Interactions Between the Soybean Cyst Nematode and *Fusarium solani* f. sp. *glycines* Based on Greenhouse Factorial Experiments

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ABSTRACT

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The soybean cyst nematode, *Heterodera glycines*, and the fungus that causes sudden death syndrome (SDS) of soybean, *Fusarium solani* f. sp. *glycines,* frequently co-infest soybean (*Glycine max*) fields. The interactions between *H. glycines* and *F. solani* f. sp. *glycines* were investigated in factorial greenhouse experiments with different inoculum levels of both organisms on a soybean cultivar susceptible to both pathogens. Measured responses included root and shoot dry weights, *H. glycines* reproduction, area under the SDS disease progress curve, and fungal colonization of roots. Both *H. glycines* and *F. solani* f. sp. *glycines* reduced the growth of soybeans. Reproduction of *H. glycines* was suppressed by high inoculum levels but not by low levels of *F. solani* f. sp. *glycines*. The infection of soybean roots by *H. glycines* did not affect root colonization by the fungus, as determined by real-time polymerase chain reaction. Although both pathogens reduced the growth of soybeans, *H. glycines* did not increase SDS foliar symptoms, and statistical interactions between the two pathogens were seldom significant.

Additional keywords: microbes, pathogen population ecology, quantitative plant pathology.

Both the soybean cyst nematode (*Heterodera glycines*) and *Fusarium solani* f. sp. *glycines* are major pathogens of soybean and can cause significant yield losses (36,46). Both pathogens are soilborne and infect soybean roots. Although the total monetary loss due to *H. glycines* damage in the United States was estimated to be 1.1 billion dollars annually (36), the nematode does not always cause obvious aboveground symptoms (44). Under some conditions, soybeans infected by *F. solani* f. sp. *glycines* may develop soybean sudden death syndrome (SDS). This disease is characterized by root necrosis or rot, vascular discoloration of the roots and stem, and foliar chlorosis, necrosis, and premature defoliation (16,29). The foliar symptoms of SDS are caused by phytotoxins produced by *F. solani* f. sp. *glycines* in the roots under favorable conditions for disease development (13,14). *F. solani* f. sp. *glycines* has been accepted as the causal organism for SDS, but recently the new name *F. virguliforme* Akoi, O'Donnell, Homma & Lattanzi has been proposed to replace *F. solani* f. sp. *glycines* on the basis of morphological and phylogenetic studies (2). This new name has yet to be fully accepted (28); therefore, we refer to the SDS pathogen as *F. solani* f. sp. *glycines* in this paper.

The relationship between *H. glycines* and *F. solani* f. sp. *glycines* has been investigated for nearly 2 decades, but the interaction is not clearly understood. *H. glycines* is frequently present when SDS occurs (9). However, there are inconsistent reports concerning the effects of *H. glycines* on SDS. In greenhouse experiments, soybean seedlings inoculated simultaneously with *F. solani* f. sp. *glycines* and *H. glycines* developed more severe foliar symptoms of SDS than soybean seedlings inoculated with the fungus alone (18,21,23,30). In field experiments, symptoms of SDS increased when *H. glycines* was present, whereas *H. glycines* cyst numbers were suppressed by *F. solani* f. sp. *glycines* (21,33). Hershman et al. (8) reported, however, that there was no correlation between cyst population density and the area under SDS disease progress curve (AUDPC). Scherm et al. (35) reported a positive but weak relationship between *H. glycines* cyst counts and SDS symptoms in the field; their cross-correlation analysis indicated that *F. solani* f. sp. *glycines* densities and the number of *H. glycines* cysts were consistently associated with SDS severity. The interaction of *H. glycines* and SDS is not fully understood, as is indicated by both positive and negative or weak correlations of *H. glycines* population densities with the disease (8,31,33,35).

Since the interaction between *H. glycines* and *F. solani* f. sp. *glycines* is still unclear, questions remain to be answered. For instance, does infestation level affect the interaction or will more sensitive detection methods, in particular real-time polymerase chain reaction (PCR) for quantification of *F. solani* f. sp. *glycines* (5), provide more insights into the interaction?

In this study, greenhouse experiments were used to (i) measure both the effects of *F. solani* f. sp. *glycines* infestation levels on *H. glycines* reproduction and final populations, and the effects of *H. glycines* infestation levels on *F. solani* f. sp. *glycines* colonization in root tissues as shown by quantification of fungal DNA, and (ii) compare the symptoms of host plants infected either singly or simultaneously with different levels of *H. glycines* and *F. solani* f. sp. *glycines*.

MATERIALS AND METHODS

***H. glycines* culture.** An Hg-type 0 isolate of *H. glycines* designated PA3 (26) was maintained in a greenhouse at 27 ± 1°C for

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30 days on soybean, *Glycines max* cv. Lee. *H. glycines* females and cysts were extracted from the roots and soil as previously described (27). Females and cysts were then placed on a 60-mesh sieve (250-µm aperture) and were manually ruptured with a rubber stopper to release the eggs, which were collected on a 500-mesh sieve (25-µm aperture) and then separated from debris by sucrose centrifugation (12). Eggs were rinsed into a 500-ml beaker, and sufficient tap water was added to dilute the suspension to 1,000 eggs per ml.

