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Influence of *Calonectria crotalariae* on Reproduction of *Heterodera glycines* on Soybean[1]

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Abstract: *Calonectria crotalariae* enhanced root penetration of Lee 74 (susceptible) and Centennial (resistant) soybeans by juveniles of race 3 of *Heterodera glycines*. Numbers of cysts in and on the roots of Lee 74 increased during the first 30 days in the presence of the fungus. Percentage of root infection by the fungus increased at 40 days in Lee 74 in the presence of the nematode. Numbers of cysts in soil at 80 and 120 days after inoculation with both organisms accounted for the significantly increased nematode population levels on Lee 74. In the presence of the fungus on the resistant cultivar, significantly increased levels of cysts were recovered from soil at 120 days. Fungus infection of Centennial roots also infected with the nematode increased from 58 to 86% at 120 days. An inoculum timing study in which Lee 74 was infested with the nematode and fungus individually, sequentially, and in combination at days 0 and 35 indicated that enhanced nematode reproduction was related more to early plant–fungus than to early plant–fungus–nematode interaction(s).

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

The soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, is widespread in the United States (18,22). Red crown rot (RCR) disease, caused by *Calonectria crotalariae* (Loos) Bell and Sobers (= *Cylindrocladium crotalariae* (Loos) Bell and Sobers), causes serious losses in soybean and peanut in the southeastern United States (4,17). Both pathogens are widespread in Louisiana, occurring in at least 13 parishes (G. T. Berggren, pers. comm.). Interactions of these two pathogens with other plant parasites have been investigated on a variety of hosts, including peanut, soybean, and kidney bean (1–3,6–8,19).

Several investigators have studied *H. glycines*–fungus complexes. *H. glycines* increases the severity of Phytophthora root rot (2) and Fusarium wilt (19) of soybean. Infection by *H. glycines* resulted in increased colonization of soybean roots by *Macrophomina phaseolina* (21). On California Light Red kidney bean, however, neither plant growth nor Fusarium root rot severity ratings were influenced by the presence of *H. glycines* (1). *H. glycines* and *Septoria glycines* Hemmi on soybean resulted in additive yield losses (3). *Meloidogyne hapla* Chitwood, *M. arenaria* (Neal) Chitwood, or *Criconemella ornata* (Raski) Luc & Raski have increased the severity of RCR disease on peanut (6–8). The influence of *H. glycines* on the severity of RCR disease of soybean has not been investigated.

Soybean *Glycine max* (L.) Merr. cv. Centennial in a commercial field near Burnside, Louisiana, had RCR disease symptoms and was also heavily infected with SCN. Subsequent laboratory and greenhouse observations revealed that root systems of many plants in this field were parasitized by *H. glycines* race 3 and *C. crotalariae*. Race 3 of *H. glycines* does not normally reproduce on Centennial (13).

Objectives of this research were 1) to determine if infection and reproduction of *H. glycines* on SCN resistant and susceptible soybean is influenced by *C. crotalariae* and 2) to evaluate the damage potential of the two pathogens, individually and in combination, to soybean.

Materials and Methods

Soybean plants which exhibited foliar symptoms of RCR disease and possessed diagnostic, dark red perithecia on the low-

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er stems were collected from the Burnside location. The lower 10 cm of stems above the soil line were removed and cut into 1-cm sections, disinfected for 1 minute in 0.5% NaOCl, dipped in sterile water, and incubated at ambient temperature (20–26 C) under continuous fluorescent light in petri dishes (100 × 15 mm) containing 2% water agar. Hyphal tips were transferred to dishes containing potato dextrose agar. Microsclerotia of *C. crotalariae* formed in darkness within 4 weeks at 28 C. They were extracted by blending 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 from the 75-μm-pore sieve were suspended in water, and aliquots necessary to obtain 50 microsclerotia/g soil were pipetted into 0.5 or 3.0 kg of a 3:2:1 mixture of steam-sterilized sandy loam soil, sand, and Weblite (Weblite Corp., Roanoke, VA) in polyethylene bags. The soil and microsclerotia were then thoroughly mixed, and 0.5 or 3.0 kg soil was placed in 10-cm-d (experiments 1, 2, 3) or 20-cm-d (experiments 4, 5) clay pots, respectively. A single 10-day-old soybean seedling was transplanted into a central depression (ca. 1.5 cm wide × 5.0 cm deep) in the soil of each pot.

