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Pathogenicity of Fungi to Eggs of *Heterodera glycines*[1]

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Abstract: Twenty-one isolates of 18 fungal species were tested on water agar for their pathogenicity to eggs of *Heterodera glycines*. An egg-parasitic index (EPI) for each of these fungi was recorded on a scale from 0 to 10, and hatch of nematode eggs was determined after exposure to the fungi on water agar for 3 weeks at 24 C. The EPI for *Verticillium chlamydosporium* was 7.6, and the fungus reduced hatch 74%. *Pyrenochaeta terrestris* and two sterile fungi also showed a high EPI and reduced hatch 42–73%. *Arthrobotrys dactyloides, Fusarium oxysporum, Paecilomyces lilacinus, Stagonospora heteroderae, Neocosmospora vasinfecta, Fusarium solani,* and *Exophiala pisciphila* were moderately pathogenic to eggs (EPI was 2.0–4.5, and hatch was reduced 21–56%). *Beauveria bassiana, Hirsutella rhossiliensis, Hirsutella thompsonii, Dictyochaeta heteroderae, Dictyochaeta coffeae, Gliocladium catenulatum,* and *Cladosporium* sp. showed little parasitism of nematode eggs but reduced hatch. A negative correlation was observed between hatch and fungal parasitism of eggs. *Fusarium oxysporum, H. rhossiliensis, P. lilacinus, S. heteroderae, V. chlamydosporium,* and sterile fungus 1 also were tested in soil in a greenhouse test. After 3 months, the nematode densities were lower in soil treated with *H. rhossiliensis* and *V. chlamydosporium* than in untreated soil. The nematode population densities were correlated negatively with the EPI, but not with the percentage of cysts colonized by the fungi. Plant weights and heights generally increased in the soil treated with the fungi.

Key words: biological control, cyst, egg parasite, egg-parasitic index, female nematode, fungus, hatch, *Heterodera glycines*, nematode, parasitism, pathogenicity, soybean cyst nematode.

At least 168 species of fungi have been isolated from cysts of the soybean cyst nematode (SCN) *Heterodera glycines* Ichinohe (3), but only a few of them have been tested for their parasitism or pathogenicity to the nematode (12,22,25). Godoy et al. (12) studied parasitism of 14 fungal species on eggs of *H. glycines* and *Meloidogyne arenaria* (Neal) Chitwood. They showed that *Verticillium lamellicola* (F. E. V. Smith) Gams, *V. leptobactrum* Gams, *Phoma macrostoma* Mont., and *P. multirostrata* (Mathur, Menon & Thirum) Dorenb. & Boerema parasitized high percentages of eggs of both nematodes. *Chaetomium indicum* Corda, *Fusarium solani* (Mart.) Sacc., *Fusarium oxysporum* Schlecht., *Neocosmospora vasinfecta* E. F. Smith, and *Thielavia terricola* (Gilman & Abbott) Emmons did not parasitize eggs of either nematode. *Dictyochaeta heteroderae* (Morgan-Jones) Carris & Glawe (synonym = *Codinaea heteroderae* Morgan-Jones) and *Stagonospora heteroderae* Morgan-Jones showed a low degree of parasitism on both nematodes. Meyer et al. (25) assayed 22 fungi in vitro for their antagonism to eggs of *H. glycines*. Only *Phoma chrysamthemicola* Hollos and one strain each of *Verticillium chlamydosporium* Goddard (synonym = *Diheterospora chlamydosporia* (Goddard) Barron & Onions) and *Verticillium lecanii* (A. Zimmermann) Viégas were shown to have antagonistic effects on eggs of *H. glycines*. Two strains of an unidentified fungus, designated as ARF18, were tested for their infectivity of SCN eggs (22). Isolate ARF18-A infected 89% of eggs in yellow females and 61% of eggs in brown cysts, whereas isolate ARF18-B infected 51% of eggs in yellow females and 57% of eggs in brown cysts.