Fungal culture. Isolate Mont-1 of *F. solani* f. sp. *glycines* was provided by the National Soybean Pathogen Collection Center at the University of Illinois and was maintained under constant fluorescent light at 24°C on water agar. To prepare inoculum, the fungus was grown on potato dextrose agar for 2 weeks and was then transferred to 1-liter flasks, each containing 200 g of red sorghum seed that had been soaked overnight and autoclaved twice. Each flask was infested with 10 plugs (8-mm-diameter) of the fungal culture and kept under constant fluorescent light at 24°C. The inoculum in the flask was mixed by manual shaking every 2 to 3 days. After 2 weeks, the quantity of *F. solani* f. sp. *glycines* DNA in the freeze-dried inoculum mixture, as determined by real-time PCR (5), was 310 ± 152 pg/mg.

Experimental design and conditions. All three experiments described in the following sections were set up in randomized complete blocks with four replications of each treatment. A silty clay loam soil (15% sand, 49% silt, 36% clay [pH 6.1], and 4.2% organic matter) and a Topedo sand (93% sand, 3% silt, 4% clay [pH 8.5], and 0.2% organic matter) were collected from Champaign Country, Illinois. A steam-pasteurized soil mix (two parts sand to one part silty clay loam, giving a final mix of 77% sand, 11% silt, and 12% clay, with pH 7.8, and 0.9% organic matter) was placed in 400-ml tri-corner beakers (350 cm^3 of soil mix per beaker) that were used as pots. All the *F. solani* f. sp. *glycines* inoculum was deposited in the middle of the pot as an evenly distributed layer. *H. glycines* eggs were pipetted into a hole (8 mm wide by 30 mm deep). Seeds of soybean cv. Williams 82 were germinated for 48 h on damp germination paper, and one seedling was planted in each pot. The soil was maintained near water-holding capacity by watering once daily or as needed.

In one repetition (trial 1) of each experiment, pots were placed in a greenhouse water bath to maintain a constant root-zone temperature of 27 ± 1°C. The other repetition (trial 2) was conducted on a bench in one of two greenhouses where the air temperature ranged from 22.7 to 28.7°C, with an average of 25.6°C during the experiments. The pots on the bench, as opposed to those in the water bath, were wrapped with aluminum foil to exclude light. Artificial light was provided for the greenhouse with the water bath (trial 1) at 14 h/day and for the other greenhouse (trial 2) at 16 h/day. The average supplemental light intensity was 21.5 ± 3.0 klux in trial 1 and 9.2 ± 2.6 klux in trial 2.

Effect of inoculum level of *F. solani* f. sp. *glycines* (experiment 1). A 2 × 3 complete factorial design included all combinations of two levels of *H. glycines* (0 or 2,000 eggs per pot) and three levels of *F. solani* f. sp. *glycines* (none, low, or high). Three levels of *F. solani* f. sp. *glycines* inoculum were defined by the volume of inoculum added per pot. The "high" treatment received 15 cm^3 of infested sorghum seed, whereas the "low" treatment received 1.5 cm^3 of infested sorghum seed plus 13.5 cm^3 of autoclaved infested sorghum seed. The "none" treatment received 15 cm^3 of autoclaved infested sorghum seed.

Effect of *H. glycines* inoculum level (experiment 2). A 4 × 2 complete factorial design included all combinations of four levels of *H. glycines* (0, 200, 2,000, or 20,000 eggs per pot) and two levels of *F. solani* f. sp. *glycines* (none or high); the "high" treatment received 15 cm^3 of infested sorghum seed, whereas the "none" treatment received 15 cm^3 of autoclaved infested sorghum seed.

Effect of high inoculum levels of *H. glycines* and *F. solani* f. sp. *glycines* (experiment 3). A 2 × 2 complete factorial design included all combinations of two levels of *H. glycines* (0 or 20,000 eggs per pot) and two levels of *F. solani* f. sp. *glycines* (none or high, i.e., 30 cm^3 of autoclaved infested sorghum seed or 30 cm^3 of infested sorghum seed per pot).

Data collection. Foliar symptoms were evaluated 12 days after planting and every 4 days thereafter with the following rating scale adapted from Hartman et al. (7): 0 = no foliar symptoms; 1 = slight symptom development, with mottling and mosaic on leaves (1 to 20% of foliage affected); 2 = moderate symptom development, with interveinal chlorosis and necrosis on foliage (21 to 50% of foliage affected); 3 = heavy symptom development, with interveinal chlorosis and necrosis (51 to 80% of foliage affected); and 4 = severe interveinal chlorosis and necrosis (81 to 100% of foliage affected). This scale was used to calculate the area under the SDS disease progress curve (AUDPC) as described by Shaner and Finney (37).