At the conclusion of each test, a 0.5-g root subsample was collected randomly from each plant to determine the presence or absence of *C. crotalariae.* In tests 2, 4, and 5, the percentage of each root system infected by *C. crotalariae* was estimated. One-centimeter-long root fragments were disinfested for 30 seconds in 0.5% NaOCl and transferred to petri dishes containing a medium selective for *C. crotalariae* (16). Dishes were incubated for 10 days at ambient temperature under continuous fluorescent light. The percentage of infection per root system was estimated from 20 root fragments per plant and was calculated by multiplying by five the number of fragments infected with *C. crotalariae.*

A single cyst selected from the Burnside population of *H. glycines* originally isolated from Centennial soybean roots was increased and maintained on Lee 74 soybean under greenhouse conditions. Cysts used for inoculum were recovered from soil by a modified centrifugal-sugar flotation technique (12) with a 150-μm-pore (100 mesh) sieve at the bottom of a stack of nested sieves. Soil in pots was infested with 100 cysts of *H. glycines*/500 cm^3 soil at transplanting by pipetting suspensions containing the desired numbers of cysts per milliliter into five depressions (1.0 cm wide × 4.0 cm deep) surrounding each seedling. Nematode population development was monitored by counting juveniles, males, females, and cysts present on and in roots and in 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. Levels of immature life stages within roots were estimated from a 0.5-g subsample of root tissue collected randomly from each root system. Root subsamples were cleared and stained (5). Nematodes were extracted from soil by the same centrifugation procedure. Juveniles and males were recovered from the 44-μm-pore sieve and cysts from the 150-μm-pore sieve. Solutions used in extraction of *H. glycines* cyst and vermiform life stages contained 615 and 454 g sucrose/liter, respectively. Extraction efficiency was estimated to be 67% for cysts and 65% for second-stage juveniles and males.

All tests were conducted in a greenhouse with temperatures averaging 18–35 C. Supplementary light from both fluorescent and incandescent sources (ca. 3,000 lux at 45 cm above bench surface) was provided to give a 16:8 hour light : dark photoperiod each day. Plants were fertilized biweekly with a liquid 20-20-20 fertilizer solution, and insect and mite populations were controlled as necessary. The experimental design used for all tests was a randomized complete block with five replications unless otherwise stated. Data were subjected to analysis of variance using the SAS general linear models procedure (20).

Experiment 1: Lee 74 and Centennial seedlings were transplanted into pots either

immediately after infesting soil with *H. glycines* and (or) *C. crotalariae* or after establishing noninfested controls. Plants were harvested at 6, 12, 18, 24, and 27 days after infestation. The density and composition of *H. glycines* populations in soil and roots were determined at each interval.

Experiment 2: Lee 74 seedlings were transplanted into pots containing soil infested with *H. glycines, C. crotalariae,* both pathogens, or neither pathogen. Plants were harvested after 30 and 40 days. Nematode population development, percentage of *C. crotalariae* infection, and root injury levels were determined. To determine if *H. glycines* and *C. crotalariae* occur in the same root fragments, 40-day coinoculated Lee 74 roots were partitioned into those symptomatic and asymptomatic for RCR disease. The number of sedentary juveniles in 20 1-cm-long root fragments from both groups was determined in each coinoculated plant. 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.

Experiment 3: Seedlings of the two soybean cultivars were transplanted into pots of soil mixture containing *H. glycines, C. crotalariae,* both pathogens, or neither pathogen. After 80 days three replications of each treatment were harvested and data collected as described for experiment 1.

