Journal of Nematology 22(4):496–505. 1990.

Interactions Between *Calonectria crotalariae* and *Heterodera glycines* on Soybean[1]

C. OVERSTREET,[2] E. C. MCGAWLEY,[3] AND J. S. RUSSIN[4]

Abstract: The interactions of *Heterodera glycines* at four egg inoculum levels (0, 100, 1,000, and 10,000 per pot) and three cyst levels (0, 100, and 200 per pot) and *Calonectria crotalariae* at 500, 5,000, and 50,000 microsclerotia per pot were evaluated on soybean. At the two lowest nematode egg levels, the presence of *C. crotalariae* did not affect nematode reproduction. At 10,000 eggs per pot, however, nematode reproduction was increased significantly at each microsclerotial level. The increase in nematode reproduction was stepwise at 500 and 5,000 microsclerotia per pot but declined at 50,000 microsclerotia per pot. Similar results were obtained when cysts rather than eggs were used as nematode inoculum. The nematode × fungus interaction significantly affected 60-day plant growth parameters of both Lee 74 and Centennial soybean. The nematode × fungus interaction was antagonistic to plant roots and significantly influenced root injury ratings. The presence of *C. crotalariae* in tissues of stock plants or plants used as race differentials did not alter the analysis of this population as race 3.

Key words: Calonectria crotalariae, disease complex, *Glycine max, Heterodera glycines,* nematode–fungus interaction, red crown rot disease, soybean cyst nematode.

Soybean cyst nematode, *Heterodera glycines* Ichinohe, is the most important nematode pest on soybean (*Glycine max* (L.) Merr.) in the southern United States (29). Yield losses caused by this nematode were estimated to be 2.6% in 1987 (19). *Calonectria crotalariae* (Loos) Bell and Sobers (= *Cylindrocladium crotalariae* (Loos) Bell and Sobers) causes serious disease in peanut (*Arachis hypogea* L.) and soybean. Red crown rot, caused by this fungus, is a major disease of soybean in Louisiana (5). Centennial soybean plants with red perithecia produced by *C. crotalariae* also are sometimes infected with *H. glycines.* Other investigators have detailed plant disease complexes in which either *H. glycines* or *C. crotalariae* (1–3,9–13,24,25,27) were significant components.

Fungal pathogens in plant roots have been shown to influence the population development of plant-parasitic nematodes, particularly those in the genera *Heterodera* and *Globodera.* Ross (24) reported increased numbers of *H. glycines* juveniles in soil when roots were infected with *Fusarium oxysporum* Schlecht. f. sp. *glycines* Armst. & Armst. The association of *Phytophthora megasperma* Drechs. var. *sojae* Hild with *H. glycines* results in significantly fewer females and cysts of the soybean cyst nematode (2).

In the presence of the stem canker fungus, *Diaporthe phaseolorum* (Cke. and Ell.) Sac. var. *caulivora* Anthow and Caldwell, fewer juveniles and cysts of *H. glycines* were found in soil and on the roots of soybean (25). Jorgenson (17) found that *F. oxysporum* inhibited the invasion and development of *H. schachtii* Schmidt in sugarbeet. Similarly, James (14,15) reported that *Pyrenochaeta lycopersicon* Schn. & Gerl. inhibited the hatch of eggs, infection by juveniles, and production of new cysts by *G. rostochiensis* (Woll.) Behrens on tomato.

Penetration by juveniles and overall population levels of *H. glycines* were increased in soybean roots coinfected with *C. crotalariae* (20). These studies, however, utilized only single levels of inocula of both pathogens. Objectives of the current study were 1) to evaluate the impact of multiple in-

Received for publication 28 August 1989.

[1] Approved for publication by the Director of the Louisiana Agricultural Experiment Station as manuscript number 89-38-3429. Portion of a thesis submitted by the senior author in partial fulfillment of the Ph.D. requirement, Louisiana State University, Baton Rouge.

[2] Assistant Specialist, Louisiana Cooperative Extension Service, Baton Rouge, LA 70803.

[3] Associate Professor, Department of Plant Pathology and Crop Physiology, Louisiana State University, Baton Rouge, LA 70803.

[4] Senior Plant Pathologist, Crop Genetics International, Route 1, Box 112A, Henderson, MD 21640.

The authors thank Mrs. Kristine Winchell, Research Associate, for technical assistance throughout the course of this study.

oculum densities of both pathogens on reproduction of *H. glycines*, root colonization by *C. crotalariae*, and soybean plant growth and 2) to determine whether the presence of *C. crotalariae* would alter the race reaction of *H. glycines* race 3.