More than 40 species of fungi have been isolated from females, cysts, and eggs of SCN collected from a soybean (*Glycine max* (L.) Merr.) field at the University of Florida, Green Acres Agronomy Farm, Gainesville (4), and from soil in pot tests in a greenhouse (5). The objective of this in-

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vestigation was to evaluate the pathogenicity to SCN eggs of selected fungi that we found associated frequently with females, cysts, and eggs of *H. glycines*.

Materials and Methods

Pathogenicity to eggs on water agar: Twenty-one isolates of 18 species were tested by exposing cysts to fungi growing on water agar. The fungi *Arthrobotrys dactyloides* Drechsler, *Dictyochaeta coffeae* (Maggi & Persiani) Cabello & Arambarri, *D. heteroderae, Exophiala pisciphila* McGinnis & Ajello, *F. oxysporum, Fusarium solani, Gliocladium catenulatum* Gilm. & Abbott, *N. vasinfecta, Paecilomyces lilacinus* (Thom.) Samson, *Pyrenochaeta terrestris* (Hansen) Gorenz, Walker & Larson, *S. heteroderae,* sterile fungus 1 (black, yeast-like fungus), and sterile fungus 2 were isolated from females, cysts, and eggs of *H. glycines* (3–5). *Paecilomyces lilacinus* and *V. chlamydosporium* were isolated from *Meloidogyne* spp. egg masses on tobacco (*Nicotiana tobacum* L.) roots collected from the agronomy farm where the soybean field was located. *Cladosporium* sp. was isolated from aphids on soybean plants in the greenhouse. In addition, the following isolates were evaluated: *P. lilacinus* obtained from Dr. P. Jatala at the International Potato Research Center, Lima, Peru; *Hirsutella rhossiliensis* Minter & Brady obtained from Dr. B. Jaffee at the University of California, Davis; *Hirsutella thompsonii* Fisher obtained from Dr. C. W. McCoy at the University of Florida, Citrus Research and Education Center, Lake Alfred; and *Beauveria bassiana* (Bals.) Vuill obtained from Dr. D. G. Boucias at the University of Florida, Gainesville.

Yellow females and light-brown cysts were extracted from roots of soybean cv. Cobb grown in a greenhouse. Females and cysts were surface-disinfested with 0.5% NaOCl for 3 minutes and placed on water agar. Females and cysts that showed no signs of either fungal or bacterial growth were used. Blocks (1 cm × 1 cm) of 2- to 3-week-old fungal cultures on cornmeal agar were transferred to 1.5% water agar in 10-cm-diam. petri dishes. Twelve to 15 fungus-free females or cysts were placed on water agar next to fungal block in each petri dish. Each fungal isolate was replicated three times, and one group of females and cysts was plated on water agar to serve as an untreated control.

After 3 weeks of incubation at 24 °C, cysts were removed, placed on slides, covered with a glass coverslip, ruptured by pressing the coverslip, and observed under a light microscope. The percentages of cysts and eggs colonized by each fungus were recorded. Colonization was based on the presence or absence of fungal mycelium in cysts and eggs. An egg-parasitic index (EPI) for each fungus was recorded based on the following 0–10 scale: 0 = no eggs colonized, 1 = 1–10%, 2 = 11–20%, 3 = 21–30%, 4 = 31–40%, 5 = 41–50%, 6 = 51–60%, 7 = 61–70%, 8 = 71–80%, 9 = 81–90%, and 10 = 91–100% eggs colonized.

The eggs were collected, suspended in water, counted, and prepared for a hatch test. They were placed on 1-cm-diam. sieves with 35 μm openings and sieves were placed in a 24-well tissue culture plate containing a 4-mM $ZnCl_2$ hatching solution (7). The nematodes that hatched were counted at days 1, 4, 7, and 14, and at each time the hatching solution was replaced. The percentage of eggs hatched within 14 days was recorded.

The experiments were repeated, and the data presented are the averages of two tests except those in which a significant interaction in fungal parasitism of eggs was observed between test and the fungal treatment.