Experiment 4: Treatments and procedures for evaluating nematode reproduction, fungal infection, and root injury were the same as in experiment 2. Additionally, fresh shoot, root, and plant weights were recorded for both cultivars. The test was terminated after 120 days. Treatments were replicated four times.

Experiment 5: To determine the influence of inoculum timing and sequence on infection and colonization of roots by *H. glycines* and *C. crotalariae,* Lee 74 soybean was infested with the nematode, the fungus, or both, either at transplanting or after 35 days. To evaluate pathogen sequence effects, seedlings infested at transplanting with the fungus were infested with the nematode 35 days later, and plants infested with the nematode at transplanting were infested with the fungus 35 days later. Post-transplant *C. crotalariae* inoculum of 50 microsclerotia/g soil was applied via pipet as described for *H. glycines* inoculum. The treatments were replicated four times. After 95 days, plant and pathogen growth and reproduction data were collected as described.

Results

Experiment 1: Soybean plants infested with *H. glycines* alone had population totals representing different life stages of 472 individuals per pot for Lee 74 and 223 individuals for Centennial, after 27 days (Table 1). When *H. glycines* was combined with *C. crotalariae,* population densities were 1,792 on Lee 74 and 1,225 on Centennial, significantly higher than with *H. glycines* alone. One influence of the fungus on nematode population development was detected at 18 days on Lee 74. There was a significant increase in the level of females on roots coupled with a threefold increase in the number of juveniles within the roots. At 24 days *H. glycines* populations in the soil and roots were significantly greater on Lee 74 in the presence of the fungus, with higher numbers of cysts in soil and roots, higher numbers of females on roots, and higher numbers of juveniles within roots. The number of juveniles in soil increased significantly at 27 days in the presence of the fungus and, when combined with the threefold increase in levels of juveniles in roots, accounted for the significantly greater 27-day population totals for Lee 74.

The influence of the fungus on nematode population levels on Centennial was not observed until 24 days after infestation. The increase was significant on this cultivar on day 27. Stained Centennial root systems contained many deteriorating nematodes in various life cycle stages at 27 days.

Heterodera glycines in root and soil population densities were different ($P < 0.05$) between Lee 74 and Centennial whether *C. crotalariae* was present or absent (Table

TABLE 1. Numbers of different stages of *Heterodera glycines* recovered from soil and roots of Lee 74 and Centennial soybeans during five intervals following infestation with *H. glycines* alone (−) and combined with *Calonectria crotalariae* (+).

Days	Treatment	Cysts		Females		Juveniles		Total nematodes		
		Soil	Roots	Soil	Roots	Soil	Roots	Soil	Roots	Pot
					Lee 74					
6	−	58 a	0 a	0 a	0 a	22 a	78 a	80 a	78 a	158 a
	+	55 a	0 a	0 a	0 a	12 a	24 a	67 a	24 b	91 a
12	−	71 a	0 a	0 a	0 a	0 a	160 a	71 a	160 a	231 a
	+	57 a	0 a	0 a	0 a	1 a	113 a	58 a	113 a	171 a
18	−	76 a	0 a	0 a	39 b	24 a	155 a	100 a	194 b	294 a
	+	75 a	0 a	0 a	182 a	43 a	486 a	118 a	668 a	786 a
24	−	57 b	20 b	170 a	114 b	9 a	104 b	236 b	238 b	474 b
	+	239 a	385 a	237 a	255 a	19 a	307 a	495 a	947 a	1,442 a
27	−	59 b	29 b	85 a	69 a	11 b	219 a	155 a	317 b	472 b
	+	198 a	464 a	197 a	297 a	30 a	606 a	425 a	1,367 a	1,792 a
					Centennial					
6	−	56 a	0 a	0 a	0 a	8 a	31 a	64 a	31 a	95 a
	+	60 a	0 a	0 a	0 a	18 a	65 a	78 a	65 a	143 a
12	−	69 a	0 a	0 a	0 a	7 a	161 a	76 a	161 a	237 a
	+	72 a	0 a	0 a	0 a	0 b	113 a	72 a	113 a	185 a
18	−	83 a	0 a	0 a	0 a	7 a	283 a	90 a	283 a	373 a
	+	64 b	0 a	0 a	0 a	18 a	186 a	82 a	186 a	268 a
24	−	72 a	2 a	7 a	3 a	7 a	225 a	86 a	230 a	316 a
	+	81 a	10 a	13 a	4 a	9 a	409 a	103 a	423 a	526 a
27	−	47 a	1 a	8 a	2 a	3 a	162 b	58 a	165 b	223 b
	+	82 a	12 a	11 a	13 a	3 a	1,104 a	96 a	1,129 a	1,225 a