The experiments were concluded after 6 weeks. The response variables evaluated included root necrosis, shoot height, flower number, root dry weight, plant dry weight, *H. glycines* eggs per pot, eggs per gram of dried root, and the AUDPC. *H. glycines* eggs were extracted as described previously (26) and stained with 0.035% acid fuchsin (10). Nematode reproduction factor (Rf) was calculated by dividing the final number of eggs per pot (Pf) by the initial number (Pi), (Pf + 1)/(Pi + 1). Root necrosis was visually rated as the proportion of necrosis in the whole root system, and flowers were counted. Plant shoots were dried at room temperature to less than 10% moisture, and the roots were freeze-dried for 3 days in a BenchTop 4K freeze dryer (VirTis, Gardiner, NY). After weighing, all of the freeze-dried roots from each replicate were ground in a cyclone sample mill (Model 3010, UDY Corp., Fort Collins, CO) (40), and the mill was cleaned manually between samples with high-pressure air. Ground roots were stored at –20°C.

Quantities of *F. solani* f. sp. *glycines* DNA in roots. DNA was extracted as described in protocol 2 from Gao et al. (5) for quantification of *F. solani* f. sp. *glycines* in roots. Protocol 2 was adapted from Malvick and Grunden (19) and produced DNA extracts free of PCR inhibitors (5). Up to 50 mg of freeze-dried root powder was used for DNA extraction. *F. solani* f. sp. *glycines* colonization levels in roots were determined with the real-time PCR protocol described previously (5).

Data analysis. Data were analyzed using version 8 of SAS (SAS Institute, Cary, NC). A two-way analysis of variance (ANOVA) model, the main statistical tool, was used to assess the effects of *H. glycines*, *F. solani* f. sp. *glycines*, and their interaction upon the growth of soybean. To assess the effect of the two trials in the greenhouse (with or without water bath control of soil temperature), a three-way ANOVA model was used; because the effect of trial was significant for most response variables other than root necrosis and AUDPC (data not shown), the data from trials 1 and 2 of each experiment were not combined. Egg numbers were transformed to $\log_{10}(x + 1)$ values before analysis. Finally, the ANOVA for experiment 2 was supplemented with *t* tests to determine whether *F. solani* f. sp. *glycines* was a significant factor at any of the four levels of nematode. A total of eight *t* tests were conducted. The means were separated with Tukey's studentized range test at $\alpha = 0.05$. To test whether the decline in *H. glycines* reproduction in *F. solani* f. sp. *glycines*-infested treatments resulted from detrimental effects of the fungus on the nematodes or from the reduction in available feeding sites as indicated by dry root weight, the number of *H. glycines* eggs per gram of dried root tissue was included as a parameter in the analysis.

Initial analysis of the DNA data was based on a mixed model in which the fixed effects were trial, *F. solani* f. sp. *glycines*, and *H. glycines*. The random effect was the pot, which was nested within the particular combination of trial, *F. solani* f. sp. *glycines* treatment, and *H. glycines* treatment. Pot was included as a ran-

dom effect because the data clearly indicated a significant pot effect. While this effect is not of primary interest, it must be included to achieve a correct analysis. The two-way and three-way interactions among trial, *F. solani* f. sp. *glycines*, and *H. glycines* were also included in the model. In cases where trial had a significant main effect or significant interactions with *F. solani* f. sp. *glycines* or *H. glycines*, an additional analysis was done separately for each of the two trials. The additional analysis employed a mixed model with *F. solani* f. sp. *glycines* and *H. glycines* as fixed effects, and pot as a random effect nested within *F. solani* f. sp. *glycines*–*H. glycines* combinations. In cases where trial had neither a significant main effect nor significant interactions with *F. solani* f. sp. *glycines* or *H. glycines*, the data were merged across trials. In these cases, the additional analysis used a mixed model with *F. solani* f. sp. *glycines* and *H. glycines* as fixed effects, and pot as a random effect within *F. solani* f. sp. *glycines*–*H. glycines* combinations.

RESULTS

Overall interaction between *H. glycines* and *F. solani* f. sp. *glycines*. Statistical interactions between *H. glycines* and *F. solani* f. sp. *glycines* were seldom significant (Table 1), indicating that the effects of *H. glycines* and *F. solani* f. sp. *glycines* can be explained by an additive model. The strongest statistical effect in all three experiments was the main effect of *F. solani* f. sp. *glycines*, i.e., the fungus significantly reduced most response variables including *H. glycines* final population density (Tables 2, 3, and 4). The effect of the fungus on nematode population density was confirmed by *t* tests with fixed *H. glycines* levels. These two-sample *t* tests showed significant differences in the numbers of eggs per pot according to *F. solani* f. sp. *glycines* inoculum level (data not shown).