Greenhouse test: The fungi tested in soil included *F. oxysporum, H. rhossiliensis, P. lilacinus, S. heteroderae, V. chlamydosporium,* and sterile fungus 1. They were cultured on corn grits (100 g of corn grits, 200 g of dry sand, and 50 ml of water). The culture medium was autoclaved twice, inoculated with each fungus, and incubated at 24 °C for 3–4 weeks. Soil used in the experiment

was collected from a soybean field and is classified as an Arredondo fine sand (91% sand, 5% silt, 4% clay; 1.6% organic matter; pH 5.7). The soil was treated by heating 1-kg lots placed in uniform layers in open plastic bags in a 650-watt microwave oven for 4 minutes. About 500 cm^3 of the treated soil was placed in autoclaved pots (10-cm-high × 10-cm-diam.) and treated with fungi and nematodes in four treatments arranged in a randomized complete block design: (i) corn grits with fungus + nematodes, (ii) corn grits without fungus + nematodes, (iii) corn grits with fungus, and (iv) corn grits only. Each pot received 5 g of dry corn grits with or without fungi, and each treatment was replicated six times.

Soybean seeds and second-stage juveniles (J2) were obtained by the procedure described previously (5). After the fungal inoculum was added, two seeds were planted in each pot on 15 December 1993, and the seedlings were thinned after 1 week to one per pot. Two weeks after planting, each pot received 2,000 J2 added to three holes about 1 cm in diameter and 2–3 cm deep around each plant. The pots were maintained in a greenhouse that had been scrubbed and washed down with 0.5% NaOCl. The plants, bench, and floor of the greenhouse were sprayed as needed with pesticides to control pests and diseases. Autoclaved fertilizer was applied at 3, 4, 5, and 6 weeks after planting at a rate of 0.5 g of 20-20-20 (N-P-K) per pot. The lowest and highest daily ambient temperatures (mean ± SD) in the greenhouse were 9.7 ± 6.2 and 30.8 ± 3.5, 13.0 ± 6.3 and 31.0 ± 3.2, 17.9 ± 2.1 and 35.5 ± 2.7, and 17.5 ± 0.9 and 34.1 ± 5.5 in late December 1993 and January, February, and early March 1994, respectively.

Three months after planting, the soybean plant heights and shoot weights were measured. A soil and root sample from one half of each pot was taken, and cysts and J2 were extracted by centrifugal flotation (5). The densities of J2 and cysts per 100 g of soil and densities of eggs per gram of soil were recorded. Eggs were extracted from cysts and counted. Fungal colonization of cysts and EPI were determined as described for the test on water agar. Cysts were collected from each treatment to determine the frequency of colonization of cysts by the original fungus and other fungi that may have contaminated the soil. The cysts were washed with sterilized water, surface-disinfested with 0.5% NaOCl for 3 minutes, rinsed three times with sterile deionized water, and suspended in a solution of 100 mg of streptomycin and 50 mg of chlortetracycline/liter of water. The surface-disinfested females and cysts were transferred to water agar (five cysts per dish) and incubated at room temperature (23–24 °C). The fungi were identified directly on the water agar and their frequencies recorded; however, a few species could not be identified.

The percentages of fungal colonization of cysts were transformed by arcsin ($\sqrt{x}$), and the nematode densities were transformed by $\log_{10}(x + 1)$ before being subjected to ANOVA. Means for each fungus were compared with the untreated control using student's *t*-test. Linear regression was used for analyzing the relationship between EPI and hatch of juveniles in the agar test and the relationship between nematode density and fungal colonization of cysts and eggs in the greenhouse test.

Results

Pathogenicity to eggs on water agar: The pathogenicity of fungi to eggs on water agar varied among species (Table 1), although all cysts exposed to the fungi were colonized. Of the fungi examined, *V. chlamydosporium, P. terrestris,* sterile fungi 1 and 2 were most pathogenic to SCN eggs, and their EPI ranged from 4.4 to 7.6. Eggs colonized by *V. chlamydosporium* were yellow to light brown in color. *Pyrenochaeta terrestris* produced red pigments, and some eggs colonized by this fungus were red. *Arthrobotrys dactyloides, E. pisciphila, F. oxysporum, F. solani, N. vasinfecta, P. lilacinus, and S. heteroderae* exhibited a moderate degree

TABLE 1. Parasitism of eggs by fungi on water agar and their effect on hatching of second-stage juveniles from eggs of *Heterodera glycines*.

