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"Colonization of *Heterodera glycines* cysts by *Fusarium solani* form A, the cause of sudden death syndrome, and other fusaria in soybean fields in the midwestern and southern United States"

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Colonization of *Heterodera glycines* cysts by *Fusarium solani* form A, the cause of sudden death syndrome, and other fusaria in soybean fields in the midwestern and southern United States

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Fusarium solani form A and other fusaria were isolated from surface-disinfested cysts of *Heterodera glycines* collected from the rhizosphere of soybean (*Glycine max*) plants symptomatic for sudden death syndrome, collected from fields in the midwestern and southern United States. Two forms of *Fusarium solani* pathogenic to soybean were isolated : form A, the cause of sudden death syndrome, and form B, the cause of seedling disease and root rot. *Fusarium solani* form B was more frequently isolated than form A. *Fusarium solani* form A occurred in cysts in 82% of the cyst-infested fields containing plants symptomatic of sudden death syndrome, with maximum and average isolation frequencies (across fields and years) of 28 and 7%, respectively. This fungus was not found in cysts from five fields lacking sudden death syndrome. Isolates of *F. solani* form B were found in high frequency in cysts from each of those fields; *F. solani* form B occurred in cysts in 94% of the cyst-infested fields containing sudden death syndrome, with maximum and average isolation frequencies (across years and fields) of 48 and 16%, respectively. *Fusarium oxysporum* (the species with the third highest average isolation frequency), *F. chlamydosporum*, *F. equiseti*, *F. moniliforme*, and *F. pallidoroseum* (syn. *F. semitectum*) were also isolated. Based on cyst population densities occurring in soil and percentages of cysts colonized by *F. solani* form A, the maximum and average numbers of colonized cysts per 500 cm^3 of rhizosphere soil were 149 and 22, respectively. *Fusarium solani* form A survived at a high rate in cysts in soil stored for 8 months at ca 10°C, indicating an ability to overwinter in cysts between soybean growing seasons. *Fusarium solani* form A had a poor survival rate in cysts in soil stored for 20 months. Compared to *F. solani* form A, maximum and average numbers of *F. solani* form B in cysts per 500 cm^3 soil were higher, at 700 and 82, respectively. Furthermore, the survival rate of cysts in soil stored for 8 and 20 months was also higher. Isolates of *F. solani* form A from cysts were as virulent on soybean as isolates from diseased roots.

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[Colonisation des kystes de l'*Heterodera glycines* par la forme A du *Fusarium solani*, l'agent responsable du syndrome de la mort subite, et d'autres fusariums dans des champs de soja du centre-ouest et du sud des États-Unis]

La forme A du *Fusarium solani* et d'autres fusariums ont été isolés de kystes de l'*Heterodera glycines* désinfectés en surface et récoltés dans la rhizosphère de plants de soja (*Glycine max*) présentant des symptômes du syndrome de la mort subite et récoltés dans des champs du centre-ouest et du sud des États-Unis. Deux formes du *Fusarium solani*, pathogènes du soja, ont été isolées : la forme A, responsable du syndrome de la mort subite, et la forme B, responsable d'une maladie des semis et d'un pourridié fusarien. La forme B du *Fusarium solani* a été isolée plus souvent que la forme A. La forme A du *Fusarium solani* était présente dans les kystes de 82 % des champs infestés de kystes et avec des plantes présentant des symptômes du syndrome de la mort subite, avec des fréquences maximale et moyenne (champs et années confondus) de respectivement 28 et 7 %. Ce champignon n'a pas été retrouvé dans les kystes de cinq champs exempts du syndrome de la mort subite. Des isolats de la forme B du *F. solani* ont été très fréquemment retrouvés dans les kystes de chacun de ces champs ; la forme B du *F. solani* était présente dans les kystes de 94 % des champs envahis de kystes et avec le syndrome de la mort subite, avec des fréquences maximale et moyenne (champs et années confondus) de respectivement 48 et 16 %. Le *Fusarium oxysporum* (l'espèce ayant la troisième fréquence moyenne d'isolement), le *F. chlamydosporum*, le *F. equiseti*, le *F. moniliforme*, et le *F. pallidoroseum* (syn. *F. semitectum*) ont aussi été isolés. En se basant sur les densités des populations de kystes dans le sol et les pourcentages de kystes colonisés par la forme A du *F. solani*, le maximum et la moyenne de kystes colonisés par 500 cm^3 de sol de la rhizosphère étaient respectivement de 149 et 22. Une grande proportion de la forme A du *Fusarium solani* a survécu dans des kystes dans du sol entreposé durant 8 mois à approximativement 10°C, ce qui montre la possibilité d'une hibernation dans des kystes entre les saisons de croissance du soja. Le taux de survie de la forme A du *Fusarium solani* était faible dans des kystes dans du sol entreposé durant 20 mois. Le maximum et la moyenne de kystes par 500 cm^3 de sol étaient plus élevés pour la forme B du *F. solani*, avec respectivement 700 et 82, que pour la forme A du *F. solani*. De plus, les taux de survie des kystes dans du sol entreposé durant 8 et 20 mois étaient aussi plus élevés. Les isolats de la forme A du *F. solani* provenant de kystes étaient aussi virulents sur le soja que des isolats provenant de racines malades.

