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Interrelationships of *Rotylenchulus reniformis* with *Rhizoctonia solani* on Cotton[1]

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Abstract: The interrelationships between reniform nematode (*Rotylenchulus reniformis*) and the cotton (*Gossypium hirsutum*) seedling blight fungus (*Rhizoctonia solani*) were studied using three isolates of *R. solani,* two populations of *R. reniformis* at multiple inoculum levels, and the cotton cultivars Deltapine 90 (DP 90) and Deltapine 41 (DP 41). Colonization of cotton hypocotyl tissue by *R. solani* resulted in increases ($P \leq 0.05$) in nematode population densities in soil and in eggs recovered from the root systems in both 40- and 90-day-duration experiments. Increases in soil population densities resulted mainly from increases in juveniles. Enhanced reproduction of *R. reniformis* in the presence of *R. solani* was consistent across isolates (1, 2, and 3) of *R. solani* and populations (1 and 2) and inoculum levels (0.5, 2, 4, and 8 individuals/g of soil) of *R. reniformis,* regardless of cotton cultivar (DP 90 or DP 41). Severity of seedling blight was not influenced by the nematode. *Rhizoctonia solani* caused reductions ($P \leq 0.05$) in cotton growth in 40- and 90-day periods. *Rotylenchulus reniformis* reduced cotton growth at 90 days. The relationship between nematode inoculum levels and plant growth reductions was linear. At 90 days, the combined effects of these pathogens were antagonistic to plant growth.

Key words: cotton, *Gossypium hirsutum,* interrelationships, interaction, nematode, reniform nematode, *Rhizoctonia solani, Rotylenchulus reniformis,* seedling blight.

In the United States, yield loss in cotton (*Gossypium* spp.) attributed to nematodes is estimated at 2.4% (2). The reniform nematode, *Rotylenchulus reniformis,* is recognized as an important pest of cotton in the United States (12). It has been reported from 47 of the 67 parishes in Louisiana and is probably responsible for most of the nematode damage done to the cotton crop (E. C. McGawley and C. Overstreet, pers. comm.). Seedling diseases in cotton account for an annual loss of 2.7% in the United States and 2% in Louisiana (2). *Rhizoctonia solani* is the fungus most commonly isolated from diseased cotton seedlings in Louisiana. Population density of *R. reniformis* in soil is consistently higher in areas of cotton fields where seedling blight symptoms are apparent, compared with areas in which seedlings do not exhibit disease symptoms (E. C. McGawley and C. Overstreet, pers. comm.).

Numerous investigators have detailed examples of relationships between *Rhizoctonia solani* and nematodes in which their association results in augmented disease incidence and altered nematode reproduction. Increased incidence of seedling disease caused by *R. solani* was noticed in the presence of *Maloidogyne* spp. on various crops, including cotton (4–6,8,24,25). Resistance to *Meloidogyne incognita* in pepper (*Capsicum annuum*) was lost in the presence of *R. solani* (11). *Rhizoctonia solani* inhibited the multiplication of *M. incognita* and *R. reniformis* in cowpea (*Vigna sinensis*) (17). In tomato (*Lycopersicon esculentum*), the presence of *R. solani* increased the ratio of males to females of *Heterodera rostochiensis* (16).

The association of *R. reniformis* with *R. solani* in disease complexes in cotton and okra (*Abelmoschus esculentus*) has been studied by a few researchers (1,18,19,26). Brodie and Cooper (1) investigated the involvement of *R. reniformis* and *R. solani* in the seedling disease complex of cotton. They found that at 40 nematodes/g of soil, susceptibility to postemergence damping-off was greater than that observed in the

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absence of the nematode. However, their study focused mainly on the severity of seedling blight in the presence of both pathogens. No information related to the reproduction of *R. reniformis* was provided. The objectives of our study were i) to determine the influence of *R. solani* on reproduction of *R. reniformis*, ii) to study the impact of *R. reniformis* on the severity of *Rhizoctonia* seedling blight of cotton, and iii) to evaluate the effect of both pathogens on cotton growth.

