilian isolates, although this conidial type was not observed in the Argentinean strains of this species, as reported in Aoki et al. (2003). Because the S-2a conidial type is not observed consistently in *F. brasiliense* and *F. tucumaniae*, and because the morphology and size of the conidia are very similar, use of the first conidial type (S-1) is required to differentiate of the four soybean SDS species.

Average sizes of the sporodochial conidia of the first type (S-1) formed by the four soybean SDS species in South America are compared in Figs. 50 and 51, together with eight isolates of *F. virguliforme* from the United States and five nonsoybean isolates of *F. cuneirostrum*. Strains of *F. tucumaniae* isolated in Brazil and Argentina are differentiated from strains of the other three species based on the size of sporodochial conidia. Conidial sizes of the Brazilian and Argentinean isolates of *F. tucumaniae* consisently exceeded 50 µm in length and they were mostly 4.5–5 µm in width on average on SNA and PDA, although S-1 conidia of the Brazilian isolates of this species are slightly shorter and wider. When average S-1 conidial sizes were compared based on the number of septa, *F. tucumaniae* is clearly differentiated from the three other *Fusarium* species that cause soybean SDS in South America. Because average conidial sizes of *F. brasiliense*, *F. cuneirostrum*, and *F. virguliforme* overlap, detailed morphology of the first sporodochial conidia type (S-1) is critical for diagnosing these species. Among the four species, *F. brasiliense* is distinguished by the production of cylindrical and gradually curved sporodochial conidia of the first type with nearly parallel dorsal and ventral lines in which the apical and basal parts are often rounded (see Figs. 1D,E,H,I, 9–15). *Fusarium cuneirostrum*, in contrast, produces falcate and gradually curved sporodochial conidia in which the apical cell is often rostrate and pointed and the basal foot cell

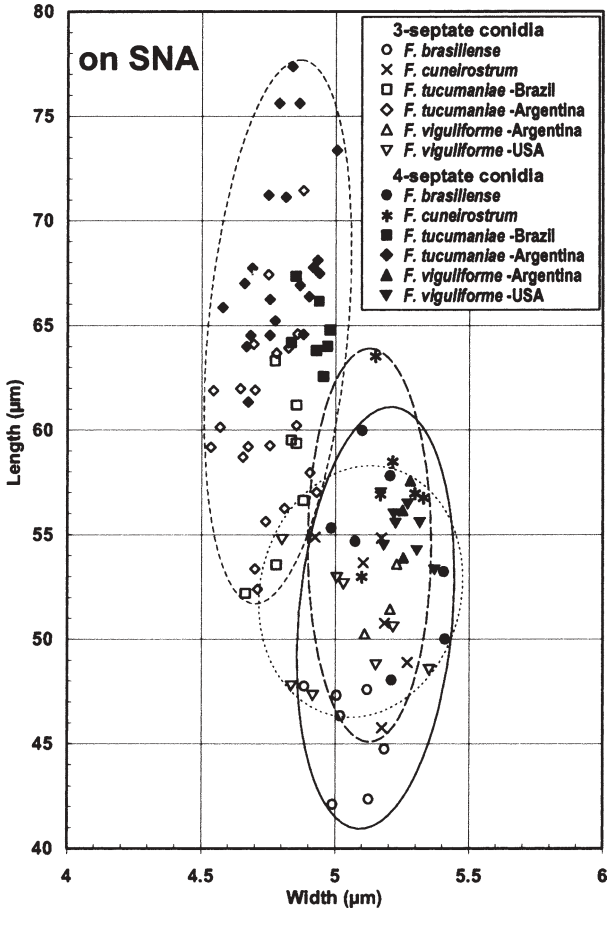


Fig. 50. Plots of mean values of length and width of 3- and 4-septate sporodochial conidia of *Fusarium brasiliense*, *F. cuneirostrum*, *F. tucumaniae* (Brazilian and Argentinean isolates), and *F. virguliforme* (Argentinean and American isolates) grown on SNA in the dark at 20°C

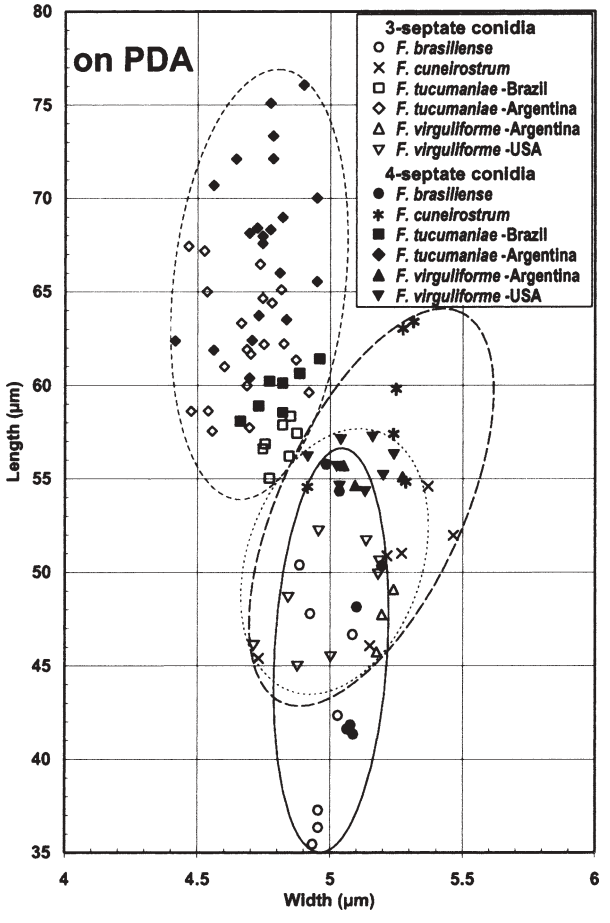


Fig. 51. Plots of mean values of length and width of 3- and 4-septate sporodochial conidia of *Fusarium brasiliense*, *F. cuneirostrum*, *F. tucumaniae* (Brazilian and Argentinean isolates), and *F. virguliforme* (Argentinean and American isolates) grown on PDA in the dark at 20°C