Fungal species	Egg-parasitic index[a]	Percentage of eggs hatched within 14 days[b]	Percentage reduction in hatch[b]
Arthrobotrys dactyloides	3.9*	20.6	50.1
Beauveria bassiana	1.0**	29.0	36.1
Cladosporium sp.	0.2 NS	39.2	12.6
Dictyochaeta coffeae	0 NS; 1.2**	24.6	34.6
Dictyochaeta heteroderae	0.2 NS; 1.3**	22.4	43.8
Exophiala pisciphila	2.4***	24.7	50.1
Fusarium oxysporum	3.7***	25.6	43.6
Fusarium solani	2.2***	18.5	52.6
Gliocladium catenulatum	0.1 NS; 0.5*	20.5	52.8
Hirsutella rhossiliensis	1.7*; 0 NS	24.1	41.1
Hirsutella thompsonii	0.7*	39.0	39.0
Neocosmospora vasinfecta isolate 1	2.0***	21.3	55.6
isolate 2	2.0**	26.1	32.6
isolate 3	2.2***	20.3	55.4
Paecilomyces lilacinus Peruvian strain	3.5***	36.3	21.4
Floridian strain	2.8***	33.2	23.2
Pyrenochaeta terrestris	4.9***	12.0	60.0
Stagonospora heteroderae	2.0*; 4.5**	20.5	47.8
Sterile fungus 1	4.7***	26.3	42.1
Sterile fungus 2	4.4***; 7.6***	11.2	73.3
Verticillium chlamydosporium	7.6***	12.8	73.8
Control (without fungus)	0	46.8	

[a]Egg-parasitic index: 0 = no eggs colonized, 1 = 1–10%, 2 = 11–20%, 3 = 21–30%, 4 = 31–40%, 5 = 41–50%, 6 = 51–60%, 7 = 61–70%, 8 = 71–80%, 9 = 81–90%, and 10 = 91–100% eggs colonized. Data with one value are means of two tests, each with three replicates. There was an interaction between test and fungal treatment where data are presented with two values that are the means of the three replicates in test 1 and test 2, respectively. The means were compared for each fungus with the control by student's *t*-test. *, **, and *** indicate the means were significantly different from the control at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively. NS = not significant.

[b]The percentage reduction in hatch = 100× (hatch in fungal treatment − hatch in control) ÷ hatch in control. Data are means of two tests with one replicate each. No statistical analysis was performed to compare the means between fungal treatment and the control.

of parasitism of eggs (EPI ranged from 2.0 to 4.5). The remaining species showed a low degree of parasitism of eggs (EPI < 2.0) (Table 1). All the fungi inhibited hatch of J2 from eggs (Table 1). *Verticillium chlamydosporium* and sterile fungus 2 reduced hatch by more than 73%. The fungi that showed little colonization of eggs, *B. bassiana, Cladosporium* sp., *D. coffeae, D. heteroderae, Gliocladium catenulatum, H. rhossiliensis,* and *H. thompsonii,* also inhibited hatch. A negative correlation was revealed between hatch and fungal parasitism (Fig. 1).

Greenhouse test: Nematode densities in all soil treatments were low. The densities of cysts and eggs in soil treated with *H. rhossiliensis* and *V. chlamydosporium* were lower than those in control soil (Table 2). Nematode densities in soil treated with the remaining fungi were not different from those in control soil (Table 2).