Effect of *F. solani* f. sp. *glycines* inoculum level (experiment 1). Results of experiment 1, in which *H. glycines* was added at two levels (0 or 2,000 eggs per pot) and *F. solani* f. sp. *glycines* at three levels (0, 1.5, or 15 cm^3 of inoculum per pot), showed similar trends in both trials (Fig. 1; Table 2). SDS increased over time in all treatments containing *F. solani* f. sp. *glycines* except the treatment with the low level of the fungus in trial 1 (Fig. 1); no SDS developed in the treatments with autoclaved fungal inoculum. The AUDPC value with *F. solani* f. sp. *glycines* at high levels was statistically similar whether or not *H. glycines* was added, though it was highest when *H. glycines* was added (Table 2). Disease symptoms progressed more rapidly and the differences between treatments were more pronounced in trial 1 (constant

TABLE 1. *P* values of the two-way (*Heterodera glycines* × *Fusarium solani* f. sp. *glycines*) analysis of variance model used to explain responses in three greenhouse experiments

	Trial 1[w]			Trial 2[x]		
Response variable	Experiment 1	Experiment 2	Experiment 3	Experiment 1	Experiment 2	Experiment 3
Root necrosis	0.4233	0.0239	0.0006	0.4053	0.1104	0.2411
Plant height (cm)	0.2992	0.3511	0.0837	0.6084	0.4402	0.7365
Flowers/plant	0.2155	0.8914	0.2149	0.4065	0.0041	0.1877
Root dry weight (g)	0.0741	0.3979	0.2583	0.8748	0.4767	0.9904
Plant dry weight (g)	0.5317	0.0642	0.1082	0.8890	0.0774	0.7633
Eggs/pot[y]	0.0143	0.0020	0.0261	0.0230	0.0031	0.1950
AUDPC[z]	0.3665	0.0859	0.8465	0.0701	0.0357	0.4676

[w] In trial 1, pots were in a water bath, which provided a constant root-zone temperature of 27 ± 1°C.
[x] In trial 2, pots sat on the greenhouse bench, and the average air temperature was 25.6°C (22.7 to 28.7°C).
[y] The number of *H. glycines* eggs per pot was determined at the end of the experiment (6 weeks after planting).
[z] Area under the SDS (soybean sudden death syndrome) disease progress curve.

TABLE 2. Effects of two levels of *Heterodera glycines* and three levels of *Fusarium solani* f. sp. *glycines* on soybean plants and nematode reproduction (experiment 1)[t]

Treatment										
H. glycines (eggs)	*F. solani* f. sp. *glycines*[u]	AUDPC[v]	Root necrosis[w]	Root dry weight (g)	Shoot dry weight (g)	Shoot height (cm)	Whole plant dry weight (g)	Flowers/ plant	Eggs/g of root	*H. glycines* Rf[x]
Trial 1[y]										
2,000	High	80.4 a	0.88 a	0.12 d	0.32 d	9.9 b	0.44 d	1.3 a	36,109 a	3.85 b
0	High	50.5 ab	0.87 ab	0.28 c	0.66 cd	12.5 ab	0.95 cd	2.8 a	0	1.00
2,000	Low	11.1 bc	0.86 ab	0.46 b	0.99 bc	12.4 ab	1.45 bc	1.5 a	100,167 a	16.92 ab
0	Low	0.0 c	0.74 ab	0.62 ab	1.49 ab	16.0 a	2.11 ab	1.5 a	0	1.00
2,000	0	0.0 c	0.40 bc	0.70 a	1.54 ab	15.6 a	2.24 a	0.5 a	102,601 a	34.55 a
0	0	0.0 c	0.11 c	0.62 ab	1.86 a	16.0 a	2.51 a	0.3 a	0	1.00
Trial 2[z]										
2,000	High	53.1 a	0.79 a	0.77 b	2.26 b	20.3 a	3.03 b	1.3 a	1,455 a	0.88 b
0	High	34.8 ab	0.85 a	0.86 b	2.69 b	22.2 a	3.66 b	1.3 a	0	1.00
2,000	Low	20.8 bc	0.65 a	1.11 b	2.00 b	20.8 a	3.11 b	1.0 a	7,599 a	4.68 ab
0	Low	29.3 ab	0.66 a	1.09 b	2.85 b	19.2 a	3.93 b	3.5 a	0	1.00
2,000	0	0.0 c	0.25 b	2.24 a	5.33 a	20.5 a	7.57 a	1.3 a	11,500 a	13.99 a
0	0	0.0 c	0.17 b	2.13 a	5.64 a	22.8 a	7.77 a	3.5 a	0	1.00

[t] Within a trial and column, treatments followed by the same letter are not significantly different according to Tukey's studentized range test at $P \leq 0.05$. Values are the means of four replicate pots. For eggs per gram of root and *H. glycines* reproduction factor (Rf), data from pots not inoculated with *H. glycines* were excluded from the analysis.
[u] Inoculum consisted of colonized sorghum seeds.
[v] Area under the SDS (soybean sudden death syndrome) disease progress curve.
[w] Root necrosis was visually rated as the proportion of necrosis in the whole root system.
[x] Rf = (final population + 1)/(initial population + 1).
[y] In trial 1, pots were placed in a greenhouse water bath, which maintained a constant root-zone temperature of 27 ± 1°C.
[z] In trial 2, pots sat on a bench in a greenhouse with an average air temperature of 25.6°C (22.7 to 28.7°C).

root-zone temperatures) than in trial 2. Presence of *H. glycines* with the low level of *F. solani* f. sp. *glycines* did not significantly affect SDS severity. Instead, SDS development was most dependent upon *F. solani* f. sp. *glycines* inoculum level (Fig. 1; Table 2).