protrudes slightly (see Figs. 22F–H,J,K, 29–38). *Fusarium virguliforme* is differentiated by apically and basally symmetrical sporodochial conidia (S-1) with an apical and a basal cell similarly pointed such that they are indistinguishable, in addition to the unique production of comma-shaped sporodochial conidia (S-2b) on PDA. Sporodochial conidia in *F. tucumaniae* are cylindrical and gently curved or sometimes falcate, with an acuate apical cell and a distinct basal foot cell, exhibiting some similarity to those produced by *F. cuneirostrum*. However, sporodochial conidia of *F. tucumaniae* are longer and narrower and the curvature of its conidia is less pronounced than in *F. cuneirostrum*.

Terminal or intercalary chlamydospores in hyphae were formed commonly by strains of the four species (see Figs. 1F, 18, 19, 22E, 42, 43) but they do not have taxonomic value. Conidial chlamydospores were also frequently observed (see Figs. 20, 21, 22N, 41) in older cultures. They were smooth- to rough-walled and occasionally became yellowish.

Colony characteristics of the four species were compared on PDA in 9-cm plastic Petri dishes at 20°C, but no clear difference was observed. Mycelial and pionnotal strains were found in each of the four species, and greater numbers of conidia were formed by the pionnotal strains. When cultures were grown in complete darkness, colony color often remained whitish to yellowish, although colonies frequently became greenish to bluish and also dark green or dark turquoise when cultured under fluorescent light or under daylight. Colony morphology and pigmentation were not useful for species recognition.

To reconfirm the original description of *F. martii* given by Appel and Wollenweber (1910), conidial morphology of two authentic specimens of the species, BPI 452384 and BPI 452385, both prepared by W. Wollenweber (dried cultures on potato stems and tubers, isolated originally from *Solanum tuberosum* L., Berlin, Germany, 1909), were also examined. Three different types of conidia were observed: (1) cylindrical to falcate, (2–)3–4(–6)-septate conidia, often with a rounded apex and a protruding (up to 1.5 µm) foot, (2) short-clavate to ellipsoidal or naviculate, straight or slightly curved, 1-septate conidia with a rounded apex and a truncate base, 14.5–28 × 2.5–5 µm, and (3) minute, oval to ellipsoidal or short clavate, 0(–1)-septate conidia, 5–14 × 1.5–3.5 µm. Three different types of conidiophores were also observed: (1) densely branched and aggregated sporodochial conidiophores, (2) tall aerial conidiophores up to 190 µm long, 2.5–3.5 µm wide, and (3) short conidiophores up to 50 µm long, 2–2.5 µm wide. Terminal or intercalary, subglobose chlamydospores were frequently observed in hyphae and in conidia. The description and illustration given by Appel and Wollenweber (1910) of falcate sporodochial conidia and conidiophores, falcate aerial conidia and conidiophores, and chlamydospores were reconfirmed precisely. The combination of morphological characters exhibited by *F. martii*, such as conidia and conidiophores types, are basically the same as observed in the *Fusarium* species causing soybean SDS and dry bean root-rot examined in the present study and by Aoki et al. (2003) (Table 4).

Table 4. Comparison of major diagnostic morphology of *Fusarium* species within the *F. solani* complex related to soybean SDS and bean root-rot

Species	Aerial conidia		First type of sporodochial conidia			Second type of sporodochial conidia	Host	Country of occurrence
	Cylindrical to falcate, septate, with a foot cell, on tall conidiophores	Short-clavate, minute, mostly aseptate, on short conidiophores	Average size (4-septate conidia on SNA)		Apical cell	Basal foot cell		
			Length	Width				
Fusarium tucumaniae	+	+	>60 µm	<5 µm	Acuate	Distinct	+/–, short-clavate, gently curved	Soybean	Argentina, Brazil
Fusarium virguliforme[a]	+	+	50–60 µm	5–5.5 µm	Acuate	Often indistinct	+, comma-shaped	Soybean	USA, Argentina[d]
Fusarium brasiliense	+	+	45–60 µm	5–5.5 µm	Often rounded	Often rounded	+/–, short-clavate, gently curved	Soybean	Brazil, USA
Fusarium cuneirostrum	+	+	50–65 µm	5–5.5 µm	Wedge-shaped beak	Distinct	+, short-clavate, gently curved	Soybean, dry bean, mung bean	Brazil, Japan, USA, Canada
Fusarium phaseoli[a]	+	+	<55 µm	5–5.5 µm	Acuate, hooked	Distinct, rounded	+, short-clavate, gently curved	Dry bean	USA (cosmopolitan?)
Fusarium martii[b]	+	+[c]	48–60 µm	5–5.5 µm[b]	Often rounded	Protruded	+, short-clavate, gently curved[c]	Potato (various hosts?)	Germany, (cosmopolitan?)