INTRODUCTION

Numerous fungi are known to colonize cysts of the soybean cyst nematode (SCN) (*Heterodera glycines* Ichinohe) in soybean (*Glycine max* (L.) Merr.) fields (Carris and Glawe 1989; Carris *et al.* 1989a; Gintis and Morgan-Jones 1983; Gintis *et al.* 1982; Morgan-Jones and Rodriguez- Kabana 1981; Morgan-Jones *et al.* 1981; Roy and Van Etten 1993; Roy *et al.* 1993; Stiles and Glawe 1989). Some of these fungi are saprophytic and others are pathogenic, including some which are pathogenic on soybean roots (Sinclair and Backman 1989) or parasitize SCN eggs (Gintis and Morgan-Jones 1983; Gintis *et al.* 1982). Among the soybean pathogens, *Fusarium solani* (Mart.) Sacc. has been a common isolate from cysts (Carris and Glawe 1989; Carris *et al.* 1989b; Gintis and Morgan-Jones 1983; Gintis *et al.* 1982; Morgan-Jones and Rodriguez-Kabana 1981; Morgan-Jones *et al.* 1981; Roy and Van Etten 1993; Roy *et al.* 1993; Stiles and Glawe 1989). In most reports of *F. solani* isolation (Carris and Glawe 1989; Carris *et al.* 1989a; Gintis *et al.* 1982; Melgar *et al.* 1994; Morgan-Jones and Rodriguez-Kabana 1981; Morgan-Jones *et al.* 1981; Stiles and Glawe 1989), no mention has been made of morphological distinctions among isolates. However, at least two morphologically and pathologically distinct forms of *F. solani* have been described from soybean : one, distinguished as form A (FSA), is the causal agent of sudden death syndrome (SDS) (Melgar and Roy 1994; Melgar *et al.* 1994; Roy *et al.* 1989a, 1989b, 1989c, 1991; Rupe 1989; Rupe *et al.* 1993); the other, distinguished as form B (FSB), is the causal agent of seedling disease and root rot of older soybean plants (Killebrew *et al.* 1988,1993; Roy *et al.* 1989a, 1989b, 1989c). Even though the FSA form is sometimes referred to as the «blue strain» of *F. solani* (Rupe 1989), the bluish color is absent or is limited to small sporodocial spots within the mycelium of FSB isolates (Rupe *et al.* 1996; Roy and Rupe, personal communication).

Sudden death syndrome, a relatively new root and lower stem disease of soybean, occurs in Alabama, Arkansas, Georgia, Illinois, Indiana, Iowa, Kansas, Kentucky, Mississippi, Missouri, and Tennessee (Hirrel 1987; Melgar and Roy 1994; Melgar *et al.* 1994; O'Donnell and Gray 1995; Roy and Ratnayake 1995; Roy and Van Etten 1993; Roy *et al.* 1989a, 1989b, 1989c, 1991, 1993, Rupe 1989; Rupe *et al.* 1993; Baird, unpublished) in the United States, and in Brazil and Argentina (J. F. Hennon, personal communication). The pathogen causing SDS is especially prevalent in irrigated fields or fields with persistently high soil moisture (Hirrel 1987; Melgar *et al.* 1994; Roy *et al.* 1989a, 1989b, 1991; Rupe 1989). The major symptoms of SDS include root rot, crown necrosis, vascular discoloration of roots and stems, interveinal chlorosis and necrosis of leaves, defoliation, and pod abortion. Leaf symptoms are the most conspicuous phase of this disease (Hirrel 1987; Melgar *et al.* 1994; Roy *et al.* 1989a, 1989b, 1991; Rupe 1989).