Values are means of five replications. Within time intervals, means in columns followed by the same letter are not significantly different ($P < 0.05$) according to Duncan's multiple-range test.

TABLE 2. Analysis of variance for effects of *Calonectria crotalariae*, soybean cultivar (Lee 74 and Centennial), and time on levels of cysts, *Heterodera glycines* females and juveniles, from soil and roots during 27 days following infestation with the nematode.

Variable	Cysts		Females		Juveniles		Total nematodes		
	Soil	Roots	Soil	Roots	Soil	Roots	Soil	Roots	Pot
Cultivar (C)	**	**	NS	**	NS	NS	NS	NS	NS
Fungus (F)	**	**	NS	*	NS	**	NS	**	**
Time (T)	NS	**	*	**	**	*	*	NS	NS
C × T	NS	**	*	**	*	NS	NS	NS	NS
F × T	*	**	NS	NS	NS	**	NS	**	**
F × C	NS	**	NS	*	NS	NS	NS	NS	NS
F × C × T	NS	**	NS	NS	NS	**	NS	NS	NS

*, ** = significant at $P = 0.05$ and $P = 0.01$ based on F-test.
NS = nonsignificant at $P = 0.05$ based on F-test.

2). Cyst population densities in the soil were influenced significantly ($P < 0.05$) by cultivar, fungus, and fungus × time interactions. Cyst populations from roots were influenced significantly by all variables, and female populations from roots were influenced significantly by all variables except fungus × time and fungus × cultivar × time. Fungus, time, fungus × time, and fungus × time × cultivar interactions influenced the levels of juveniles in roots significantly. The number of juveniles in soil was influenced significantly by both time and cultivar × time interaction. Soil totals that included juveniles, females, and cysts, however, were influenced by time only. Population totals for both roots and nematodes per pot were effected ($P < 0.01$) by both fungus and fungus × time interaction.

Experiment 2: At 30 but not 40 days after infestation, the total number of nematodes per pot increased significantly in coinoculated Lee 74 plants (Table 3). Total nematode population density increased approximately fourfold with or without the fungus between 30 and 40 days primarily because of a 14-fold increase in the number of juveniles in roots. The number of cysts present on coinoculated roots was significantly ($P < 0.05$) higher at 30 but not at 40 days. The presence of *C. crotalariae* increased the

TABLE 3. Life-stage distribution of *Heterodera glycines* recovered from soil and roots of Lee 74 soybean at 30 and 40 days following infestation with *H. glycines* alone (−) and combined with *Calonectria crotalariae* (+).