SNA, synthetic low-nutrient agar
[a] Aoki et al. (2003)
[b] Based principally on Appel and Wollenweber (1910) and the average size for 4-septate conidia is under various culture conditions
[c] Based on examination of BPI 452384 and BPI 452385
[d] Reported first by Scandiani et al. (2004b)

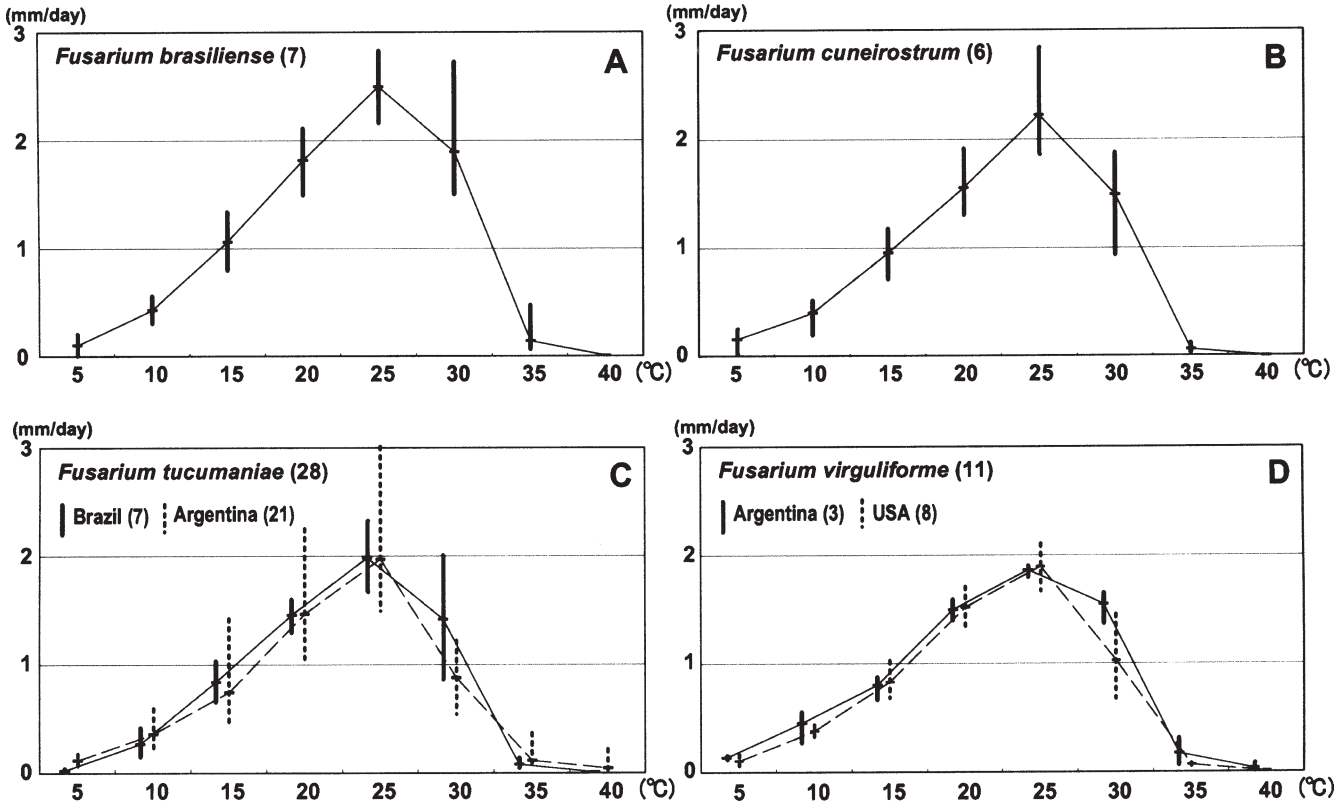


Fig. 52. Comparison of radial growth rates per day on PDA of *Fusarium brasiliense* (**A**), *F. cuneirostrum* (**B**), *F. tucumaniae* (**C**), and *F. virguliforme* (**D**) at different temperatures from 5° to 40°C. *Thick horizontal and vertical bars* indicate means and total ranges, respectively, among the strains of each species (number of strains examined in *parentheses*)