H. glycines plus the high level of *F. solani* f. sp. *glycines* reduced root dry weight more than either pathogen alone (Table 2). For the other variables, the combination of two pathogens had similar effects as single pathogen treatments. The high level of *F. solani* f. sp. *glycines* significantly reduced *H. glycines* reproduction, but the low level of the fungus did not (Table 2). Neither the high nor the low level of the fungus reduced the number of *H. glycines* eggs per gram of root (Table 2).

Effect of *H. glycines* inoculum level (experiment 2). In treatments with *F. solani* f. sp. *glycines*, SDS increased over time in both trials (data not shown). AUDPC was not significantly greater in the presence of *H. glycines* and was independent of the three nematode levels (Table 3). Disease development was more rapid and the differences between symptom severities in the treatments were more pronounced in trial 1 (constant root-zone temperature) than in trial 2.

Dual infestation with a high level of *F. solani* f. sp. *glycines* and 20,000 *H. glycines* eggs caused severe root necrosis, but symptoms were not greater in this treatment than when 2,000 or 200 eggs were used with fungal inoculum in either trial (Table 3).

TABLE 3. Effects of four levels of *Heterodera glycines* and two levels of *Fusarium solani* f. sp. *glycines* on soybean plants and nematode reproduction (experiment 2)[t]

Treatment										
H. glycines (eggs)	*F. solani* f. sp. *glycines*[u]	AUDPC[v]	Root necrosis[w]	Root dry weight (g)	Shoot dry weight (g)	Shoot height (cm)	Whole plant dry weight (g)	Flowers/ plant	Eggs/g of root	*H. glycines* Rf[x]
Trial 1[y]										
20,000	High	76.7 ab	0.96 a	0.09 c	0.18 c	9.4 b	0.27 d	1.3 a	1,089,892 a	1.56 b
2,000	High	88.5 a	0.83 a	0.11 c	0.18 d	11.2 b	0.29 d	1.0 a	135,600 a	4.66 b
200	High	49.3 ab	0.85 a	0.13 bc	0.37 cd	10.3 b	0.50 d	2.7 a	9,951 b	5.99 b
0	High	38.8 abc	0.68 ab	0.09 c	0.28 bc	10.2 b	0.37 d	1.3 a	0	1.00
20,000	0	0 c	0.82 a	0.36 ab	0.76 ab	12.8 ab	1.12 bc	1.3 a	438,641 a	6.81 b
2,000	0	0 c	0.35 bc	0.55 a	1.26 ab	16.3 a	1.81 ab	2.8 a	102,175 a	28.20 a
200	0	0 c	0.08 c	0.59 a	1.56 a	17.5 a	2.15 a	3.0 a	8,931 b	25.30 a
0	0	0 c	0.08 c	0.40 ab	1.58 a	16.6 a	1.98 a	2.8 a	0	1.00
Trial 2[z]										
20,000	High	55.7 a	0.96 a	0.65 b	1.52 c	16.0 a	2.17 c	0 b	24,562 ab	0.81 c
2,000	High	49.7 a	0.93 ab	0.76 b	2.08 bc	21.3 a	2.84 c	2.5 a	3,501 ab	1.32 c
200	High	45.4 a	0.91 ab	0.72 b	1.77 c	19.4 a	2.49 c	0.3 b	34 c	0.14 c
0	High	38.3 a	0.89 ab	0.72 b	1.89 c	17.6 a	2.60 c	0.3 b	0	1.00
20,000	0	0 b	0.56 bc	1.87 a	3.27 b	18.3 a	5.14 b	0 b	73,948 a	7.45 b
2,000	0	0 b	0.41 dc	2.12 a	4.66 a	21.0 a	6.78 b	0 b	17,288 ab	18.02 a
200	0	0 b	0.13 d	2.35 a	5.04 a	22.3 a	7.38 a	0.3 b	779 b	9.29 ab
0	0	0 b	0.19 dc	2.14 a	4.96 a	22.0 a	7.10 a	0.3 b	0	1.00

[t] Within a trial and column, treatments followed by the same letter are not significantly different according to Tukey's studentized range test at $P \leq 0.05$. Values are the means of four replicate pots. For eggs per gram of root and *H. glycines* reproduction factor (Rf), data from pots not inoculated with *H. glycines* were excluded from the analysis.
[u] Inoculum consisted of colonized sorghum seeds.
[v] Area under the SDS (soybean sudden death syndrome) disease progress curve.
[w] Root necrosis was visually rated as the proportion of necrosis in the whole root system.
[x] Rf = (final population + 1)/(initial population + 1).
[y] In trial 1, pots were placed in a greenhouse water bath, which maintained a constant root-zone temperature of 27 ± 1°C.
[z] In trial 2, pots sat on a bench in a greenhouse with an average air temperature of 25.6°C (22.7 to 28.7°C).