Materials and Methods

An isolate of *C. crotalariae* was obtained from infected soybean plants near Burnside, Louisiana. Microsclerotia used as fungal inoculum were produced on potato dextrose agar and extracted by comminuting cultures in water for 1 minute and passing the resultant slurry through nested 425-μm-pore (40 mesh) and 75-μm-pore (200 mesh) sieves. Microsclerotia collected on the 75-μm-pore sieve were suspended in water, and aliquots necessary to obtain 1, 10, 50, or 100 per g of soil were pipetted into 0.5 kg of 3:2:1 mixture of methyl bromide-treated loamy sand (80.8% sand, 4.7% silt, 14.5% clay), sand, and Weblite in polyethylene bags. The soil and microsclerotia were thoroughly mixed and placed in 10-cm-d clay pots. Soybean seed were sown in a 42 × 20 × 7.5-cm galvanized tray containing soil mixture and dusted with a commercial preparation of *Bradyrhizobium japonicum*. A single 10-day-old soybean seedling was transplanted into the soil in each pot.

At harvest, the percentage of root colonization by *C. crotalariae* was estimated. A randomly collected 0.5-g root subsample from each plant was disinfected for 30 seconds in 0.5% NaOCl, and 1-cm-long root fragments were transferred to culture dishes containing a medium selective for *C. crotalariae* (21). Cultures were incubated for 10 days at ambient temperature under continuous fluorescent light. The percentage of colonization per root system was estimated from 20 root fragments per plant.

A race 3 population of *H. glycines* was obtained from the same field as the isolate of *C. crotalariae* and increased on Lee 74 soybean in a greenhouse. Cysts were extracted from soil using a modified centrifugal-sugar flotation technique (16) with nested 425-μm-pore (40 mesh) and 150-μm-pore (100 mesh) sieves. Cysts were blended in water for 30 seconds to release eggs. Nematode inoculum, as either eggs or cysts, was introduced into soil by pipetting equal portions of the inoculum suspension into five depressions (1.0 cm wide × 4.0 cm deep) surrounding each seedling. At harvest, nematode population development was monitored by counting juveniles, males, females, and cysts on and in roots and soil. Cysts and females were dislodged from roots by washing them over nested 425-μm-pore and 150-μm-pore sieves with a strong stream of water. Immature life stages within roots were estimated from a 0.5-g subsample of root tissue collected randomly from each root system that had been cleared and stained with acid fuchsin (7). Nematodes were extracted from soil by the modified centrifugal-sugar flotation technique.

Root injury was rated on a 1–5 scale where 1 = no visible symptoms, 2 = slight (≤ 20%), 3 = moderate (21–50%), 4 = severe (51–75%), and 5 = > 75% necrosis. All tests were conducted in a greenhouse with temperatures across all experiments averaging 18–35 C and provided with supplementary light from both fluorescent and incandescent sources (ca. 3,000 lux at 45 cm above bench surface). The experimental design used for all tests was a randomized complete block. Data were analyzed using PROC GLM (26) to test for main treatment effects and interactions. When the number of treatment levels exceeded two, orthogonal contrasts were used to test for differences between levels.

Experiment 1: Treatments consisted of two levels of both nematode (0 and 10,000 eggs per pot) and fungus (0 and 50 microsclerotia per gram of soil—25,000 per pot) and one cultivar (Lee 74); a total of four treatment combinations, each replicated five times. The experiment was terminated after 80 days. The population density and life stage composition of *H. glycines* populations in soil and roots were estimated.

Experiment 2: Treatments consisted of four inoculum levels of the nematode (0,

TABLE 1. Influence of *Calonectria crotalariae* on reproduction of *Heterodera glycines* on Lee 74 soybean 80 days after inoculation with 10,000 eggs of the nematode.

Fungus level†	Cysts in soil	Females in roots	Juveniles		Males in soil	Total nematodes		
			Soil	Roots		Soil	Roots	Pot
0	283 b	104 a	15 a	272 a	15 a	312 b	376 a	689 a
1	516 a	117 a	15 a	227 a	32 a	563 a	345 a	908 a

Values are means of five replications. Means in columns followed by the same letter are not different ($P < 0.05$) according to ANOVA.

† Levels 0 and 1 correspond to microsclerotial densities of 0 and 50,000 per pot.

100, 1,000, and 10,000 eggs per pot) and the fungus (0, 500, 5,000, and 50,000 microsclerotia per pot) and one cultivar (Lee 74); a total of 16 treatment combinations, each replicated five times. The experiment was terminated after 60 days. Soil and root populations of the nematode, plant fresh weights, percentage of *C. crotalariae* colonization, and root injury ratings were determined.

Experiment 3: Treatments consisted of three inoculum levels of the nematode (0, 100, and 200 cysts per pot) and the fungus (0, 5,000, and 50,000 microsclerotia per pot) and two cultivars (Lee 74, susceptible and Centennial, resistant to *H. glycines* race 3), for a total of 18 treatment combinations, each replicated four times. The experiment was terminated after 60 days and data were collected as in experiment 2.