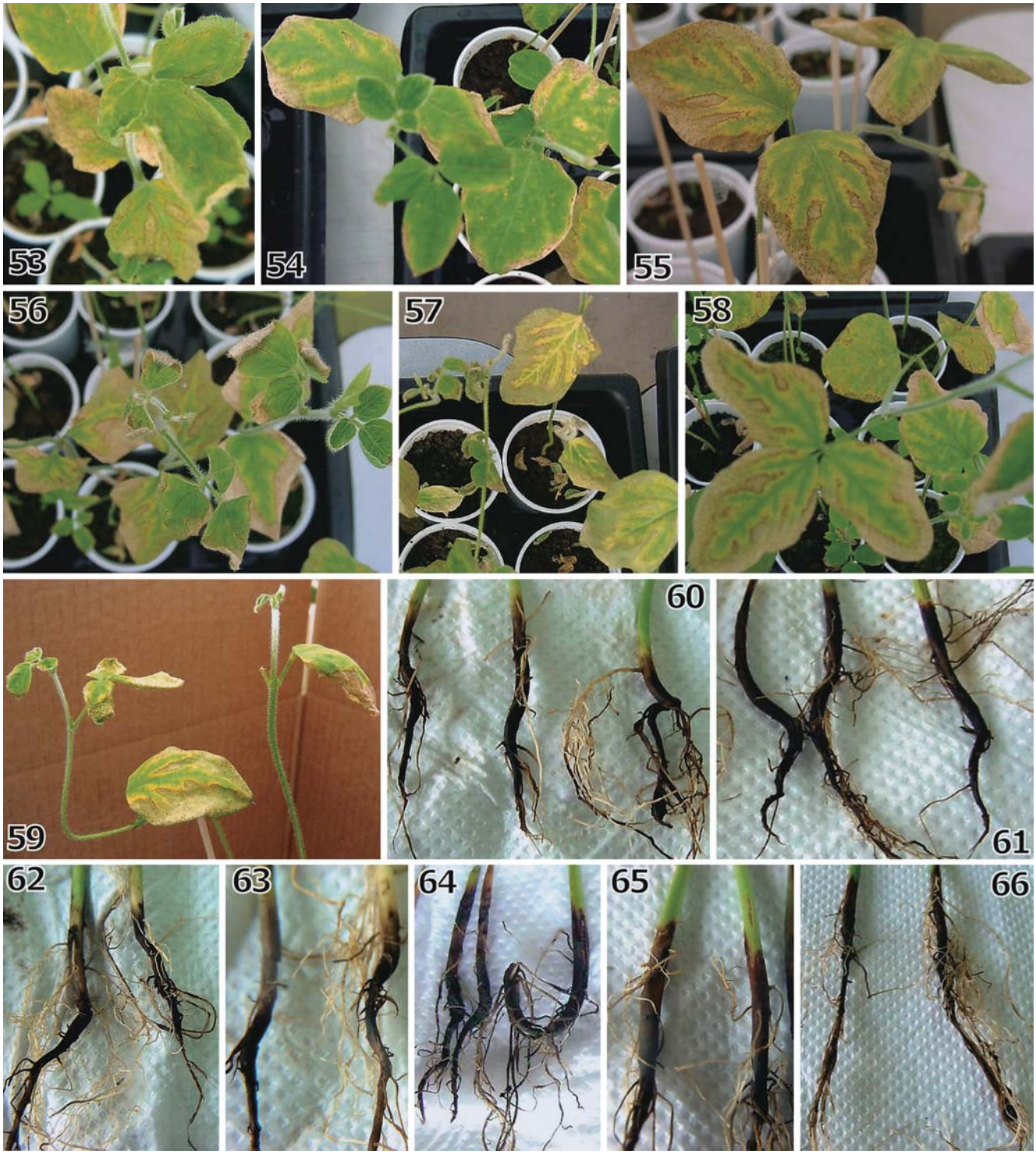
Radial mycelial growth rates of four *Fusarium* species causing soybean SDS in South America

Average radial mycelial growth rates on PDA in the dark at eight different temperatures at 5°C intervals from 5° to 40°C were calculated for 7 strains of *F. brasiliense*, 6 strains of *F. cuneirostrum*, 28 strains of *F. tucumaniae*, and 11 strains of *F. virguliforme* (Fig. 52). In addition, values for the strains of *F. tucumaniae* and *F. virguliforme* from different countries are shown separately.

Optimal temperature for mycelial growth was 25°C for all species: 2.2–2.8 mm/day for *F. brasiliense*; 1.9–2.8 mm/day for *F. cuneirostrum*; 1.7–2.3 mm/day for the Brazilian strains; 1.5–3.0 mm/day for the Argentinean strains and for all strains of *F. tucumaniae*; 1.8–1.9 mm/day for the Argentinean strains; and 1.7–2.1 mm/day for the American strains and for all strains of *F. virguliforme*. Growth rates of the four species causing soybean SDS in Brazil and Argentina were nearly the same and approximately half that reported for the representative biological species (i.e., mating populations) of the *F. solani* species complex (Aoki et al. 2003). This ratio was nearly the same for the other temperatures tested.

Pathogenicity of selected strains of five *Fusarium* species on soybean plants

Pathogenicity of 15 strains representing the soybean SDS and dry and mung bean root-rot pathogens were tested using soybean cultivar Pioneer 9492RR (Table 2; Figs. 53–66). The ANOVA revealed that there was a significant strain effect for disease incidence (DI), disease severity (DS), plant height, shoot fresh weight, plants with basal stem rot, root-rot, and root dry weight. Only mild foliar symptoms were caused by some strains under greenhouse conditions, which are similar to the early stage of typical SDS foliar symptoms and may be associated with the root-rot observed. However, only typical foliar symptoms were evaluated for the disease incidence in the present study. Variability in pathogenicity among the strains tested is shown in Table 2. All strains of *F. tucumaniae* and *F. virguliforme* tested produced typical SDS foliar symptoms, root-rot, basal stem rot, and stunting (see Figs. 57, 58, 64, 65), except for *F. tucumaniae* NRRL 34549, which was weakly pathogenic to soybean. Among the strains of *F. cuneirostrum* examined, NRRL 31949, isolated from a soybean exhibiting SDS symptoms in Brazil, was highly pathogenic (see Figs. 54, 61), NRRL 31157 (from dry bean; see Figs. 55, 62) and NRRL 36024 (from mung bean; see Fig. 56) showed intermediate pathogenicity, and NRRL 36023