Days	Treatment	Cysts		Females	Juveniles		Total nematodes		
		Soil	Root	Root	Soil	Root	Soil	Root	Pot
30	−	54 a	32 b	454 b	85 a	131 a	343 a	617 b	960 b
	+	88 a	58 a	584 a	212 a	129 a	450 a	771 a	1,221 a
40	−	250 a	239 a	664 b	765 a	1,797 a	1,239 a	2,700 a	3,939 a
	+	267 a	357 a	1,037 a	874 a	1,803 a	1,346 a	3,197 a	4,543 a
					Fungus				
		NS	NS	*	NS	NS	NS	NS	NS
					Time				
		**	**	NS	*	**	*	**	**
					Fungus × time				
		NS	NS	NS	NS	NS	NS	NS	NS

Values are means of 10 and 5 replications, respectively. Within time intervals, means in columns followed by the same letter are not significantly different ($P < 0.05$) according to Duncan's multiple-range test.
*, ** = significant at $P = 0.05$ and $P = 0.01$ based on F-test.
NS = nonsignificant at $P = 0.05$ based on F-test.

TABLE 4. *Heterodera glycines* numbers and life stages recovered from soil and roots of the soybean cultivars Lee 74 and Centennial 80 days after infestation with *H. glycines* alone (−) and combined with *Calonectria crotalariae* (+).

Treatment	Cysts		Juveniles		Males	Total nematodes		
	Soil	Roots	Soil	Roots	Soil	Soil	Roots	Pot
				Lee 74				
−	160 b	361 a	124 a	40 a	16 b	300 b	401 a	701 b
+	1,156 a	288 a	184 a	51 a	24 a	1,364 a	339 a	1,703 a
				Centennial				
−	72 a	5 a	0 a	111 a	0 a	72 a	116 a	188 a
+	32 a	11 a	4 a	80 a	0 a	36 a	91 a	127 a
				Cultivar				
	*	**	**	*	NS	**	**	**
				Fungus				
	*	NS	NS	NS	NS	*	NS	NS
				Cultivar × fungus				
	*	NS	NS	NS	NS	*	NS	NS

Values are means of three replications. Within cultivars, means in columns followed by the same letter are not significantly different ($P < 0.05$) according to Duncan's multiple-range test.
*, ** = significant at $P = 0.05$ and $P = 0.01$ based on F-test.
NS = nonsignificant at $P = 0.05$ based on F-test.

number of females on roots at both time intervals. Population levels of all nematode life stages except females on roots were significantly affected by time. The fungus × time interactions were not significant.

The nematode and fungus did not appear to colonize the same tissue. Eighty-two percent of the sedentary nematode life stages observed in the roots of coinoculated plants were found in areas where no signs of the fungus or root lesions characteristic of RCR disease were visible.

The percentage of fungus infection and injury ratings for roots at 30 days were not significantly affected by the nematode. At 40 days, root infection by the fungus was 19% in the absence of the nematode and 57%, significantly ($P < 0.05$) greater, in the presence of the nematode. The nematode × fungus interaction on 40-day root injury rating was significant ($P < 0.01$) and averaged 3.5 for plants infested with both organisms together and significantly less (2.0 and 1.0, respectively) for separate infestations with the fungus and nematode.

Experiment 3: Populations of *H. glycines* were different ($P < 0.05$) between cultivars at 80 days (Table 4). In the presence of *C. crotalariae,* significantly greater numbers of nematodes were recovered per pot of Lee 74 but not of Centennial. There was an approximate sevenfold increase in levels of cysts in soil when the fungus was present on Lee 74. The fungus and cultivar × fungus significantly ($P < 0.05$) affected soil cyst and total nematode numbers.

Experiment 4: Population trends of *H. glycines* on both cultivars were similar to those in experiment 3. That is, in the presence of *C. crotalariae,* the numbers of nematodes recovered were significantly higher on Lee 74 but not on Centennial (Table 5). The higher population levels on Lee 74 reflected primarily more cysts recovered from soil. On the SCN-resistant cultivar Centennial also, the number of cysts in the soil was significantly higher in the presence of the fungus. There was a significant cultivar effect ($P < 0.01$) on cyst numbers and total nematodes from soil and roots. There were also significant effects ($P < 0.01$) of fungus and cultivar × fungus interactions on densities of soil cysts, soil nematodes, and population totals per pot.