Experiment 4: This test was conducted to evaluate the influence of *C. crotalariae* on the race reaction of *H. glycines* when inoculum was produced on stock culture plants (Lee 74) infected with the fungus. Twenty pots, each containing 3.0 kg soil mixture in which one Lee 74 plant was growing, served as stock cultures. Ten of the stock pots received 50 microsclerotia of the fungus per gram of soil, and all 20 received 10,000 eggs of the nematode. Eighty days after establishment of the stock cultures, cysts were collected from the soil. Eggs from cysts that developed on roots not parasitized or parasitized by *C. crotalariae* were used to inoculate (2,000 eggs per pot) the soybean differentials Peking, PI 88788, PI 90763, and Centennial (instead of the usual differential cultivar Pickett). In a comparison study using our population of race 3, Pickett and Centennial had female indices (23) of 1.8 and 2.7, respectively. The test was terminated after 40 days, and a female index was calculated for each differential.

Experiment 5: This experiment was to evaluate the direct influence of *C. crotalariae* on race differential reaction to *H. glycines.* Seedlings of the four race differentials described in experiment 4 were transplanted into 10-cm-d pots containing soil infested with 5,000 microsclerotia per pot and (or) 10,000 eggs of *H. glycines.* Plants were harvested after 45 days, and female indices were calculated for each differential.

RESULTS

Experiment 1: Results in this test, in which inoculum consisted of nematode eggs plus 50 microsclerotia per gram soil, are in agreement with those we reported previously when comparable and identical numbers of cysts and microsclerotia, respectively, were used. In the presence of *C. crotalariae,* the numbers of cysts in soil and total nematodes in soil were greater ($P = 0.058$) than in pots containing only the nematode (Table 1).

Experiment 2: Increasing numbers of *H. glycines* eggs as inoculum resulted in a stepwise increase ($P < 0.01$) in all nematode life stages in both soil and roots (Table 2). The presence of *C. crotalariae* caused an increase ($P < 0.01$) in the number of cysts recovered from soil. Numbers of soil juveniles, soil population totals, and total nematodes per pot decreased ($P < 0.05$) at the highest fungal inoculum level (50,000 microsclerotia per pot). Nematode × fun-

TABLE 2. Main and interactive effects of *Heterodera glycines* and *Calonectria crotalariae* on nematode reproduction on Lee 74 soybean after 60 days.

Treatment	Level†	Cysts		Females in roots	Juveniles		Males		Total nematodes		
		Soil	Roots		Soil	Root	Soil	Root	Soil	Root	Pot
Nematode	1	2	1	3	17	5	2	0	21	8	29
	2	11	4	7	101	18	5	2	117	30	147
	3	102	67	40	2,912	382	77	24	3,092	513	3,604
Contrast											
1 vs. 2 + 3		**	**	**	**	**	**	**	**	**	**
1 vs. 3		**	**	**	**	**	**	**	**	**	**
Fungus	0	8	17	17	982	185	40	15	1,030	235	1,264
	1	32	24	18	976	141	23	9	1,031	192	1,223
	2	78	29	19	1,359	109	36	9	1,472	166	1,638
	3	35	25	12	725	105	13	3	773	144	916
Contrast											
0 vs. 1 + 2 + 3		**	NS	NS	NS	NS	NS	NS	NS	NS	NS
1 vs. 2 + 3		*	NS	NS	NS	NS	NS	NS	NS	NS	NS
3 vs. 1 + 2		NS	NS	NS	*	NS	NS	NS	*	NS	*
Source											
Nematode		**	**	**	**	**	**	**	**	**	**
Fungus		**	NS	NS	NS	NS	NS	NS	NS	NS	NS
N × F		**	NS	NS	NS	NS	NS	NS	*	NS	NS

*, ** = significant at $P = 0.05$ and $P = 0.01$, respectively, based on ANOVA.

† Nematode levels 1, 2, and 3 correspond to egg inoculum densities of 100, 1,000, and 10,000 per pot, respectively. Fungus levels 0, 1, 2, and 3 correspond to microsclerotial densities of 0, 500, 5,000 and 50,000 per pot, respectively.

gus interaction significantly ($P < 0.01$) affected the numbers of cysts recovered from the soil (Fig. 1). Examination of individual treatment means showed that at the highest nematode inoculum density (10,000 eggs per pot), the numbers of cysts in the soil increased as levels of microsclerotia were increased to 5,000 per pot. The number of cysts in soil declined, however, when the number of microsclerotia per pot was 50,000. No such fungus influence was observed when the number of nematode eggs per pot was either 100 or 1,000. A significant nematode × fungus interaction was detected for nematode population totals in soil. This was largely a reflection of the augmented numbers of cysts.