Figs. 53–66. Soybean sudden death syndrome (SDS) symptoms induced by artificial inoculation tests with strains of five *Fusarium* species using the susceptible soybean cultivar Pioneer 9492RR. **53–59** Foliar symptoms caused by *F. brasiliense* NRRL 31756 (**53**), *F. cuneirostrum* NRRL 31949 (**54**), *F. cuneirostrum* NRRL 31157 (**55**), *F. cuneirostrum* NRRL 36024 (**56**), *F. tucumaniae* NRRL 34550 (**57**), *F. virguliforme* NRRL 34551 (**58**), and *F. phaseoli* NRRL 31156 (**59**). **60–66** Root-rot symptoms caused by *F. brasiliense* NRRL 31756 (**60**), *F. cuneirostrum* NRRL 31949 (**61**), *F. cuneirostrum* NRRL 31157 (**62**), *F. cuneirostrum* NRRL 36023 (**63**), *F. tucumaniae* NRRL 34550 (**64**), *F. virguliforme* NRRL 34552 (**65**), and *F. phaseoli* NRRL 31156 (**66**). Note typical soybean SDS symptoms consisting of interveinal chlorosis and necrosis, and root-rot. **59** shows a petiole remaining attached to the stem after leaf dropping, as a typical symptom of soybean SDS

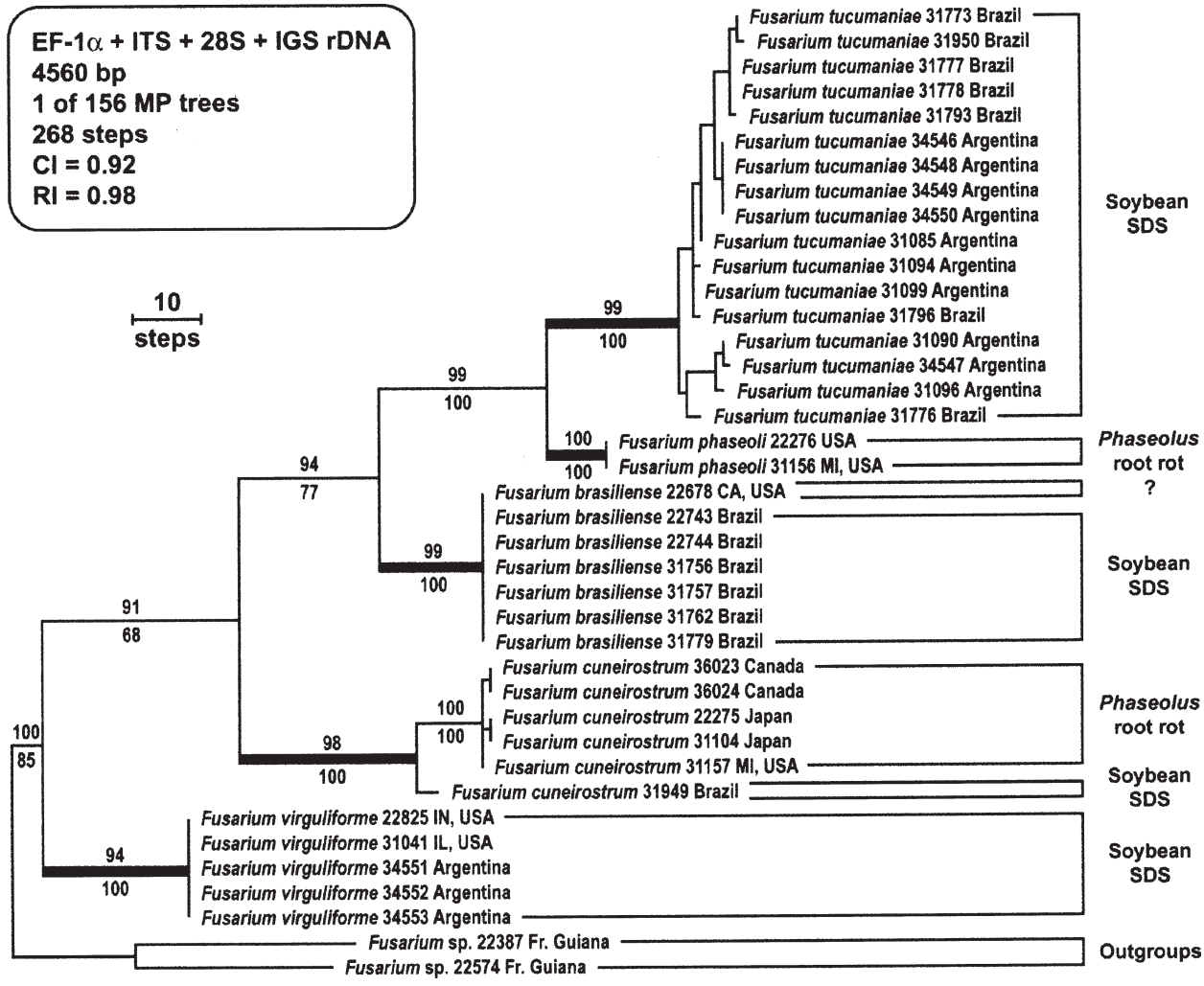


Fig. 67. One of 156 most parsimonious (MP) phylograms inferred from the combined data set consisting of 4560 aligned base pairs rooted with sequences of *Fusarium* spp. NRRL 22387 and NRRL 22574. Note that internodes (in *bold*) identify five ingroup species that are strongly supported (bootstrap = 94%–100%)