Fungal colonization of cysts and eggs by *H. rhossiliensis* and *V. chlamydosporium* was not determined because no nematodes were found in soil treated with *H. rhossiliensis* or in four of six pots treated with *V. chlamydosporium.* The percentages of cysts colonized by fungi in soil treated with *F. oxysporum, P. lilacinus, S. heteroderae,* or sterile fungus 1 were higher than those in control soil, although 77–86% of cysts in control soil were colonized (Tables 2,3). Fungi recovered from cysts, however, differed among the treatments (Table 3). In soil infested with *F. oxysporum,* the fungus was recovered from 100% of cysts plated on water agar. Sixty-six percent of cysts in soil treated with *P. lilacinus* were colonized, and the remaining cysts were colonized by *F. oxysporum, Chaetomium cochliodes* Pall., and *Curvularia lunata* (Wakker) Boedijn. In soil treated with *S. heteroderae,* 92% of cysts were colonized by *S. heteroderae,* and the remaining cysts were colonized by *F. oxysporum* and other fungi. In the soil treated with sterile fungus 1, 36% of cysts were colonized by the fungus, 26% of cysts were colonized by *C. cochliodes,* and 19% by other fungi. Although no fungus was added to the control soil, 86% of cysts were colonized by fungi. Fungi recovered from the cysts in the control soil included *F. oxysporum* (18%), *Trichoderma lignorum* (Tode

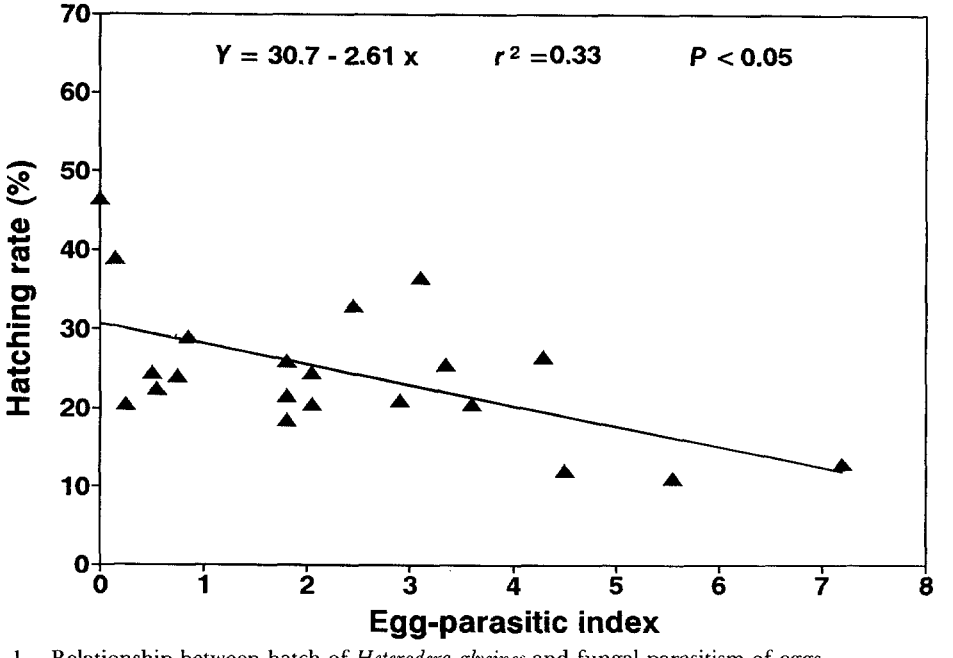

FIG. 1. Relationship between hatch of *Heterodera glycines* and fungal parasitism of eggs.

TABLE 2. Densities and fungal colonization of cysts and eggs of *Heterodera glycines* in soil infested with the nematode and nematophagous fungi in a greenhouse pot test.

Fungi	Second-stage juveniles/100 g soil	Cysts/100 g soil	Eggs/g soil	Percentages of cysts colonized by fungi	Egg-parasitic index
Fusarium oxysporum	2	22	22	95*	7.3*
Hirsutella rhossiliensis	0*	0*	0*		
Paecilomyces lilacinus	34	29	29	97*	8.3*
Stagonospora heteroderae	7	20	24	94*	4.7
Sterile fungus 1	4	44	47	97*	6.8*
Verticillium chlamydosporium	1	19*	25*		
Control (without fungus)	4	68	80	77	2.5

Data are means of six replicates, except that the percentage data for *S. heteroderae* are based on five replicates. The nematode densities were transformed by $\log_{10}(x + 1)$, and the percentages were transformed by arcsin ($\sqrt{x}$) before being subjected to ANOVA. Student's *t*-test was performed to compare each fungus with the control (without fungus). * indicates significant difference from control at $P \leq 0.05$.