Fresh root weights of plants inoculated with *H. glycines* were reduced ($P < 0.01$) below those of controls (Table 3), but there was no influence of nematode inoculum level. Plant growth parameters also were reduced ($P < 0.05$) by the fungus. There was a stepwise reduction ($P < 0.01$) in fresh root weights as the densities of microsclerotia in soil were increased. The nematode × fungus interaction affected ($P < 0.05$) plant growth parameters measured. Examination of individual root and plant weight means revealed an antagonistic interaction in which the reduction caused by both nematode and fungus together was less than the sum of reductions caused by each alone.

At all inoculum levels of *H. glycines*, root colonization by the fungus decreased and root injury ratings increased (Table 3). The

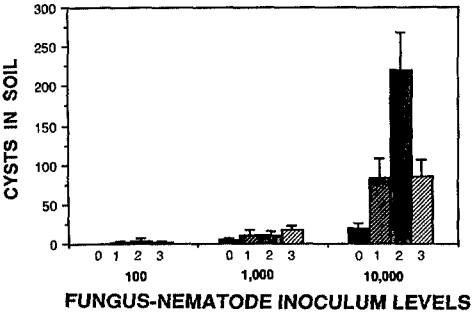

FIG. 1. Treatment means for the interaction between *Heterodera glycines* and *Calonectria crotalariae* on cysts recovered from soil in experiment 2. Vertical lines delimit standard errors of means. Fungus levels of 0, 1, 2, 3 = 0, 500, 5,000, and 50,000 microsclerotia per pot, respectively. Nematode levels of 100, 1,000, and 10,000 eggs per pot.

TABLE 3. Effects of *Heterodera glycines* and *Calonectria crotalariae* on plant fresh weights, root colonization by the fungus, and root injury ratings (RIR) on Lee 74 soybean 60 days after inoculation.

Treatment	Level†	Fresh weight (grams)			Root colonization (%)	RIR‡
		Root	Shoot	Plant		
Nematode	0	1.9	7.4	9.3	27	2.3
	1	1.5	7.5	9.0	15	2.4
	2	1.5	7.8	9.3	18	2.2
	3	1.5	7.8	9.3	22	2.7
Contrast						
0 vs. 1 + 2 + 3		**	NS	NS	**	*
1 vs. 2 + 3		NS	NS	NS	NS	NS
3 vs. 1 + 2		NS	NS	NS	*	**
Fungus	0	1.7	8.1	9.9	0	1.3
	1	1.7	7.6	9.3	7	1.9
	2	1.5	7.3	8.8	36	3.0
	3	1.4	7.5	8.9	39	3.4
Contrast						
0 vs. 1 + 2 + 3		**	*	**	**	**
1 vs. 2 + 3		**	NS	NS	**	**
3 vs. 1 + 2		**	NS	NS	**	**
Source						
Nematode		**	NS	NS	**	**
Fungus		**	NS	*	**	**
N × F		*	**	**	**	**

*, ** = significant at $P = 0.05$ and $P = 0.01$, respectively, based on ANOVA.
† Nematode levels 0, 1, 2, and 3 correspond to egg inoculum densities of 0, 100, 1,000, and 10,000 per pot, respectively. Fungus levels 0, 1, 2, and 3 correspond to microsclerotial densities of 0, 500, 5,000 and 50,000 per pot, respectively.
‡ Root injury rating = 1–5 (1 = no symptoms, 5 = > 75% necrosis).

percentage of root colonization by the fungus increased with increasing nematode inoculum; however, these values were lower than those observed in the absence of the nematode. Both the percentage of root colonization and the root injury ratings increased as the level of microsclerotia in soil increased. In both cases, there was a significant ($P < 0.01$) nematode × fungus interaction. Inspection of individual treatment means revealed an antagonistic relationship (Fig. 2). Root colonization by the fungus followed a similar trend. The presence of the nematode reduced the percentage of root colonization at all egg densities.

Experiment 3: Numbers of cysts extracted from soil decreased ($P = 0.05$) when nematode inoculum density was increased from 100 to 200 cysts per pot (Table 4). Numbers of cysts in soil increased ($P = 0.01$) when roots also were colonized by *C. crotalariae,* an effect that was most evident at 5,000 microsclerotia per pot. Nematode fungus interaction also influenced ($P < 0.05$) the numbers of cysts extracted from both soil and roots. At 100 cysts per pot (Fig. 3), numbers of cysts extracted from the soil increased when the fungal inoculum level was 5,000 microsclerotia per pot but declined at 50,000 per pot. This pat-

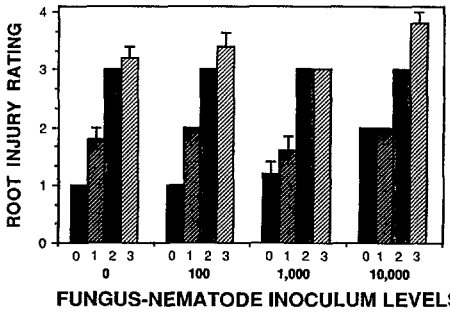

FIG. 2. Treatment means for the interaction between *Heterodera glycines* and *Calonectria crotalariae* with root injury ratings in experiment 2. Vertical lines delimit standard errors of means. Where standard error bars are not shown, the standard error is zero. Root injury rating: 1 = no symptoms, 2 = < 20, 3 = 21–50, 4 = 51–75, 5 = > 75% necrosis. Fungus levels of 0, 1, 2, 3 = 0, 500, 5,000, and 50,000 microsclerotia per pot, respectively. Nematode levels of 100, 1,000, and 10,000 eggs per pot.