(from mung bean; see Fig. 63) showed low pathogenicity to soybean. All isolates of this species produced typical SDS foliar symptoms, root-rot, basal stem rot, and stunting. *Fusarium brasiliense* NRRL 31756 was highly pathogenic to soybean (see Figs. 53, 60), whereas isolates NRRL 31762 and NRRL 31779 showed low pathogenicity. For *F. phaseoli*, NRRL 31156 (see Figs. 59, 66) induced moderate disease severity of 32% (exhibiting interveinal chlorosis and necrosis and 21%–50% foliage affected), but only mild SDS foliar symptoms were observed with NRRL 22276. To fulfill Koch's postulates, microscopic and molecular analyses demonstrated that the same species of *Fusarium* inoculated in the pathogenicity experiments were reisolated from diseased roots (root-rot) of selected soybean plants exhibiting typical SDS symptoms.

Molecular phylogenetic relationships among strains

Tree statistics and sequence data from the individual and combined partitions are summarized in Table 3. Aligned sequences from each partition were analyzed individually (see Table 3) and as a combined data set (Fig. 67). Outgroup sequences (*Fusarium* spp. NRRL 22387 and NRRL 22574) were chosen to root the trees based on more inclusive phylogenetic analyses (O'Donnell 2000; Aoki et al. 2003). All the ingroup shared identical partial 28S rDNA sequences. Within the ITS rDNA gene tree, the five ingroup taxa formed the following three groups: *F. phaseoli* + *F. tucumaniae*, *F. virguliforme* + *F. cuneirostrum*, and *F. brasiliense*. Although strains of *F. brasiliense*, *F. phaseoli* and one soybean SDS-causing strain of *F. cuenirostrum* NRRL 31949 shared an identical EF-1α allele, strains of *F. tucumaniae*, *F. virguliforme* and the other strains of *F. cuneirostrum*, which cause *Phaseolus* and mung bean root-rot, each possess unique EF-1α alleles. All five ingroup taxa were resolved as exclusive groups within the IGS rDNA gene tree (see Table 3; tree not shown) and in the species phylogeny inferred from the combined data set (see Fig. 67; parsimony bootstrap support for each species = 94%–100%). Infraspecific DNA polymorphism was detected only within *F. tucumaniae* and *F. cuneirostrum*, with the

basal most strain of the latter species (NRRL 31949) being the most phylogenetically divergent (Fig. 67). Furthermore, it is noteworthy that strains of *F. tucumaniae* from Brazil and Argentina did not form subclades according to geographic origin. Because the soybean SDS pathogens appeared to have independent or polyphyletic evolutionary origins based on the combined phylogeny, a topological constraint was enforced on the combined data set, forcing the monophyly of the four soybean SDS pathogens. When these trees were compared in PAUP* (Swofford 2002) using the two-tailed Kishino–Hasegawa test, the probability of obtaining a more extreme *T* value under the null hypothesis of no difference between the constraint (314 steps) and the most parsimonious trees (268 steps, or 46 steps shorter than the SDS monophyly constraint) was significant at $P < 0.0001$.

Discussion

Four morphologically and phylogenetically distinct species within clade 2 of the *F. solani* species complex were detected among soybean SDS pathogens in South America, i.e., *F. brasiliense*, *F. cuneirostrum*, *F. tucumaniae*, and *F. virguliforme*. Among them, *F. brasiliense* and *F. cuneirostrum* were described as new species. *Fusarium tucumaniae* and *F. virguliforme* are reported as new records of the species from Brazil and Argentina, respectively, as soybean SDS pathogens, after their first descriptions from Argentina or the United States (Aoki et al. 2003). *Fusarium brasiliense* is characterized by septate cylindrical sporodochial conidia with rounded ends. In contrast, *F. cuneirostrum* produces sporodochial conidia with a rostrate and pointed apical cell and a distinct basal foot cell. *Fusarium virguliforme* is differentiated by the production of apically and basally symmetrical sporodochial conidia and comma-shaped sporodochial conidia of the second type. Because *F. tucumaniae* produces longer and narrower sporodochial conidia (on average, cultured on SNA, 3-septate: exceeding 50 µm long and mostly 4.5–5 µm wide; 4-septate: exceeding 60 µm long and mostly 4.5–5 µm wide) than those of the other three species, conidial morphology can be used to diagnose all four of these putatively South American endemics.