TABLE 4. Effects of high inoculum levels of *Heterodera glycines* and *Fusarium solani* f. sp. *glycines* on soybean plants and nematode reproduction (experiment 3)[t]

Treatment										
H. glycines (eggs)	*F. solani* f. sp. *glycines*[u]	AUDPC[v]	Root necrosis[w]	Root dry weight (g)	Shoot dry weight (g)	Shoot height (cm)	Whole plant dry weight (g)	Flowers/ plant	Eggs/g of root	*H. glycines* Rf[x]
Trial 1[y]										
20,000	Very high	100.8 a	0.95 a	0.03 b	0.08 b	8.8 b	0.10 a	0 b	9,500 a	0.02 b
0	Very high	96.9 a	0.96 a	0.05 b	0.09 b	7.3 b	0.13 a	0 b	0	1.00
20,000	0	0.0 c	0.66 b	0.33 a	0.81 a	16.3 a	1.13 b	2.5 ab	7,061 a	0.13 a
0	0	0.0 c	0.13 c	0.44 a	1.28 a	12.9 a	1.72 b	4.5 a	0	1.00
Trial 2[z]										
20,000	Very high	48.6 a	0.95 a	0.37 b	0.84 b	15.9 b	1.20 b	0 a	378 a	0.01 a
0	Very high	38.7 a	0.92 a	0.41 b	1.00 b	16.0 b	1.40 b	0 a	0	1.00
20,000	0	1.5 c	0.59 ab	1.23 ab	3.13 a	21.4 a	4.36 a	0.5 a	26,005 a	1.92 a
0	0	0.6 c	0.32 b	1.28 a	3.62 a	22.5 a	4.89 a	0 a	0	1.00

[t] Within a trial and column, treatments followed by the same letter are not significantly different according to Tukey's studentized range test at $P \leq 0.05$. Values are the means of four replicate pots. For eggs per gram of root and *H. glycines* reproduction factor (Rf), data from pots not inoculated with *H. glycines* were excluded from the analysis.
[u] Inoculum consisted of colonized sorghum seeds.
[v] Area under the SDS (soybean sudden death syndrome) disease progress curve.
[w] Root necrosis was visually rated as the proportion of necrosis in the whole root system.
[x] Rf = (final population + 1)/(initial population + 1).
[y] In trial 1, pots were placed in a greenhouse water bath, which maintained a constant root-zone temperature of 27 ± 1°C.
[z] In trial 2, pots sat on a bench in a greenhouse with an average air temperature of 25.6°C (22.7 to 28.7°C).

Root and shoot dry weights were reduced in the treatments containing high levels of *F. solani* f. sp. *glycines*. Nematode reproduction was reduced by the fungus and by the high initial level of nematode infestation. Plant dry weight was reduced and root necrosis was increased by increasing *H. glycines* population densities, although not always significantly.

Nematode reproduction was significantly greater in trial 1 (constant root-zone temperature) than in trial 2 (Table 3). Nematode reproduction only occurred in *H. glycines*-infested treatments and was reduced by *F. solani* f. sp. *glycines* inoculum regardless of nematode inoculum level. As in experiment 1, however, *F. solani* f. sp. *glycines* did not affect numbers of nematode eggs per gram of root (Table 3). Thus, the fungus reduced nematode reproduction by reducing root mass.