Both cultivars infested with both organ-

TABLE 5. *Heterodera glycines* numbers and life stages recovered from soil and roots of the soybean cultivars Lee 74 and Centennial at 120 days after infestation with *H. glycines* alone (−) and in combination with *Calonectria crotalariae* (+).

Treatment	Cysts		Juveniles		Males	Total nematodes		
	Soil	Roots	Soil	Roots	Soil	Soil	Roots	Pot
				Lee 74				
−	2,980 b	603 a	305 a	123 a	45 a	3,330 b	726 a	4,055 b
+	7,967 a	816 a	475 a	149 a	50 a	8,492 a	965 a	9,456 a
				Centennial				
−	595 b	70 a	115 a	124 a	15 a	725 a	194 a	919 a
+	1,065 a	130 a	160 a	123 a	40 a	1,265 a	254 a	1,519 a
				Cultivar				
	**	**	NS	NS	NS	**	**	**
				Fungus				
	**	NS	NS	NS	NS	**	NS	**
				Cultivar × fungus				
	**	NS	NS	NS	NS	**	NS	**

Values are means of four replications. Within cultivars, means in columns followed by the same letter are not significantly different ($P < 0.05$) according to Duncan's multiple-range test.
*, ** = significant at $P = 0.05$ and $P = 0.01$ based on F-test.
NS = nonsignificant at $P = 0.05$ based on F-test.

isms had significantly lower plant weights than did noninfested controls (Table 6). Weights of roots of Lee 74 but not Centennial were reduced significantly more by the two organisms combined than by either alone. Infested and noninfested Lee 74 shoot weights did not differ significantly. Centennial plants that received both *H. glycines* and *C. crotalariae* had significantly lower shoot weights than those of plants that received nematodes only. There was no fungus × nematode or cultivar × fungus × nematode interaction.

Infection of Lee 74 roots by *C. crotalariae* was not affected by *H. glycines*. Infection of Centennial roots was greater with *H. glycines* ($P < 0.05$) than without. Root injury ratings for both cultivars increased nu-

TABLE 6. Plant fresh weights, root infection by *Calonectria crotalariae*, and root injury ratings (RIR) of Lee 74 and Centennial soybeans 120 days after infestation with *Heterodera glycines* (N) and *C. crotalariae* (F) alone and combined.

Treatment†	Plant fresh weights (g)			Root infection (%)	RIR‡
	Shoot	Root	Plant		
			Lee 74		
N	90.1 a	19.4 b	109.4 ab		2.6 a
F	106.2 a	23.8 ab	130.1 ab	90 a	2.3 a
N + F	87.0 a	11.3 c	98.3 b	70 a	2.8 a
C	110.5 a	26.7 a	139.3 a		
			Centennial		
N	119.6 a	25.1 a	144.7 a		1.4 a
F	106.0 ab	16.6 b	122.6 bc	58 b	2.0 a
N + F	95.2 b	16.7 b	111.8 c	86 a	3.0 a
C	110.3 ab	22.7 ab	133.1 ab		

Values are means of four replications. Within cultivars, means in columns followed by the same letter are not significantly different ($P < 0.05$) according to Duncan's multiple-range test.
† N = *H. glycines*; F = *C. crotalariae*; C = noninoculated control.
‡ RIR rating scale = 1–5 (1 = no symptoms; 5 = > 75% necrosis).

TABLE 7. Influence of timing, association with *Calonectria crotalariae,* and infestation sequence on the numbers and life stages of *Heterodera glycines* recovered from soil and roots of Lee 74 after 95 days.