As early as 1985, anecdotal evidence indicated that soybean fields containing SDS-symptomatic plants were almost invariably infested with the nematode (Hirrel 1987). This invited speculation that cysts might be involved in some way in disease development and perhaps even in dissemination of the SDS causal agent. Speculation regarding the possible association of SCN with spread of SDS was heightened with the finding in 1986 that FSA-colonized cysts internally (Roy, unpublished, SDS Workshop, American Soybean Association, St. Louis, 1987). It was later found that SDS severity was increased in the presence of SCN (Melgar *et al.* 1994; Roy *et al.* 1989a, 1989b) and that the population density of SCN juveniles was positively correlated with SDS severity (Rupe *et al.* 1993). Evidence reported in 1993 linked SCN with new outbreaks of SDS in Midwest fields closely proximate to others having a history of the disease (Roy *et al.* 1993). Since FSA has not been found in soybean seeds (Melgar *et al.*1994; Roy *et al.* 1989c), which is one possible means of FSA dispersal, the 1994 evidence (Melgar *et al.* 1994), combined with other compatible evidence (Hirrel 1987; Melgar *et al.* 1994; Roy and Van Etten 1993; Roy *et al.* 1989a,

1989b, 1993; Rupe *et al.* 1993; Roy, unpublished), points to SCN as a more probable agent of dispersal.

Artificial infestation of sterilized greenhouse potting soil with a combination of FSA and SCN produced FSA-colonized cysts, and the fungus was found capable of surviving in these cysts when soil was stored for 6 mo at reduced temperatures (Lawrence *et al.* 1988). However, frequency of occurrence and survival of FSA in cysts in field soils containing native microflora, in which competition between FSA and other fungi would be likely, would be more representative of the ability of FSA to colonize cysts and survive in them under natural conditions.

The ability of FSA and other pathogens to survive in cysts is relevant to their primary inoculum potential in fields in the spring. In this regard, in addition to FSA and FSB, *Fusarium oxysporum* Schl. causes a soybean wilt and seedling disease (Sinclair and Backman 1989) and a parasite of SCN eggs (Gintis and Morgan-Jones 1983; Gintis *et al.* 1982). Both FSA, FSB, and *F. oxysporum* must be capable of surviving in cysts, soybean root debris, or soil from the end to the beginning of the next growing season if they are to be successful parasites in the spring and beyond. From a practical standpoint, these fungi also must occur in cysts at reasonably high frequencies and be widely distributed geographically with soybeans if they are to play a substantial role in parasitism.

Stiles and Glawe (1989) demonstrated that some plant pathogenic fungi borne within SCN cysts, including *F. solani*, are capable of colonizing roots when inoculated on soybean seedlings. Our paper emphasizes and reports findings on the frequency of occurrence, geographic distribution, and survival of FSA in cysts in field soil, as related to the occurrence of SDS. The ability of FSA isolates from cysts to incite the symptoms of SDS is demonstrated. Frequency of occurrence, geographic distribution, and survival of FSB in cysts, and frequency of occurrence and geographic distribution of *F. oxysporum* in cysts, are also reported.

MATERIALS AND METHODS

Survey of soybean fields for occurrence and geographic distribution of FSA, FSB, *F. oxysporum*, and other fusaria in cysts

From 1988 to 1995 during August and September, soil samples were collected from 56 soybean fields (Table 1), the fields were located in Arkansas, Illinois, Indiana, Kentucky, Mississippi, Missouri, and Tennessee. Growth stage of soybean plants in the fields sampled ranged from R3 to R7, but most were in the R5 to R6 stage (Fehr *et al.* 1971). To

Table 1. Location, number, and characteristics of soybean fields from which soil was collected and *Heterodera glycines* cysts were extracted for fungal assays, 1988-1995