TABLE 3. Frequencies of fungi recovered from cysts collected from soil infested with nematophagous fungi.

Fungus treatment	Number of cysts examined	Percentage of cysts colonized						
		Total	Original fungus	*Fusarium oxysporum*	*Trichoderma lignorum*	*Chaetomium cochliodes*	*Curvularia lunata*	Other fungi
Fusarium oxysporum	30	100	100	100	0	0	0	
Hirsutella rhossiliensis	0							
Paecilomyces lilacinus	27	100	66	4	0	15	15	0
Stagonospora heteroderae	39	100	92	5	0	0	0	3
Sterile fungus 1	43	81	36	0	0	26	0	19
Verticillium chlamydosporium	0							
Control (without fungus)	28	86		18	18	0	0	50

& Harz) (18%), and other fungi (50%) (Table 3). Egg-parasitic indices in soil treated with *F. oxysporum, P. lilacinus,* or sterile fungus 1 were higher than those in the control (Table 2). Cyst and egg densities were correlated with the fungal parasitism of eggs, but not the percentage of cysts colonized by the fungi (Table 4).

No differences were observed in plant growth of soybean inoculated with nematodes compared with uninoculated plants (Table 5). Fresh-shoot weights of plants growing in soil treated with all fungi and heights of plants from soil treated by *F. oxysporum, H. rhossiliensis, P. lilacinus,* and *V. chlamydosporium* were greater than those in control soil (Table 5). No interaction on plant growth was observed between nematode and fungal treatments.

Discussion

The EPI (percentage of eggs colonized by fungi) is a useful means of measuring fungal parasitism of nematode eggs. It is difficult to determine whether or not a fungus is an actual parasite of nematode eggs even though the eggs may be colonized. Some fungi may colonize dead eggs after the eggs died from other causes. Irving and Kerry (13) reported that *H. schachtii* eggs that were heat-killed were more susceptible to colonization by *V. chlamydosporium* than untreated eggs. In our tests the percentages of eggs colonized by fungi varied among the fungi studied.

Table 4. Pearson's product moment correlation coefficient (r) between nematode density and fungal colonization of cysts and eggs of *Heterodera glycines* on soybean grown in soil infested with nematophagous fungi.

Nematode density	Independent variable: Percentage of cysts colonized by fungi	Independent variable: Egg-parasitic index
Cysts	−0.17 NS	−0.46*
Eggs	−0.17 NS	−0.55**
Second-stage juveniles	−0.09 NS	−0.13NS

n = 36. * and ** indicate the correlation is significant at $P \leq 0.05$ and 0.01, respectively. NS = not significant.

Table 5. Fresh-shoot weights and heights of soybean plants growing in soil containing nematophagous fungi and either infested with *Heterodera glycines* or not infested in a greenhouse.

Fungi (Fu)	Fresh-shoot weight (g/plant): Nematode (N) +	Nematode (N) −	ANOVA Fu	ANOVA N	ANOVA Fu × N	Plant height (cm): Nematode +	Nematode −	ANOVA Fu	ANOVA N	ANOVA Fu × N
Fusarium oxysporum	3.7	2.7	***	NS	NS	16.6	15.0	***	NS	NS
Hirsutella rhossiliensis	2.1	2.2	*	NS	NS	13.2	11.7	*	NS	NS
Paecilomyces lilacinus	2.7	3.1	***	NS	NS	13.8	13.4	***	NS	NS
Stagonospora heteroderae	2.3	3.2	***	NS	NS	11.6	13.4	[a]	NS	NS
Sterile fungus 1	1.5	1.8	*	NS	NS	10.8	11.1	NS	NS	NS
Verticillium chlamydosporium	4.8	4.7	***	NS	NS	16.2	15.4	***	NS	NS
Control (without fungus)	0.9	1.0				9.3	11.4			

[a]Significant at $P \leq 0.10$.