Time sequence of infestation								
At transplant†	After 35 days	Nematodes in roots			Nematodes in soil			Total nematodes per pot
		Cysts	Juveniles	Total	Cysts	Juveniles	Total	
N		308 a	57 b	365 a	3,180 b	300 b	3,480 b	3,845 b
N + F		608 a	26 b	634 a	4,920 a	450 ab	5,370 a	6,004 a
	N	292 a	6 b	298 a	1,785 c	270 b	2,055 c	2,353 b
	N + F	287 a	521 a	808 a	1,320 c	225 b	1,545 c	2,353 b
N	F	518 a	156 ab	737 a	2,310 bc	120 b	2,430 bc	3,167 b
F	N	538 a	16 b	554 a	5,160 a	930 a	6,090 a	6,644 a
				Fungus				
		NS	NS	NS	**	*	**	**
				Time				
		NS	NS	NS	NS	*	NS	NS
				Fungus × time				
		NS	NS	NS	NS	NS	NS	NS

Values are means of four replications. Means in columns followed by the same letter are not significantly different ($P < 0.05$) according to Duncan's multiple-range test.
*, ** = significant at $P = 0.05$ and $P = 0.01$ based on F-test.
† N = *H. glycines*; F = *C. crotalariae.*
NS = nonsignificant at $P = 0.05$ based on F-test.

merically, but nonsignificantly, with combined inoculations.

Experiment 5: At-transplant coinfestation of Lee 74 with *H. glycines* and *C. crotalariae* resulted in nematode population levels significantly greater than those from Lee 74 infested with the nematode alone (Table 7). The number of cysts in the soil was significantly higher in the presence of the fungus. Numbers of cysts on roots and juveniles in soil also were higher, but not significantly, in the presence of the fungus. The weights of roots, shoots, and whole plants infested with the nematode alone or both nematode and fungus at transplanting were significantly lower than those of noninfested controls (Table 8). Weights of roots of plants infested with both organisms were not significantly less than weights of plant roots infested with nematodes alone. Root injury ratings significantly ($P < 0.05$) increased when the nematode was combined with the fungus.

The significant nematode population increase that occurred when the nematode and fungus were added at transplanting was not observed when the pathogens were added 35 days after transplanting (Table 7). The number of juveniles in roots, however, increased significantly in the presence of the fungus. Root weights of infested plants were significantly lower than those of noninfested controls (Table 8). There were no significant differences between root weights of plants that received either organism alone or both. Shoot and plant weights followed similar trends, but only weights of plants that received the nematode alone were significantly lower than those of controls. Infection percentage of roots by the fungus and root injury ratings were not significantly affected by treatment.

When infestation with one organism was 35 days after transplanting, the order of inoculation significantly influenced the development and final population density of *H. glycines.* Despite the fact that populations of the nematode added 35 days after infestation with the fungus had less time (60 days) in which to reproduce, the final population was approximately double (6,644/plant) that observed when the nematode was added alone at transplanting

TABLE 8. Plant weights, root infection by *Calonectria crotalariae,* and root injury ratings (RIR) of Lee 74 soybeans as influenced by timing, association, and infestation sequence of *Heterodera glycines* and *C. crotalariae* after 95 days.

Time sequence of infestation		Plant fresh weights (g)			Root infection	
At transplant†	After 35 days	Root	Shoot	Plant	(%)	RIR‡
N		2.9 cd	16.8 b	19.6 b		1.1 b
F		5.8 b	28.4 ab	34.2 ab	18 a	1.5 b
N + F		2.1 d	16.9 b	19.8 b	38 a	3.8 a
	N	3.7 bcd	19.2 b	22.9 b		1.1 b
	F	3.9 bcd	23.9 ab	27.6 ab	13 a	2.5 ab
	N + F	4.2 bcd	23.4 ab	27.6 ab	14 a	2.5 ab
F	N	5.2 bc	27.4 ab	32.7 ab	28 a	2.0 ab
N	F	3.7 bcd	22.2 ab	25.9 b	10 a	3.0 ab
C		8.9 a	32.5 a	41.4 a		

Values are means of four replications. Means in columns followed by the same letter are not significantly different ($P < 0.05$) according to Duncan's multiple-range test.

† N = *H. glycines*; F = *C. crotalariae*; C = noninoculated control.