TABLE 4. Effects of *Heterodera glycines, Calonectria crotalariae,* and cultivar on nematode reproduction on two soybean cultivars 60 days after inoculation.

Treatment	Level†	Cysts		Females in roots	Juveniles		Males in soil	Total nematodes		
		Soil	Roots		Soil	Root		Soil	Root	Pot
Nematode	1	286	69	564	699	268	143	1,128	706	1,834
	2	237	55	323	160	225	31	428	602	1,027
Fungus	0	217	73	392	714	162	109	1,040	626	1,661
	1	326	58	661	321	403	117	764	831	1,595
	2	241	54	276	254	175	36	531	505	1,036
Contrast										
0 vs. 1 + 2		**	NS	NS	NS	NS	NS	NS	NS	NS
1 vs. 2		**	NS	NS	NS	NS	NS	NS	NS	NS
Cultivar										
Lee		478	113	870	844	450	168	1,491	1,238	2,729
Centennial		46	10	17	14	44	6	66	70	132
Source										
Nematode		*	NS	NS	NS	NS	NS	*	NS	NS
Fungus		**	NS	NS	NS	NS	NS	NS	NS	NS
Cultivar		**	**	**	**	**	**	**	**	**
N × F		**	*	NS	NS	NS	NS	NS	NS	NS
N × C		*	NS	NS	NS	NS	NS	*	NS	NS
F × C		**	NS	NS	NS	NS	NS	NS	NS	NS
N × F × C		**	**	NS	NS	NS	NS	*	NS	NS

*, ** = significant at $P = 0.05$ and $P = 0.01$, respectively based on ANOVA.

† Nematode levels 1 and 2 correspond to cyst inoculum densities of 100 and 200 per pot, respectively. Fungus levels 0, 1, and 2 correspond to microsclerotial densities of 0, 5,000, and 50,000 per pot, respectively.

tern of cyst extraction is similar to that when 10,000 eggs per pot were used as inoculum (Fig. 1). When the cyst level was increased to 200 per pot (Fig. 3), however, numbers of cysts extracted from soil were similar when fungal inoculum levels were 5,000 and 50,000 microsclerotia per pot. A similar pattern for treatment means was obtained for recovery of cysts from roots. The nematode × cultivar interaction affected ($P < 0.05$) both the numbers of cysts in soil and the life stage totals for soil. Treatment mean patterns for both cultivars revealed a reduction in numbers of cysts in the soil at the higher level of nematode inoculum on susceptible Lee 74 but not on resistant Centennial. Fungus × cultivar interaction ($P < 0.01$) also impacted cyst recovery from soil and resulted from enhanced reproduction in the presence of the fungus on Lee 74 but not Centennial. Nematode × fungus × cultivar interaction was also significant with respect to the numbers of cysts extracted from soil and roots as well as the totals for nematode populations present in the soil.

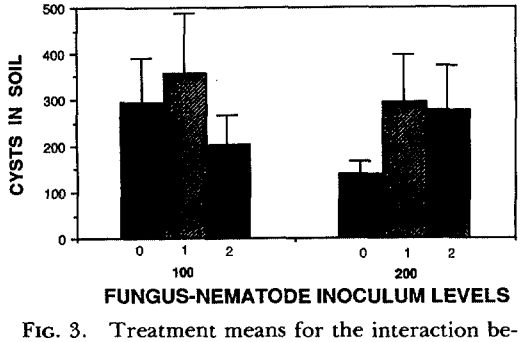

FIG. 3. Treatment means for the interaction between *Heterodera glycines* and *Calonectria crotalariae* on cysts recovered from soil in experiment 3. Vertical lines delimit standard errors of means. Fungus levels of 0, 1, 2 = 0, 5,000, and 50,000 microsclerotia per pot, respectively. Nematode levels of 100 and 200 cysts per pot.

Individually, nematode and fungus treatments reduced ($P < 0.01$) root, shoot, and plant fresh weights, but there was no inoculum level influence (Table 5). Shoot and plant weights for Centennial were lower ($P < 0.05$) than those for Lee 74. The nematode × fungus interaction affected ($P < 0.05$) all growth parameters and was antagonistic. The sum of the reductions in plant growth when the nematode and fungus were together was less than those for

TABLE 5. Effects of *Heterodera glycines, Calonectria crotalariae,* and cultivar on plant fresh weights, root colonization by the fungus, and root injury ratings (RIR) on two soybean cultivars 60 days after inoculation.