Data are means of six replicates. "+" nematodes added, "−" without addition of nematodes. Student's t-test was performed to compare each fungus with control (without fungus). Fu × N = interaction between nematode and fungus. * and *** indicate significant differences at $P \leq 0.05$ and $P \leq 0.001$, respectively. NS = not significant.

A fungus may not be an actual parasite if the EPI is low, whereas fungi that showed a high EPI are likely egg parasites. Interaction in the EPI between tests and treatments was frequently observed for fungi with low EPI. This may be attributed to variation in the viability of eggs from different cysts or from our use of different batches of cysts.

We tested fungal pathogenicity on water agar and in pasteurized soil to eliminate or reduce the effects of background organisms. However, for fungi to be used in the biological management of nematodes they must be able to compete with other microorganisms in natural soil. Stiles et al. (30) evaluated *V. chlamydosporium, F. oxysporum, F. solani, Paraphoma radicina* (McAlp.) Morgan-Jones & White, *P. terrestris,* and *S. heteroderae* in nonsterile field soil for their ability to reduce reproduction of the first generation of *H. glycines*. Although all of the fungi were isolated from soybean roots (29) and all of them except for *V. chlamydosporium* were isolated from cysts, none of the fungi reduced the nematode population density of the first generation. Further research is needed to determine whether or not the fungi reported by Stiles et al. (30) were less pathogenic to *H. glycines* than were fungi of corresponding species used in our study.

We tested only one isolate for each fungus, except for *P. lilacinus* and *N. vasinfecta.* Although the pathogenicity was similar between the two isolates of *P. lilacinus* or among the three isolates of *N. vasinfecta,* it is possible that pathogenicity of isolates from other sources would differ from those reported herein. Variations in virulence to nematode eggs have been observed among nematophagous fungal strains (13). Irving and Kerry (13) reported that strains of *V. chlamydosporium* differed in their pathogenicity to *H. avenae* and *H. schachtii,* but all were capable of colonizing viable eggs including those containing J2. Thus, many fungal isolates of each species should be evaluated before their potential as biological control agents can be realized.

Eggs containing J2 are generally resistant to fungal attack (13). The percentages of eggs colonized by fungi in our greenhouse test were higher than those of the corresponding agar test. The yellow females and light-brown cysts used in the agar test may have contained more developed eggs than would occur in young females on roots. Yellow females that are 30–35 days old and contain eggs of varying maturity are probably the best developmental stage to use in pathogenicity tests. We found 2–3 weeks of incubation to be optimum for observation of fungal parasitism on eggs. If the incubation period exceeded 4 weeks, some eggs, especially those parasitized by *V. chlamydosporium,* were consumed by the fungus.

Some fungi that exhibited little parasitism of eggs also were associated with reduced hatch. Although hatch was correlated negatively with index of fungal parasitism of eggs, the coefficient of determination was low, indicating that other factors may be involved in reducing hatch. Several fungi produce metabolites that kill eggs or inhibit hatch of juveniles from eggs (1,8,9,32).

The pathogenicity of fungi to eggs did not correspond to the frequency of occurrence in cysts in natural soil. Although *V. chlamydosporium* was highly pathogenic to SCN, the fungus was not isolated either from cysts of *H. glycines* in Florida (4) or Alabama (27) soybean field soil. *Verticillium chlamydosporium* has been reported in Europe as a suppressive parasite of *Heterodera avenae* Woll. (19–21), but it may not be an important parasite of cyst nematodes in subtropical climates, despite its pathogenicity to nematodes. Isolates of *P. lilacinus* have been tested widely for control of root-knot and cyst nematodes (18). The Floridian and Peruvian strains of *P. lilacinus* tested herein were only moderately pathogenic to SCN. This fungus was recovered frequently in egg masses of *Meloidogyne* spp. from a nearby tobacco field where the fungus was originally isolated (unpubl. data), but was encountered in only 1 of 1,711 cysts in the soybean field (4). *Paeci*

lomyces lilacinus also has been isolated from cysts at low frequencies in other locations (2,11,23,28). *Fusarium oxysporum* was encountered frequently in the soybean field (4) and also isolated from cysts in other studies (2,10,11,23,27). Our results suggest that this fungus is a good soil and cyst colonizer. Although this fungus exhibited only moderate pathogenicity to SCN, it should be evaluated further as a potential biological control agent of SCN.