State	No. fields	No. fields	
		Sudden death syndrome present	Cysts present
Arkansas	8	7	8
Illinois	17	16	15
Indiana	7	7	7
Kentucky	12	11	12
Mississippi	8	6	6
Missouri	2	2	2
Tennessee	2	2	2
Total :	56	51	52

obtain cysts, soil from the rhizosphere of 15 randomly selected SDS-symptomatic plants was sampled to a depth of 12-15 cm with a 2-cm-diam soil probe, which was inserted into the soil at an angle and directed towards the root system. If fields did not show any obvious disease symptoms, rhizosphere soil was sampled from 15 randomly selected, asymptomatic plants. From each location, the 15 subsamples were composited and stored at 10°C for 4-6 wk prior to assay. Cysts were extracted from 250 cm^3 of the composite soil sample by wet-sieving and numbers were determined as described previously (Melgar *et al.* 1994; Southey 1970). From each soil sample, 25 cysts were surface-disinfested in 95% ethanol for 2 sec and in 0.5% NaOCl for 1 min, rinsed twice in sterile water, and cultured on potato-dextrose agar (PDA) amended with streptomycin B sulphate (100 mg L^{-1}) and aureomycin (20 mg L^{-1}) (PDASA). Five cysts were placed on the agar in each of five 9-cm-diam plastic Petri dishes. Cultures were incubated at 25°C for 1 wk, during which fungi growing from cysts were identified.

Because the identity of FSB as a form of *F. solani* was recently questioned (O'Donnell and Gray 1995), morphological and colony characteristics of *F. solani* isolates from the American Type Culture Collection (ATTC) were compared with those of FSB isolates. Culture no. 46472 (ATCC) was originally isolated from a cyst of *H. glycines* by G. Morgan-Jones, and culture no. 11712 (ATCC) from squash (*Cucurbita pepo* L.) by W.C. Snyder. For comparison, the ATCC and FSB isolates were grown for 1-2 wk on PDA and modified Bilay's medium at 25°C for observation of colony and conidial characteristics, respectively. Colonies on Bilay's medium were exposed to continuous fluorescent light (163 W m^{-2}).

General references were used for identification of other *Fusarium* species (Booth 1971; Carris and Glawe 1989; Nelson *et al.* 1983) grown on PDASA and modified Bilay's medium.

Survival of FSA and FSB in cysts

Three soil samples that were previously assayed during the survey for incidence of FSA and FSB in cysts were used to determine the survival of these fungi in cysts. The samples were from fields in Gallatin County, Illinois; Union County, Kentucky; and Coahoma County, Mississippi. The percents of cysts colonized by FSA in the respective samples were 28, 24, and 20%, and those of FSB were 48, 40, and 36%. The soil samples were stored at ca. 10°C for 8 and 20 mo. Eight mo was chosen based on the length of time cysts would be expected to overwinter from harvest to planting of soybean maturity groups 3 to 7. Twenty mo represented the time elapsed from a soybean harvest through an intervening corn or sorghum rotation, which is a common practice among growers. A subsample from each of the three soil samples was drawn at 8 and 20 mo, and 50 cysts were assayed per subsample to determine incidence of the two fungi. Extraction and assay methods described previously were used to determine survival rates of the two fungi, where survival rate was calculated as percentage reisolation frequency divided by percentage original isolation frequency.

Pathogenicity and virulence on soybean seedlings of FSA isolates from cysts and roots

Isolates of FSA were recovered from surface-disinfested cysts (Table 2) and roots of SDS-symptomatic plants collected in Arkansas, Kentucky, Illinois, Indiana, and Mississippi from 1989 to 1994. Only young cultures of FSA which had not been serially transferred were used for inoculation. Two methods of inoculation, including injection of spores into hypocotyls and infestation of soil, were used in pots containing cv. Lee 74, which is susceptible to SDS (Killebrew *et al.* 1993; Melgar *et al.* 1994). To increase macroconidia, isolates were grown on modified Bilay's medium (Booth 1971) under continuous fluorescent light (150 W m^{-2}) for 1 wk. Cultures were flooded with sterile water containing one drop of Tween-20 100^{-1} mL, and spores were dislodged with a bent glass rod. Spore concentration was adjusted to 1 x 10^6 conidia ml^{-1} with a hemacytometer. With a hypodermic syringe, ca. 0.05 to 0.10 mL spore suspension

Table 2. Maximum and average frequencies of isolation, over years (1988-1995) and fields, of *Fusarium solani* form A, *F. solani* form B and *F. oxysporum* from surface-disinfested *Heterodera glycine* cysts obtained from the rhizosphere of plants symptomatic for sudden death syndrome