Treatment	Level†	Fresh weight (grams)			Root colonization (%)	RIR‡
		Root	Shoot	Plant		
Nematode	0	12.4	40.6	53.1	20.0	1.7
	1	7.8	27.2	35.1	20.6	3.4
	2	6.8	23.9	30.7	16.3	3.4
Contrast						
0 vs. 1 + 2		**	**	**	NS	**
1 vs. 2		NS	NS	NS	NS	NS
Fungus	0	11.0	33.9	44.8	0	1.8
	1	8.1	28.4	36.5	25.6	3.1
	2	8.1	29.5	37.6	31.3	3.6
Contrast						
0 vs. 1 + 2		**	**	**	**	**
1 vs. 2		NS	NS	NS	NS	*
Cultivar						
Lee		9.4	33.2	45.6	22.4	2.8
Centennial		8.7	28.0	36.7	15.6	2.9
Source						
Nematode		**	**	**	NS	**
Fungus		**	*	**	**	**
Cultivar		NS	**	*	NS	NS
N × F		**	*	*	NS	**
N × C		NS	NS	NS	NS	NS
F × C		NS	NS	NS	NS	NS
N × F × C		NS	NS	NS	NS	NS

*, ** = significant at $P = 0.05$ and $P = 0.01$, respectively based on ANOVA.
† Nematode levels 1 and 2 correspond to cyst inoculum densities of 100 and 200 per pot, respectively. Fungus levels 0, 1, and 2 correspond to microsclerotial densities of 0, 5,000, and 50,000 per pot, respectively.
‡ Root injury rating = 1–5 (1 = no symptoms, 5 = > 75% necrosis).

each individually. Root injury ratings increased ($P < 0.01$) with inoculation with *H. glycines,* but there was no inoculum density influence. A stepwise increase ($P < 0.05$) in root injury ratings was observed in response to increases in densities of microsclerotia in soil. The nematode × fungus interaction was antagonistic and influenced ($P < 0.01$) root injury ratings.

Experiment 4: When the nematode reproduced on Lee 74 infected with *Calonectria crotalariae* for 80 days, female in-

TABLE 6. Average number of females and cysts on Lee 74 and female indices on differentials inoculated with *Heterodera glycines* from Lee 74 grown in the presence or absence of *Calonectria crotalariae.*

Fungus†	Females and cysts on Lee 74	Female indices on differentials			
		Centennial	Peking	PI 88788	PI 90763
0	70	2.3	1.1	0	0
1	48	1.7	0	0.8	0
Source					
Fungus		NS	NS	NS	NS

Values are averages of five replications.
NS = nonsignificant at $P = 0.05$ based on *F*-test.
† Fungus levels 0 and 1 correspond to microsclerotial densities of 0 and 150,000 per pot, respectively.

TABLE 7. Average number of females and cysts on Lee 74 and female indices on differentials inoculated with *Heterodera glycines* alone or in combination with *Calonectria crotalariae.*

Fungus†	Females and cysts on Lee 74	Female indices on differentials			
		Centennial	Peking	PI 88788	PI 90763
0	312	9.0	7.7	6.8	0.5
1	450	7.5	5.0	5.1	0
Source					
Fungus		NS	NS	NS	NS

Values are averages of five replications.
NS = nonsignificant at $P = 0.05$ based on *F*-test.
† Fungus levels 0 and 1 correspond to microsclerotial densities of 0 and 5,000 per pot, respectively.

dices of the race differentials were no different from nematode inoculum from Lee 74 without the fungus (Table 6). Female indices were less than 10% on all differentials as expected for race 3.

Experiment 5: Female indices were similar on SCN race differentials infected or not infected with *C. crotalariae* (Table 7). In both cases, the race reaction was that of race 3 of *H. glycines.*

Discussion

Population development by *H. glycines* was influenced by inoculum density of both *C. crotalariae* and *H. glycines.* At low nematode levels (100 or 1,000 eggs per pot), the fungus had no measurable influence on nematode reproduction. This lack of influence was probably related to numbers necessary to establish the nematode populations in root systems. However, when nematode levels were 10,000 eggs, or 100 or 200 cysts per pot, the fungus influence on nematode reproduction was significant. As microsclerotia densities increased to 100 per gram of soil (50,000 per pot), the influence on nematode reproduction diminished. The lesion nematode, *Pratylenchus penetrans* Cobb, and *Verticillium albo-atrum* Reinke and Berth. had a similar inoculum-dependent relationship on eggplant: lesion nematode reproduction was enhanced at low but not at high levels of *V. albo-atrum,* and at higher levels of fungus inoculum, *V. albo-atrum* may have competed with *P. penetrans* for available nutrients (18). In our studies, increased root injury ratings and percentage of colonization by *C. crotalariae* suggest that the fungus may be more competitive than the nematode at microsclerotia levels of 100 per gram, rendering the tissue unsuitable for the nematode. Whether the influence is direct or indirect is not known. We reported previously, and have observed subsequently, however, that the nematode and fungus are rarely located in juxtaposition in the root tissue, irrespective of microsclerotial inoculum level.