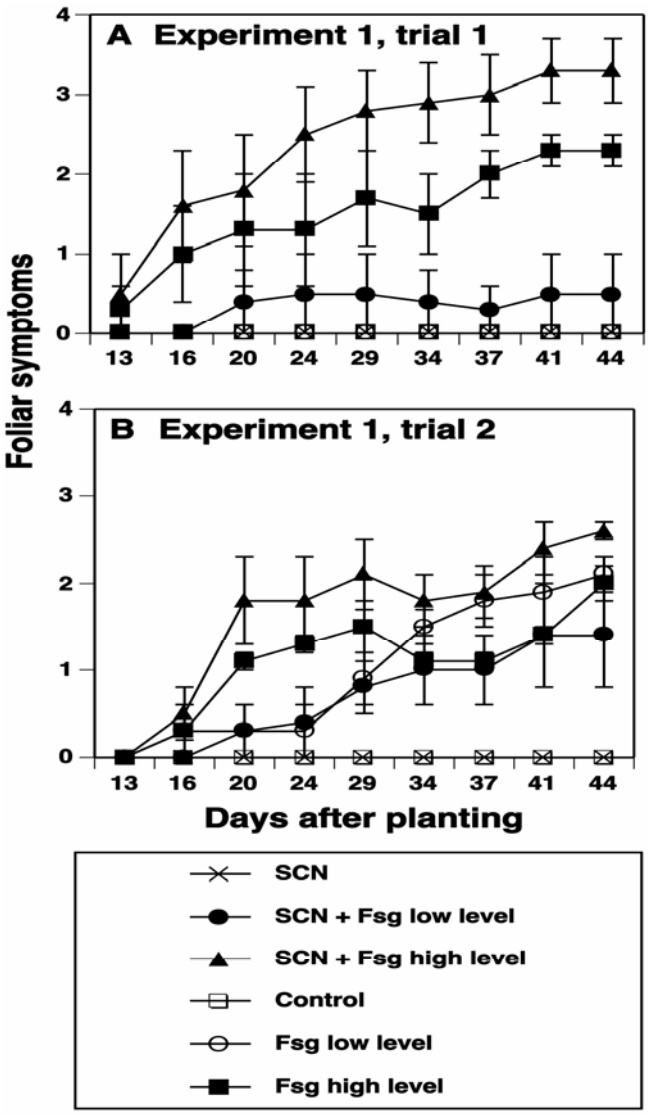

Fig. 1. Development of foliar symptoms of soybean sudden death syndrome on soybean plants infested with three levels of *Fusarium solani* f. sp. *glycines* (Fsg) and two levels of *Heterodera glycines* (SCN) in experiment 1: **A,** trial 1, with a constant root-zone temperature of 27 ± 1°C; and **B,** trial 2, with an average air temperature of 25.6°C (22.7 to 28.7°C). Foliar symptoms were rated with the following scale: 0 = no foliar symptoms; 1 = slight symptom development, with mottling and mosaic on leaves (1 to 20% of foliage affected); 2 = moderate symptom development, with interveinal chlorosis and necrosis (21 to 50% of foliage affected); 3 = heavy symptom development, with interveinal chlorosis and necrosis (51 to 80% of foliage affected); and 4 = severe interveinal chlorosis and necrosis (81 to 100% of foliage affected). Values are the means of four replications. For trial 1, there were no foliar symptoms with the noninfested control, SCN alone, or Fsg low level alone. For trial 2, there were no foliar symptoms with the noninfested control or SCN alone.

Effect of high levels of *H. glycines* and *F. solani* f. sp. *glycines* (experiment 3). In *F. solani* f. sp. *glycines*-infested treatments, AUDPC was not significantly affected by *H. glycines* in either trial (Table 4). Some foliar necrosis was observed in the two treatments lacking *F. solani* f. sp. *glycines* in trial 2, and although the cause was unknown, the mean AUDPC was significantly less than in the *F. solani* f. sp. *glycines*-infested treatments. SDS development was less severe in trial 2 than in trial 1 (Table 4).

When added separately, the fungus increased root necrosis (Table 4). In addition, root, shoot, and whole plant dry weights were reduced by *F. solani* f. sp. *glycines* (Table 4). Plants did not flower in the *F. solani* f. sp. *glycines*-infested treatments. Nematode reproduction was similar in trial 1 and trial 2 of experiment 3 but was less than in experiments 1 and 2. *H. glycines* reproduction was reduced by *F. solani* f. sp. *glycines* in trial 1 but not in trial 2.

Quantities of *F. solani* f. sp. *glycines* DNA in roots. To analyze the DNA data for the three experiments, both nested and averaged models were initially considered. Because it maintained the full structure of the data set and did not require data averaging, the nested model was selected.

In experiment 1, trial was not a significant main effect for either *F. solani* f. sp. *glycines* or *H. glycines*, and therefore the data from the two trials were merged. In the analysis, the only significant main effect was that of *F. solani* f. sp. *glycines* level; neither *H. glycines* nor its interaction with *F. solani* f. sp. *glycines* was significant (Table 5). *F. solani* f. sp. *glycines* DNA was unexpectedly detected in roots of noninoculated control plants but an additional ANOVA indicated that the quantity of DNA was less (P = 0.0214) in control plants than in plants inoculated with *F. solani* f. sp. *glycines*.

TABLE 5. Quantities of *Fusarium solani* f. sp. *glycines* DNA in soybean roots as determined by real-time polymerase chain reaction in experiment 1

Treatment		*F. solani* f. sp. *glycines* DNA (pg/mg of root)[z]	Standard error
Heterodera glycines (eggs)	*F. solani* f. sp. *glycines*[y]		
2,000	High	16,100 ab	9,088
0	High	23,784 ab	7,676
2,000	Low	35,236 b	7,181
0	Low	24,701 ab	7,181
2,000	0	11,786 a	7,183
0	0	5,717 a	8,291

[y] Inoculum consisted of colonized sorghum seeds.

[z] Least squares means of DNA quantity of *F. solani* f. sp. *glycines* in soybean roots. For each column, means followed by the same letter are not significantly different according to the Tukey pairwise comparison at $\alpha = 0.05$ in mixed procedure. Values are the means of 24 replications (trials 1 and 2 combined).

TABLE 6. Quantities of *Fusarium solani* f. sp. *glycines* DNA in soybean roots as determined by real-time polymerase chain reaction in experiment 2

Treatment		*F. solani* f. sp. *glycines* DNA (pg/mg of root)[z]			
		Trial 1		Trial 2	
Heterodera glycines (eggs)	*F. solani* f. sp. *glycines*	Mean	Standard error	Mean	Standard error
20,000	High	32,903 b	3,601	20,532 b	5,420
2,000	High	16,910 a	5,092	25,721 bc	5,420
200	High	13,000 a	5,092	44,483 d	5,420
0	High	16,761 a	5,092	37,334 cd	5,420
20,000	0	7,418 a	5,092	2,260 a	5,420
2,000	0	6,665 a	3,601	3,672 a	5,420
200	0	5,043 a	3,601	910 a	6,258
0	0	4,606 a	3,601	1,704 a	6,258

[z] Least squares means of DNA quantity of *F. solani* f. sp. *glycines* in soybean roots. Within each trial and column, means followed by the same letter are not significantly different according to the Tukey pairwise comparison α = 0.05 in mixed procedure. Values are the means of 12 replications.