Fungus	No. cyst-infested fields, of 47 sampled, with cysts colonized by fungus	Incidence (%) of fungus in surface-disinfested cyst		Estimated no. cysts colonized by fungus per 500 cm^3 soil[a]	
		Maximum	Mean	Maximum	Mean
Fusarium solani					
Form A	39	28	7	149	22
Form B	44	48	16	700	82
F. oxysporum	21	16	2	17	8

[a] Percent of cysts colonized X number of cysts/500 cm^3 soil. Cyst populations : range 12 - 1458, average 236.

were inoculated into hypocotyls of each of five 10-d-old seedlings growing in nonsterile soil in 10-cm-diam plastic pots. There were four replications for each of nine isolates evaluated, and four replications of control seedlings, which were injected with water containing Tween-20. The experiment was arranged in a completely random design in the greenhouse. For soil infestation, FSA inoculum was increased on a cornmeal-sand medium, and this was added to sterilized soil in 10-cm-diam plastic pots to provide an inoculum concentration of 0.05% w:w (Melgar *et al.* 1994; Roy *et al.* 1989a). The soil was a sandy loam greenhouse potting mix. Ten seeds were planted per pot, and an equal number of seeds planted in noninfested soil served as controls. After 1 wk, seedling numbers were reduced to five per pot. The experiment contained four replications and was arranged in a completely random design.

Disease severity was determined 2 wk after inoculation, in the spore injection test, and 6 wk after inoculation, in the soil infestation test. Disease severity was rated on a 0 - 6 scale, where 0 = no symptoms, 1 = mild leaf mottling, 2 = extensive leaf mottling, 3 = leaf interveinal chlorosis, 4 = leaf interveinal necrosis, 5 = defoliation, and 6 = premature death. Temperature in the greenhouse ranged from 19-34°C. Data for the different isolates were compared by analysis of variance and means were separated with the LSD test.

RESULTS

In the current study, colonies of *F. solani* form A and *F. solani* form B were easily distinguishable as they grew from cysts onto PDA. FSA isolate A-5 and FSB isolate B-19 are shown growing from cysts in Figure 1. Typically, no other fungi grew from cysts that were colonized by either of these two fungi or by *F. oxysporum*. Colonies and conidial characteristics of FSB isolates and *F. solani* ATCC no. 46472 and ATCC no. 11712 were similar and typical for *F. solani* (Booth 1971; Carris and Glawe 1989; Domsch *et al.* 1980; Nelson *et al.* 1983).

Isolates of FSA and FSB which superficially appear to be similar in culture can now be separated on selective media. On PDASA (media described in Materials and Methods), FSA produced a grayish white aerial mycelium which was more appressed than that of FSB (Roy, unpublished). A bluish color in the central part of the colony was most typical of wild-type colonies of FSA. With aging, colonies of some isolates became almost entirely blue. In contrast, FSB produced a floccose, grayish white aerial mycelium and a cream to tan color was imparted to the medium (Roy, unpublished data). Isolates of FSA had a much slower growth rate than FSB. On modified Bilay's medium (Booth 1971), macroconidia of FSA were longer than those of FSB. Slimy mass-

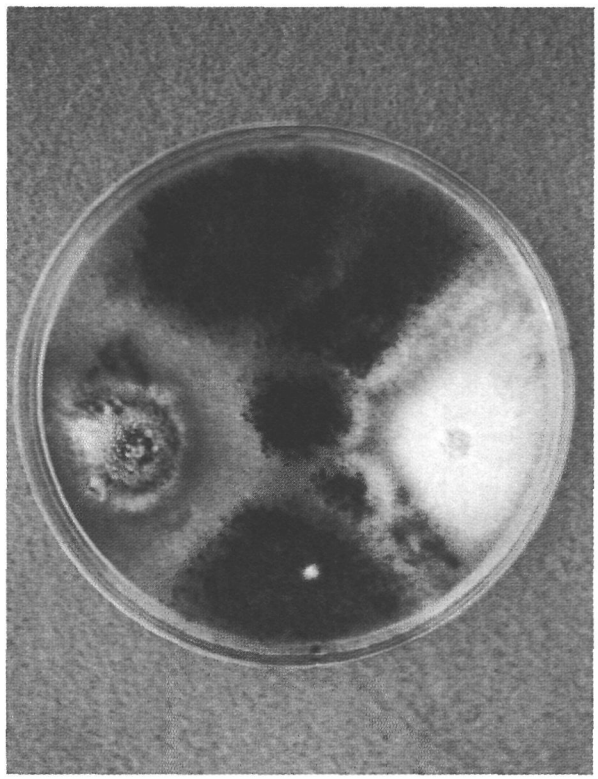

Figure 1. Colonies of blue-pigmented *Fusarium solani* form A isolate A-5 (left) and *F. solani* form B isolate B-19 (right) each growing from separate surface-disinfested *H. glycines* cysts cultured on potato-dextrose agar. Fast-growing dark colonies are *Chaetomium cochliodes*, which grew from two other cysts. Colonies are 5 days old.