Sterile fungus 2 was a slow-growing species that was highly pathogenic to SCN. Its frequency in cysts taken from the soybean field was not determined. Sterile fungus 1 also was highly pathogenic and parasitized both nematode eggs and J2 within cysts. Sterile fungus 1 was encountered at a high frequency in cysts collected from natural soil (4). Further study is needed to determine whether it can be introduced into soil for management of SCN. *Exophiala pisciphila,* which shares some characteristics, such as colony features and growth rate, with sterile fungus 1, was encountered in cysts at a moderate rate of frequency (4) and was less pathogenic to eggs than was sterile fungus 1.

Pyrenochaeta terrestris was highly pathogenic to SCN eggs and was recovered in cysts at a high rate of frequency (4); however, this fungus is believed to be a plant pathogen. When evaluated in the greenhouse, the soybean plants became diseased and died; thus, additional work was not done with the species. It has been reported previously to cause lesions on soybean roots (29,30).

Hirsutella rhossiliensis is an endoparasite of vermiform nematodes (6,16) including J2 of *H. avenae* (31) and *Heterodera schachtii* Schmidt (15). On agar this fungus infected J2 of SCN (data not shown) but showed little pathogenicity to eggs. Some studies have shown that *H. rhossiliensis* is a weak soil competitor (17). In natural soil the population of this fungus develops slowly and is nematode-density dependent (14). In the present study *H. rhossiliensis* suppressed nematode population densities completely in pasteurized soil.

The three isolates of *N. vasinfecta* varied in colony and perithecium color. In a previous study, *N. vasinfecta* was the species most frequently isolated from cysts (4). The pathogenicity of the three isolates evaluated herein may be similar at modererate levels of infestation. The frequency of *F. solani* in cysts (4) and its pathogenicity to eggs were similar to those of *N. vasinfecta.*

Dictyochaeta heteroderae was reported as weakly pathogenic to eggs (12). The present study agrees with that result. The first observation of *Dictyochaeta coffeae* in cysts of the SCN was made in the present study. This fungus is similar to *D. heteroderae* in pathogenicity to eggs. Although these two fungi were not highly pathogenic to eggs, they were recovered from cysts with a relatively high frequency (4). The isolate of *S. heteroderae* used in the present study was moderately pathogenic to the eggs, whereas an isolate of this fungus was shown to have a low degree of parasitism on the nematode in a previous study (12). This fungus was encountered frequently in cysts taken from field soil (2, 4,26).

Beauveria bassiana and *H. thompsonii* were weak parasites of SCN eggs. *Cladosporium* sp. was not pathogenic to SCN.

In the greenhouse study, the percentage of cysts colonized by fungi varied among fungal species. All cysts in soil receiving *F. oxysporum* were colonized by the fungus. Although sterile fungus 1 was recovered at high frequencies from cysts in a field with a known infestation of SCN (4) and from other sites (5), this fungus colonized only 37% of the cysts in soil to which the fungus had been added. Probably the low growth rate of this fungus (data not shown) allowed other faster-colonizing fungi to compete better in the pasteurized soil. Some cysts in pots treated with *P. lilacinus* and *S. heteroderae* also were colonized by fungi other than those added. Some of the fungi isolated from cysts in control pots were pathogenic to eggs.

The improvement of plant growth in the fungal treatments could not be attributed

to control of nematodes because the total nematode densities were low and there were no differences in growth between plants with and without nematodes. The greater plant growth in fungus-treated pots may be attributed to either decomposition of the carriers, which may have provided nutrients, or fungi, such as *P. lilacinus* that produce substances that stimulated plant growth (24).

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