The severity of disease caused by *C. crotalariae* is increased in the presence of plant-parasitic nematodes. Disease ratings are higher on peanut in the presence of *Meloidogyne hapla* Chitwood and *Criconemella ornata* (Raski) Luc & Raski (9). Root necrosis is greater when soybean is inoculated simultaneously with *M. incognita* and *C. crotalariae* (12). Root rot severity of a peanut cultivar resistant to *C. crotalariae* increases as the microsclerotia density of the fungus and levels of *M. arenaria* (Neal) Chitwood increase (11). Root rot severity also increases in a significant, but additive manner in the presence of the nematode (11). Our root injury ratings data indicate a similar, but antagonistic, pattern for *C. crotalariae* and *H. glycines.*

Disease complexes may be categorized as antagonistic, additive, or synergistic. The interaction of *H. schachtii* and *Fusarium oxysporum* is antagonistic on sugarbeets (17). When *H. schachtii* is combined with *Rhizoctonia solani* on the same host, however, the interaction is synergistic (22). Reports also document cases in which such interactions between pathogens differ between species within a single fungal genus. For example, *H. schachtii* combined with *Pythium aphanidermatum* has an additive effect in damping off of sugarbeets, but with *P. ultimum* on the same host, disease severity is synergistic (28).

Globodera rostochiensis increases wilt symptoms caused by *Verticillium dahliae* when 10 or more eggs are added per gram of soil around potato plants (8). In our tests the highest root injury ratings occurred at nematode levels of 20 eggs, or 0.2 or 0.4 cysts per gram soil.

Plant growth responses are related to initial nematode populations (4). Growth reduction was evident with *H. glycines* as a significant main effect on root weights in experiment 2 and on root, shoot, and plant weights in experiment 3. *Calonectria crotalariae* alone did not reduce soybean shoot weights in tests by Fortnum and Lewis (12) but did reduce shoot weights when combined with *M. incognita.* The nematode × fungus interactions in our nematode egg inoculum density studies all had an antagonistic effect on plant growth. A similar antagonistic interaction has been observed

between *H. glycines* and the stem canker fungus (*Diaporthe phaseolum* var. *caulivora*) on soybean stem dry weights (25).

Our results indicated that association with the fungus either before or during race determination did not influence the parasitic capability of the nematode population. Adeniji et al. (2) reported that the presence of *Phytophthora megasperma* var. *sojae* in root tissue did not alter cultivar resistance to *H. glycines.*

Threshold levels for soybean cyst nematode have been established, and control recommendations are based upon population levels present in soil. These thresholds do not take into account the presence of other plant pest species. An abundance of published research indicates that thresholds based on multiple pest densities will be superior to those based on the incidence and abundance of a single pest. Establishment of such thresholds will require additional research. However, results of this study demonstrate that the red crown rot fungus, *C. crotalariae,* has a marked influence on reproduction of the soybean cyst nematode, *H. glycines.* Although there is an antagonistic interaction between these two pathogens on plant growth and root injury, producers with fields known to be infested with propagules of both should follow cultural practices designed to reduce disease by each of these organisms.

Literature Cited

1. Abawi, G. S., and B. J. Jacobsen. 1984. Effect of initial inoculum densities of *Heterodera glycines* on growth of soybean and kidney bean and efficiency as hosts under greenhouse conditions. Phytopathology 74:1470–1474.

2. Adeniji, M. O., D. I. Edwards, J. B. Sinclair, and R. R. Malek. 1975. Interrelationships of *Heterodera glycines* and *Phytophthora megasperma* var. *sojae* in soybeans. Phytopathology 65:722–725.

3. Appel, A., G. R. Noel, D. I. Edwards, and S. M. Lim. 1984. Interrelationships of *Septoria glycines, Xanthomonas campestris* pv. *glycines,* and *Heterodera glycines* on soybeans in Illinois. Phytopathology 74:873 (Abstr.).

4. Barker, K. R., and T. H. A. Olthof. 1976. Relationships between nematode population densities and crop responses. Annual Review of Phytopathology 14:327–353.

5. Berner, D. K., G. T. Berggren, M. E. Pace, E. P. White, J. S. Gershey, J. A. Freedman, and J. P. Snow. 1986. Red crown rot: Now a major disease of soybeans. Louisiana Agriculture 29:4–5.

6. Berner, D. K., G. T. Berggren, J. P. Snow, and E. P. White. 1988. Distribution and management of red crown rot of soybeans in Louisiana. Applied Agricultural Research 3:160–166.