es of macroconidia of FSA, ranging in color from cream to blue to blue-green to bluish purple, in the central part of the colony. Macroconidia of FSB typically were cream-colored in mass, but small green to blue-green sporodochia were produced with aging in some isolates. Microconidia were absent or extremely rare in FSA but typically were produced in profusion by FSB. These characteristics agreed with previous descriptions of the two *F. solani* forms (Roy *et al.* 1989a, 1989b, 1989c; Rupe 1989).

Forty-seven of 51 fields with SDS were infested with SCN. Isolates of FSA were identified from cysts in 39 of these fields (Tables 1, 2). The maximum frequency of FSA isolation was 28%; average frequency of isolation was 7% (Table 2). The fungus most frequently isolated from cysts was FSB. This fungus occurred in cysts in 44 fields and had maximum and average frequencies of isolation of 48 and 16%, respectively. *Fusarium oxysporum* occurred in cysts in 21 fields and had maximum and average frequencies of 16 and 2%, respectively (Table 2). Other fusaria isolated from cysts were *F. chlamydosporum* Wollenweb. and Reinking, *F. equiseti* (Corda) Sacc., *F. moniliforme* J. Sheld., and *F. pallidoroseum* (Cooke) Sacc. (syn. *F. semitectum* auct. non Berk. & Ravenel.) (Farr *et al.* 1989), all of which had average frequencies (across fields and years) of less than 1%, and maximum frequencies of 4, 8, 4, and 4%, respectively.

None of the cysts from five 1995 fields lacking SDS contained FSA. In contrast, FSB occurred in cysts in each of these fields, with maximum and average frequencies of 44 and 29%, respec-

tively. In a separate study in Mississippi, FSB was isolated from cysts from two other non-SDS fields, but FSA could not be isolated (Roy, unpublished).

The survival rate for FSA in cysts was 63% after 8 mo storage in soil at ca. 10°C, and 3% after 20 mo storage. Isolates of FSB had a higher survival rate at 76% 8 mo after storage, and at 29% after 20 mo.

Each of the FSA isolates from cysts and roots was pathogenic on soybean seedlings when macroconidial suspensions were injected into seedling hypocotyls (Table 3). Generally, the isolates from cysts were as virulent as the isolates from diseased roots. There were some differences in virulence among the isolates with disease ratings ranging from 2.2 to 5.8 (mean = 4.4). Among the cyst isolates, isolate A-9 was significantly ($P \leq 0.05$) less virulent than isolates A-1, A-3, and A-4. The soil infestation inoculation technique did not show similar pathogenicity levels for all isolates including the cyst isolates. Isolates A-1, A-3, and A-9 were pathogenic, but disease ratings were lower and ranged from 0.2 to 1.5 (mean = 0.6). This disparity between the two testing techniques may have been due to high greenhouse temperature which exceeded 33°C on numerous occasions during the incubation period. At temperatures exceeding 33°C, infectivity and growth *in vitro* of FSA was drastically reduced (Roy and Van Etten 1993; Roy, unpublished data).

DISCUSSION

If SCN cysts represent a source of dispersal for FSA in the SDS disease cycle, there must be an association between FSA occurrence in cysts and the occurrence of SDS. Furthermore, FSA must occur in cysts in relatively high frequency and with a geographically widespread distribution, and the fungus must be capable of surviving in cysts in the field from one growing season to the next. Cumulative evidence from previous observations (Roy *et al.* 1993) and this investigation show that FSA can survive in cysts for at least 8 mo. Also, virulence to soybean of FSA isolates from cysts, establishes that cysts are sources of FSA. The maximum levels

Table 3. Disease reaction of cultivar Lee 74 soybean seedlings to inoculation with isolates of *F. solani* form A from *Heterodera glycines* cysts or soybean roots[a]