Materials and Methods

Single egg masses of *R. reniformis* originally isolated from cotton roots collected from Morehouse and Avoyelles Parishes, Louisiana, were increased and maintained on Rutgers tomato in a greenhouse and are referred to as populations 1 and 2, respectively. Both populations were identified as *R. reniformis* (9) and typed as race A (10). Vermiform stages of *R. reniformis* used for inoculum were recovered from soil by a modified centrifugal-sugar flotation technique (14) with nested 425-µm-pore (40 mesh) and 45-µm-pore (325 mesh) sieves. Five-day-old cotton seedlings were transplanted singly into 10-cm-d (experiments 1 and 2) or 15-cm-d (experiments 3 and 4) clay pots that contained 500 or 1,000 g, respectively, of a 3:2:1 mixture of methyl bromide-treated loamy soil (80.8% sand, 4.7% silt, 14.5% clay), autoclaved sand, and Weblite (Weblite Corp., Roanoke, VA). Soil pH was adjusted to 6.5 by adding aluminum sulfate (4 g/kg of soil). Fifteen-day-old seedlings were inoculated by pipetting suspensions containing the desired numbers of nematodes into two depressions (1.0 cm wide × 4.0 cm deep) 2.5 cm from each seedling. At harvest, plant stems were cut 2.5 cm above the soil surface and fresh weights of shoots recorded. Root systems were separated from soil by agitating the root ball in a 6-liter graduated pitcher containing 4 liters of water. An additional liter of water was poured over the root system as it was removed from the pitcher. The soil–water suspension was stirred and nematodes present in 500 ml of the suspension were extracted (14), counted, and total numbers per pot and rates of reproduction (R) were computed, where R = Pf/Pi and Pf equals final population density and Pi equals inoculum level (7). Each root system was blotted with paper towels and the weight recorded. Eggs were extracted from root pieces that were randomly selected and weighed to give a 3.0-g subsample (13). Numbers of sessile females were counted on 10 (experiments 2, 3, and 4) randomly selected 2.5-cm root segments.

Three isolates (1, 2, and 3) of *R. solani* were obtained from cotton seedlings exhibiting seedling blight symptoms in fields in Richland, Rapides, and Franklin Parishes, respectively. Hypocotyl regions were cut into 2-cm sections, disinfested for 3 minutes in 0.5% NaOCl, rinsed in sterile water, and incubated on 2% water agar at ambient temperature (22–26 C) for 24 hours. Hyphal tips were transferred to potato dextrose agar for maintenance. The three isolates were typed as members of Anastomosis Group 4 (23). Whole grain oats were soaked overnight in water (1.5 ml water/g dry grain) in wide-mouth Mason jars, autoclaved (120 C at 1.09 kg-force/cm^2) for 45 minutes on two successive days, and inoculated with two potato dextrose agar discs (1 cm-d) cut from the growing edge of a 3-day-old fungal culture. After 20 days, the contents of the jars were removed, mixed, air dried for a week, and stored in plastic bags at 5 C until use (3). Preliminary studies conducted using 1, 2, or 3 oat grains colonized by *R. solani* per pot as inoculum showed that a single infested oat grain would elicit a sublethal level of infection. A depression (1.5-cm-d, 1.0 cm deep) was made in soil at a distance of 2.5 cm from the 15-day-old cotton seedling and a single infested oat grain was added and covered with soil. At harvest, disease severity was indexed on a 0–3 scale where 0 = no hypocotyl necrosis or root discoloration; 1 = hypocotyl necrosis, slight root discoloration; 2 = hypocotyl necrosis, moderate root discoloration; and

3 = hypocotyl necrosis, marked root discoloration. The hypocotyl regions were cut into 2-cm sections, disinfested for 3 minutes in 0.5% NaOCl, rinsed in sterile water, and incubated on 2% water agar at ambient temperature for 48 hours to verify the presence of the fungus.