7. Byrd, D. W., T. Kirkpatrick, and K. R. Barker. 1983. An improved technique for clearing and staining plant tissues for detection of nematodes. Journal of Nematology 15:142–143.

8. Corbett, D. C. M., and G. A. Hide. 1971. Interactions between *Heterodera rostochiensis* Woll. and *Verticillium dahliae* Kleb. on potatoes and the effect of CCC on both. Annals of Applied Biology 68:71–80.

9. Diomande, M., and M. K. Beute. 1981. Effects of *Meloidogyne hapla* and *Macroposthonia ornata* on Cylindrocladium black rot of peanut. Phytopathology 71:491–496.

10. Diomande, M., and M. K. Beute. 1981. Relation of *Meloidogyne hapla* and *Macroposthonia ornata* populations to Cylindrocladium black rot in peanuts. Plant Disease 65:339–342.

11. Diomande, M., M. C. Black, M. K. Beute, and K. R. Barker. 1981. Enhancement of *Cylindrocladium crotalariae* root rot by *Meloidogyne arenaria* (Race 2) on a peanut cultivar resistant to both pathogens. Journal of Nematology 13:321–327.

12. Fortnum, B. A., and S. A. Lewis. 1983. Effects of growth regulators and nematodes on Cylindrocladium black rot of soybean. Plant Disease 67:282–284.

13. Francl, L. J., T. D. Wyllie, and S. M. Rosenbrock. 1988. Influence of crop rotation on population density of *Macrophomina phaseolina* in soil infested with *Heterodera glycines.* Plant Disease 72:760–764.

14. James, G. L. 1966. Effect of the causal fungus of brown root rot of tomatoes on the hatch of the potato root eelworm, *Heterodera rostochiensis* Woll. Nature 212:1466.

15. James, G. L. 1968. The interrelationships of the causal fungus of brown root rot of tomatoes and potato root eelworm, *Heterodera rostochiensis* Woll. Annals of Applied Biology 61:503–510.

16. Jenkins, W. R. 1964. A rapid centrifugal flotation technique for separating nematodes from soil. Plant Disease Reporter 48:692.

17. Jorgenson, E. C. 1970. Antagonistic interaction of *Heterodera schachtii* Schmidt and *Fusarium oxysporum* (Woll.) on sugarbeets. Journal of Nematology 2:393–397.

18. McKeen, C. D., and W. B. Mountain. 1960. Synergism between *Pratylenchus penetrans* (Cobb) and *Verticillium albo-atrum* R. & R. in eggplant wilt. Canadian Journal of Botany 38:789–794.

19. Mulrooney, R. P. 1988. Soybean disease loss estimate for southern United States in 1987. Plant Disease 72:915.

20. Overstreet, C., and E. C. McGawley. 1988. Influence of *Calonectria crotalariae* on reproduction of *Heterodera glycines* on soybean. Journal of Nematology 20:457–467.

21. Phipps, P. M., M. K. Beute, and K. R. Barker. 1976. An elutriation method for quantitative isolation of *Cylindrocladium crotalariae* microsclerotia from peanut field soil. Phytopathology 66:1255–1259.

22. Polychronopoulos, A. G., B. R. Houston, and B. F. Lownsbery. 1969. Penetration and development of *Rhizoctonia solani* in sugar beet seedlings infected with *Heterodera schachtii.* Phytopathology 59: 482–485.

23. Riggs, R. D., D. P. Schmitt, and G. R. Noel. 1988. Variability in race tests with *Heterodera glycines.* Journal of Nematology 20:565–572.

24. Ross, J. P. 1965. Predisposition of soybeans to Fusarium wilt by *Heterodera glycines* and *Meloidogyne incognita.* Phytopathology 55:361–364.

25. Russin, J. S., M. B. Layton, D. J. Boethel, E. C. McGawley, J. P. Snow, and G. T. Berggren. 1989. Development of *Heterodera glycines* on soybean damaged by soybean looper and stem canker. Journal of Nematology 21:108–114.

26. SAS Institute. 1985. SAS user's guide: Statistics, version 5 ed. Cary, NC: SAS Institute.

27. Todd, T. C., C. A. Pearson, and F. W. Schwenk. 1987. Effect of *Heterodera glycines* on charcoal rot severity in soybean cultivars resistant and susceptible to soybean cyst nematode. Supplement to the Journal of Nematology, Annals of Applied Nematology 1:35–40.

28. Whitney, E. D. 1971. Synergistic effect between *Heterodera schachtii* and *Pythium ultimum* on damping-off of sugarbeet vs. additive effect of *H. schachtii* and *P. aphanidermatum.* Phytopathology 61: 917.

29. Wrather, J. A., S. C. Anand, and V. H. Dropkin. 1984. Soybean cyst nematode control. Plant Disease 68:829–833.