Aoki et al. (2003) investigated soybean SDS pathogens from Argentina and the United States, together with a pathogen responsible for bean root-rot disease in the United States. Three different taxa were recognized and described, i.e., *F. tucumaniae* from Argentina, and *F. virguliforme* and *F. phaseoli* from the United States, as two new species and a new combination. *Fusarium tucumaniae* and *F. virguliforme* are soybean SDS pathogens whereas *F. phaseoli* is a root-rot pathogen of dry bean. By comparison, *F. phaseoli* has relatively short sporodochial conidia that gradually widen upward with an acuate apical cell and a rounded but distinct basal foot cell. Apical and basal parts of the conidia in this species are often curved ventrally and asymmetrically. Together with *F. phaseoli*, described for a dry bean root-rot pathogen in the United States, all known species capable of inducing soybean SDS and dry bean root-rot can be diagnosed using conidial morphology. A comparison of the major diagnostic morphological features of these species is shown in Table 4, together with *F. martii*, which was included in the study because of its close morphological similarity to the soybean and dry bean pathogens. Although *F. tucumaniae*, *F. virguliforme*, and *F. brasiliense* are only known to be pathogenic to soybean (*G. max*) and *F. phaseoli* to dry bean (*P. vulgaris*), isolates of *F. cuneirostrum* are pathogenic to multiple fabaceous hosts (i.e., soybean, dry bean, and mung bean). Isolates of the latter species included three dry bean isolates from Japan and the United States, a single strain that caused soybean SDS in Brazil, and two mung bean isolates from Canada. Pathogenicity of two of the dry bean isolates of *F. cuneirostrum* (NRRL 22275 and NRRL 31157) was reported previously as *F. solani* f. *phaseoli* (Tanaka and Kitazawa 1956, 1958; Abumiya and Kitazawa 1963; Schneider et al. 2001). In the present study, *F. cuneirostrum* isolate NRRL 31157 (from dry bean), and NRRL 31949 (from soybean), and NRRL 36023 and NRRL 36024 (from mung bean), were all shown to induce a range of SDS symptoms on soybean cultivar Pioneer 9492RR (see the earlier text, Table 2, Figs. 54–56, 61–63).

Soybean SDS has been reported from every state in which soybeans are grown within the United States (Hershman et al. 1990; Jardine and Rupe 1993; Yang and Rizvi 1994; Hartman et al. 1995; Roy 1997b; Roy et al. 1997; Li et al. 1998; Rupe and Hartman 1999; Pennypacker 1999; Mulrooney et al. 2002; Kurle et al. 2003), Ontario, Canada (Anderson and Tenuta 1998), Argentina (Ivancovich et al. 1992; Botta et al. 1993; Ploper 1993; Scandiani et al. 2003; Aoki et al. 2003), Brazil (Nakajima et al. 1993, 1996; Wrather et al. 1997), Paraguay (Yorinori 1999), from north of Santa Cruz de la Sierra, Bolivia (Yorinori 2002), and Uruguay (Ploper et al. 2003). The causal pathogen of soybean SDS has been called and described as *F. solani* f. sp. *glycines*, based primarily on its pathogenicity to this host (Roy 1997a; Roy et al. 1997). As discussed in Aoki et al. (2003) and demonstrated in the present study, soybean SDS and dry bean root-rot pathogens (designated *F. solani* f. sp. *phaseoli*) comprised four and two species, respectively, all of which are closely related phylogenetically.

Results of the present study provide baseline data on the geographic distribution of each soybean SDS species: *F. virguliforme* (United States and Argentina); *F. brasiliense* (United States and Brazil); *F. cuneirostrum* (United States, Brazil, Canada, and Japan); *F. tucumaniae* (Brazil and Argentina); and *F. phaseoli* (United States) (see Table 4). In contrast to North America, two soybean SDS pathogens are present within Argentina (Scandiani et al. 2004a,b), and, as reported here, three are present within Brazil. Although all four species are present within North America, our preliminary survey suggests that *F. virguliforme* may be the only one responsible for soybean SDS within the United States (Aoki et al., unpublished data). Unfortunately, published PCR-based methods for the molecular detection of the etiological agent of soybean SDS within the United States, which were based on nuclear ribosomal DNA

(O'Donnell and Gray 1995), mitochondrial small subunit ribosomal DNA (Li et al. 2000), and the single-copy nuclear gene translation elongation factor 1-α (Li and Hartman 2003), all targeted loci that are too conserved to distinguish between any of the soybean SDS and dry and mung bean root-rot pathogens included in the present study. This result emphasizes the importance of critically investigating species limits before attempting to develop species-specific molecular diagnostics.

Although these species are closely related to *F. martii* morphologically, they are quite distinct from typical isolates of *F. solani* (Aoki et al. 2003). These species are nested within independent and distinct morphological and phylogenetic groups, i.e., clades 2 and 3 of the *F. solani* species complex (O'Donnell 2000). The phylogenetic position of *F. martii*, which has long been incorrectly synonymized with *F. solani* (Snyder and Hansen 1941; Booth 1971; Gerlach and Nirenberg 1982; Nelson et al. 1983), should be investigated further based on new isolates of this species.

In the present study, pathogenicity tests using soybean cultivar Pioneer 9492RR were performed on 17 strains selected from five species: *F. brasiliense*, *F. cuneirostrum*, *F. tucumaniae*, *F. virguliforme*, and *F. phaseoli* (see Table 2). Strains of each species differed significantly ($P < 0.05$) in disease incidence, stunting, root- and crown-rot, and plant mass. All strains of *F. brasiliense*, *F. cuneirostrum*, *F. tucumaniae*, and *F. virguliforme* and one strain of *F. phaseoli*, NRRL 31156, produced typical SDS foliar symptoms on soybean plants (see Table 2; Figs. 53–66). Except for *F. tucumaniae* NRRL 34549, all strains of *F. tucumaniae* and *F. virguliforme* tested consistently induced typical SDS foliar symptoms, root-rot, basal stem rot, and stunting. However, strains of *F. brasiliense* and *F. cuneirostrum* varied in pathogenicity based on the disease incidence. Isolates of these two species either were highly pathogenic (*F. brasiliense* NRRL 31756 and *F. cuneirostrum* NRRL 31949), intermediately pathogenic (*F. cuneirostrum* NRRL 31157 and NRRL 36024), or only weakly pathogenic (*F. brasiliense* NRRL 31762 and NRRL 31779).