F. solani form A isolate	Origin		Disease severity rating[b]	
	Substrate	Geographic	Inoculation of hypocotyls by injection with macroconidia suspension[c]	Inoculation by infestation of soil[d]
A-1	Cyst	Arkansas	5.8	1.5
A-2	Root	Indiana	5.2	0.8
A-3	Cyst	Mississippi	5.2	0.8
A-4	Cyst	Illinois	4.8	0.2
A-5	Cyst	Mississippi	4.0	0.0
A-6	Root	Indiana	3.5	0.5
A-7	Root	Kentucky	3.5	0.0
A-8	Root	Kentucky	3.2	0.5
A-9	Cyst	Illinois	2.2	0.8
Control			0.0	0.0
		$LSD_{0.05:}$	1.9	0.8

[a] Cutivar Lee 74 is susceptible to sudden death syndrome (Hirrel 1987; Melgar and Roy 1994).
[b] 0 = no symptoms, 1 = mild leaf mottling, 2 = extensive leaf mottling, 3 = leaf interveinal chlorosis, 4 = leaf interveinal necrosis, 5 = defoliation, and 6 = premature death.
[c] Injection of ca. 0.05-0.10 ml $1x10^6$ of macroconidia in sterile water containing Tween-20 (one drop 100^{-1} ml).
[d] Inoculum of *F. solani* form A, grown on corn-meal sand, was added to replicate pots containing sandy loam greenhouse potting mix at an inoculum concentration of 0.05% w:w.

of cysts colonized by FSA (Table 2) appear to represent substantial amounts of inoculum, especially considering cyst population densities which can occur in the field. Populations exceeding 1000 to 2000 cysts per 500 cm^3 soil are not uncommon (Riggs and Schmitt 1987; Schmitt and Riggs 1989), and, in the present study, some fields had 900 to 1400 cysts per 500 cm^3. Also, because each cyst is capable of producing 200-600 eggs in the field (Anonymous 1984; Riggs and Schmitt 1987), and FSA has been observed to colonize eggs (McLean *et al.* 1990), the number of FSA propagules associated with cysts is probably even higher. Thus, in soybean fields affected by SDS and SCN, the potential exists for high levels of FSA-colonized cysts, and perhaps eggs, and therefore substantial levels of FSA inoculum associated with cysts.

Typically, FSA was found growing alone from individual cysts, indicating little competition with other microorganisms for nutrients and space within the cyst. In such an environment, the probability of FSA survival in overwintering cysts until spring would seem to be much greater than its survival free in soil or in soybean debris, where competition among microorganisms would exist (Domsch *et al.* 1980). FSA, which has been shown to exist, at least in part, as chlamydospores in cysts (Melgar *et al.* 1994), likely would be afforded some protection by the cyst, protection similar to that received by SCN eggs (Wallace 1964), and it would thereby escape much of the competition from other soilborne microorganisms. Moreover, nutrients within cysts might provide a food base for germination of chlamydospores in the spring.

The involvement of cysts in local or distant dispersal of FSA seems quite possible, given the generally acknowledged role of seeds in dispersal of fungi, coupled with the fact that, compared to seeds, there are many more ways that cysts can be disseminated (Anonymous, 1984; Riggs and Schmitt 1987; Wallace 1964). Cysts may be spread locally or longer distances by almost any agent capable of moving soil, including soil peds in seed lots shipped by man, farm machinery, dust storms, surface water, and animals. SCN has spread from a point of origin to more than 20 states in the United States, and from Asia to numerous soybean-producing countries, including the United States (Riggs and Schmitt 1987). By virtue of its ability to colonize cysts, FSA could be disseminated concomitantly with the dissemination of SCN.

In contrast to what was reported by O'Donnell and Gray (1995), the isolates we recorded as FSB were indeed *F. solani*. A preliminary report (Roy and Ratnayake 1995) presenting likely reasons for the claim that FSB was not *F. solani* (O'Donnell and Gray 1995) has been filed. FSB was the most frequently isolated species of *Fusarium* from cysts, and it survived for 8 and 20 mo at a relatively high rate. The involvement of SCN cysts as sources and agents of dispersal for this fungus is possible. However, further studies will be required to provide conclusive evidence. With regards to *F. oxysporum* and other fusaria found colonizing cysts, their ability to survive in cysts will need to be determined before any epidemiological role can be ascribed to *H. glycines* cysts.

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