‡ RIR rating scale = 1–5 (1 = no symptoms; 5 = > 75% necrosis).

(3,167/plant) (Table 8). Cyst and juvenile levels in the soil were significantly higher when the nematode followed the fungus by 35 days than when the fungus followed the nematode. Root weights did not reflect the order of infestation, but weights of both inoculated groups were significantly lower than the noninfested control. Values for shoot and plant weights followed similar trends. Root infection by *C. crotalariae* averaged 28% when the fungus was added at transplanting and 10% when it was added after 35 days. Root injury ratings averaged 2.0 when the fungus preceeded the nematode and 3.0 when the nematode preceded the fungus.

DISCUSSION

Investigators have found that *C. crotalariae* can significantly influence nematode reproduction on soybean (10,11) and peanut (6). Populations of *Hoplolaimus columbus* Sher on soybean were increased significantly by the presence of *C. crotalariae,* whereas populations of *Pratylenchus scribneri* Steiner and *Meloidogyne incognita* (Kofoid&White)Chitwoodwereinhibited.Ross (19) reported enhanced *H. glycines* reproduction on soybean in the presence of *Fusarium.* Other investigators have reported that nematodes can influence *C. crotalariae.* Diomande et al. (8) found that *M. arenaria* race 2 enhanced disease ratings by *C. crotalariae* on a cultivar of peanut resistant to both pathogens.

Results of the experiments reported here suggest the following: 1) The presence of *C. crotalariae* increases penetration of Lee 74 and Centennial roots by juveniles of *H. glycines* during the first generation (18–27 days) after infestation. 2) When the nematode and fungus are together on Lee 74, increased root penetration by the nematode results in significantly higher population densities and greater root injury than that caused by either pathogen alone. 3) On Centennial, the fungus-related increase in root penetration by, and maturation of, juveniles enhances the potential for adaptation of the nematode to the resistant cultivar.

Other investigators (14,15) have hypothesized that physiological changes in fungus-infected root tissues make them more attractive to nematodes. Data from experiments 1 and 5 may support this hypothesis; however, *H. glycines* may be stimulated directly by some metabolite of the fungus, before or after infection. Whatever the mechanism, the timing and sequence data (experiment 5, Table 6) suggest that the interaction between the fungus and plant

during the early stages (V2–V3) of plant development (9) has a marked influence on *H. glycines.*

The increased penetration by *H. glycines* into roots of *C. crotalariae*-infected Lee 74 soybean was a major factor contributing to greater final population densities of the nematode in experiments 2, 3, 4, and 5. The increased levels of both root and soil juvenile and adult stages of *H. glycines* during the first 30–40 days after infestation suggests that, in addition to increasing the penetration rate, the maturation rate of the nematode also was substantially increased by *C. crotalariae.* Hence, on this cultivar, cyst numbers that exceeded the inoculum level (100/500 g soil) were first detected 24 days after inoculation in the presence of the fungus and not until 40 days in its absence. Earlier maturation may have resulted from 1) the increased root injury resulting from the presence of both the nematode and the fungus, 2) the lowered food supply which results at higher nematode population levels, or 3) a combination of these factors.

Enhanced penetration of Centennial roots by *H. glycines* accounted almost entirely for the significant difference in the final population density. Few of the juveniles that penetrated Centennial roots matured, and population levels above inoculum levels were not detected until 120 days in experiment 4. Cyst numbers in soil and total *H. glycines* population levels per pot suggest a shift in SCN races or a decline in the resistance of this cultivar to race 3. Subsequent race analysis with progeny of females recovered from root systems from this experiment, and others conducted during this research, indicates that the population remains race 3 (unpubl.).

Root injury ratings paralleled root infection percentages and reflected the root injury resulting from the activities of both organisms. The significant increase in the percentage of root infection by *C. crotalariae* on Centennial suggests that the primary influence of *H. glycines* may be to enhance colonization of soybean roots by the fungus.

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