In previous reports from the United States (Achenbach et al. 1996; Roy 1997a; Rupe et al. 2001; Cho et al. 2001), difference in pathogenicity among soybean SDS-causing isolates were reported under the name of *F. solani* f. sp. *glycines*. These isolates may all have been *F. virguliforme* based primarily because this species is the only soybean SDS pathogen we have detected within North America (Aoki et al., unpublished data). Achenbach et al. (1996) reported that the pathogenic strains NRRL 22823, NRRL 22825 (=*F. virguliforme*; Aoki et al. 2003), and FSA-1 did not cause leaf symptoms and another strain, Mo1792, only caused 38% leaf symptoms even when they were inoculated onto the highly susceptible cultivar Spencer. In separate studies, however, these strains have been shown to be pathogenic to soybean (Roy 1997a; Rupe et al. 2001). Gray and Achenbach (1996) reported that strains NRRL 22825 and AR269 caused significantly fewer leaf symptoms than other soybean SDS-causing isolates including NRRL 22292 (Mont-1 = *F. virguliforme*; Aoki et al. 2003). In addition, strains of *F. solani* f. sp. *glycines*, 12, 1B, Coomer-B, Kansas, and NRRL 22292 rated 50% or less disease incidence (Rupe et al. 2001). Loss of virulence and/or loss of toxin production may help explain the observed pathogenic variation among the SDS-causing isolates. These findings suggest that cultures used for pathogenicity tests ideally should have been recently isolated from diseased plant material and maintained on a low-nutrient culture medium (Nelson et al. 1983, 1986; Burgess et al. 1988; Nirenberg 1990; Windels 1992).

In the present study, isolates of the dry bean root-rot pathogen *F. phaseoli* also exhibited different levels of pathogenicity to soybean in the greenhouse experiments. Although *F. phaseoli* NRRL 22276 induced only mild SDS foliar symptoms on soybean plants, *F. phaseoli* NRRL 31156 caused interveinal chlorosis and necrosis on 32% of the soybean plants tested (see Table 2; Fig. 59), confirming previously reported results (Achenbach et al. 1996; Gray and Achenbach 1996; Rupe et al. 2001). Even though some *F. phaseoli* isolates have the ability to cause SDS symptoms on soybean in greenhouse conditions, this result does not appear to be indicative of their ability to induce SDS under natural field conditions (Roy et al. 1997; Rupe et al. 2001).

Although Melgar et al. (1994) reported that *F. solani* f. sp. *glycines* FSA could only infect wounded soybean plants, Gray et al. (1999) demonstrated that typical dry bean root-rot (extensive lateral root and taproot necrosis) and soybean SDS symptoms were induced by strains of *F. solani* f. sp. *glycines* (=*F. virguliforme*; Aoki et al. 2003), using a soil infestation method with green bean cultivar Top Crop and soybean cultivar Spencer. Based on the yellowing, browning, and dessication of the green bean leaves, Gray et al. (1999) concluded that *F. solani* f. sp. *glycines* has a broader host range than just soybean. The results of our pathogenicity studies parallel those of Gray et al. (1999) in that we have shown that two dry bean root-rot pathogens, *F. cuneirostrum* and *F. phaseoli*, can also induce typical SDS symptoms on soybean in a greenhouse experiment.

One of the major results of the present study was the discovery that isolates of four of the five species were able to induce high levels of soybean SDS in greenhouse experiments and in the field. Moreover, SDS monophyly constraints were 46 steps longer and significantly less parsimonious than the most parsimonious trees, supporting the interpretation that pathogenicity to soybean and green or dry bean may have evolved convergently within this predominately South American clade of the *Fusarium solani* species complex (O'Donnell 2000). Because soybeans have been cultivated extensively within South America only during the past 50 years, it seems likely that the soybean SDS pathogens represent four very recent and independent host expansions from unknown hosts within South America. Given the putative polyphyly of the soybean SDS and green or dry bean root-rot pathogens (formerly known as *F. solani* f. sp. *glycines* and *F. solani* f. sp. *phaseoli*, respectively), the results presented here add to a growing list of studies that challenge the predictive value of the forma specialis naming system (O'Donnell et al. 1998; O'Donnell 2000; Baayen et al. 2000).

Lastly, because the single strain of *F. cuneirostrum* NRRL 31949 from a diseased soybean possesses some morphological and phylogenetic differences from other strains of the species isolated from dry bean and mung bean, and because pathogenicity of these species of *Fusarium* on different hosts could have great agricultural importance, further intensive phylogenetic analyses based on GCPSR (Taylor et al. 2000) are in progress on these pathogenic species, together with detailed pathogenicity experiments using different host plants including soybean, dry bean, and mung bean.

Acknowledgments Thanks are due to Dr. José Tadashi Yorinori, Embrapa Soja, Londrina, Brazil for supplying Brazilian soybean SDS isolates for inclusion in the present study; Dr. Takashi Nakajima, Kyushu-Okinawa Agricultural Research Center for providing unpublished information on soybean SDS in Brazil; Dr. Walter Gams, CBS, for preparing the Latin diagnoses; Ms. Erin B. McCray, Herbarium Manager, U.S. National Fungus Collections (BPI) for the loan of the *F. martii* specimens; and Dr. Terry Anderson, Agriculture and Agri-food Canada, for providing isolates of *F. cuneirostrum* from mung bean.

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