In experiment 2, trial had significant two-way and three-way interactions with *F. solani* f. sp. *glycines* and *H. glycines*, and therefore separate analyses were conducted for each trial. As in experiment 1, the only significant main effect was that of *F. solani* f. sp. *glycines*, and neither *H. glycines* nor its interaction with *F. solani* f. sp. *glycines* was significant (Table 6). Again, *F. solani* f. sp. *glycines* DNA was detected in the noninoculated controls but the quantity was less ($P = 0.0004$ in trial 1 and 0.0001 in trial 2) in control plants than in the inoculated plants.

In experiment 3, it was only possible to run the initial model with main effects and two-way interactions because of missing data. Because the main effect of trial was significant, two additional analyses were done: the data from the two trials were merged or analyzed separately. In both analyses, neither of the main effects for *F. solani* f. sp. *glycines* and *H. glycines* nor their interactions were significant (data not shown). DNA was again detected in the noninoculated controls but there were no significant differences in DNA quantities ($P = 0.3184$ in trial 1 and 0.6219 in trial 2) in control and inoculated plants, probably due to the severe root necrosis in the inoculated plants (Table 4).

DISCUSSION

Plant-parasitic nematodes in the rhizosphere co-exist and may interact with numerous microbes and other organisms (15). Those nematodes, like *H. glycines*, that enter roots are also likely to interact with plant-pathogenic fungi and bacteria within the roots of common host plants (3,15,34,43). Because *H. glycines* and *F. solani* f. sp. *glycines* are common and important soilborne pathogens of soybean, understanding how the plant responds to combinations of these pathogens is important. Contrary to previous reports (18,21,30,32), our greenhouse studies indicated that *H. glycines* did not increase severity of foliar symptoms of SDS caused by *F. solani* f. sp. *glycines*. This may be due to the use of different isolates and inoculum levels of *H. glycines* and *F. solani* f. sp. *glycines*, and/or different varieties of soybean. A number of reports have indicated that the nematode populations, pathogenic fungal isolates, plant cultivars, and experimental conditions can affect the interactions between nematodes and other plant pathogens (20,38,45).

Sikora and Carter (39) emphasized that a range of inoculum densities of both pathogens should be used in various environmental conditions to determine the overall influence of normal and extreme conditions on the interaction. For example, inoculum densities affected the interactions between *Meloidogyne incognita* and *F. oxysporum* f. sp. *vasinfectum* on cotton (6,41). Previous studies of interactions between *H. glycines* and *F. solani* f. sp. *glycines* have not included different inoculum levels. We found that *F. solani* f. sp. *glycines* suppressed *H. glycines* egg populations only when the *F. solani* f. sp. *glycines* inoculum was high, indicating that fungal population level plays an important role in the interaction with the nematode. Because the fungus did not reduce the number of eggs produced per gram of dried root, the reduced *H. glycines* reproduction per plant probably resulted from the reduction in root material available for nematode feeding.

Understanding how nematodes and fungal pathogens interact depends on advances in research methods and tools. Although *H. glycines* can be conveniently enumerated with a conventional microscope, *F. solani* f. sp. *glycines* cannot. Use of semiselective media to enumerate the colony forming units of *Fusarium* spp. *glycines* in roots (4,11,25) is time-consuming and labor intensive. We used a real-time PCR assay for quantification of *F. solani* f. sp. *glycines* and found that the infection of soybean roots by *H. glycines* did not significantly affect the colonization of plant tissues by *F. solani* f. sp. *glycines*.

The use of real-time PCR to quantify a plant pathogen's DNA was very useful but there is limited information available on how to statistically analyze these kinds of data. In our statistical analysis of *F. solani* f. sp. *glycines* DNA quantities, we selected a nested model after examining both nested and averaged models. The nested model was considered more appropriate and powerful than the averaged one because it captured the full structure of the data and did not sacrifice information, i.e., it did not require averaging of the individual data points.

Small quantities of *F. solani* f. sp. *glycines* DNA were detected in some of our control plants. Such fungal contamination is not uncommon in greenhouse experiments and usually results from experimental manipulation or greenhouse insects (1,17,24,42). Although sometimes contaminated with *F. solani* f. sp. *glycines*, the control plants in our study remained useful as controls because they contained much less fungal DNA than treated plants, at least when the inoculum levels were not extremely high, and were usually symptom free.

In summary, two major conclusions can be drawn from our greenhouse experiments. First, the effect of *H. glycines* on *F. solani* f. sp. *glycines* root colonization was not significant. Second, *H. glycines* did not increase SDS symptom severity under our experimental conditions. Further studies on the interaction between *H. glycines* and SDS should include a wider range of *F. solani* f. sp. *glycines* inoculum levels at planting.

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