On the basis of data collected in preliminary host suitability studies involving reniform nematode and six cotton cultivars popular in Louisiana, the cotton cultivars Deltapine 90 (DP 90) and Deltapine 41 (DP 41) were selected for use in these studies. Deltapine 90 is a much better host for reniform nematode than is DP 41. All tests were conducted in a greenhouse with temperatures ranging from 22–35 C. Plants were fertilized every 2 weeks, commencing at 5 days after transplanting, with a 23-19-17 fertilizer solution (800 ppm N, 700 ppm P, and 450 ppm K). The experimental design for all the tests was a randomized complete block with factorial treatment arrangement. Unless stated otherwise, all experiments were repeated at least once. Data were subjected to analysis using the SAS General Lineal Model procedure (28). When the number of treatment levels exceeded two, single-degree-of-freedom orthogonal contrasts were used to test for differences between levels.

Experiment 1: Effects of *R. solani* isolates 1, 2, and 3 on reproduction by population 1 of *R. reniformis* were examined. Treatments consisted of two nematode infestation levels (0 and 4,000/pot; 87% juveniles, 9% males, and 4% infective females), four fungal inocula (autoclaved oat grain, oat grain colonized by *R. solani* isolate 1, 2, or 3) and one cultivar (DP 90). Inocula were added at transplanting as described above. There were a total of eight treatment combinations, each replicated five times. The experiment was terminated after 40 days.

Experiment 2: Individual and interactive effects of populations 1 and 2 of *R. reniformis* and *R. solani* isolates 1 and 2 were studied on two cotton cultivars. Treatments consisted of two infestation levels of the nematode (0 and 4,000/pot each of the population 1 or 2; 83% juveniles, 11% males, and 6% infective females of population 1; 81% juveniles, 13% males, and 6% infective females of population 2), three fungal inocula (autoclaved oat grain, oat grain colonized by *R. solani* isolate 1 or 2), and two cotton cultivars (DP 90 and DP 41). There were a total of 18 treatment combinations, each replicated five times. The experiment was terminated after 40 days.

Experiment 3: The individual and interactive effects of multiple inoculum levels of *R. reniformis* population 1 and *R. solani* isolates 1 and 2 were examined on two cotton cultivars. Treatment consisted of three infestation levels of the nematode (0, 500, and 4,000/pot; 82% juveniles, 11% males, and 7% infective females), three fungal inocula (autoclaved oat grain, oat grain colonized by *R. solani* isolate 1 or 2), and the cultivars DP 90 and DP 41. Each treatment combination was replicated five times, and the experiment was terminated after 90 days.

Experiment 4: Individual and interactive effects of multiple inoculum levels of reniform nematode population 1 and *R. solani* isolate 1 were examined in this study. The duration of this experiment was 90 days. Treatments consisted of four infestation levels of the nematode (0, 500, 2,000, and 8,000/pot; 90% juveniles, 7% males, and 3% infective females), two fungal inocula (autoclaved oat grain or oat grain colonized by isolate 1 of *R. solani*) and the cultivar DP 90, for a total of eight treatment combinations, each replicated five times.

Results

Experiment 1: Colonization of cotton seedlings by *R. solani* resulted in an increase ($P \leq 0.01$) in reniform juveniles and total nematodes recovered from soil, Pf/Pi ratios, and eggs/g of root (Table 1). There were differences ($P \leq 0.05$) between *R. solani* isolates 1 and 2 with respect to their impact on juveniles and total nematodes recovered from soil and Pf/Pi ratios. Fungus isolates 2 and 3 differed regarding their influence on egg production ($P \leq$

TABLE 1. Effects of *Rhizoctonia solani* isolates on reproduction of *Rotylenchulus reniformis* on Deltapine 90 cotton at 40 days after inoculation.

Fungus	Nematodes/500 g soil				Pf/Pi†	Eggs/g root
	Juveniles	Males	Females	Total		
None	2,963	211	73	3,247	0.8	1,342
Isolate 1	4,100	264	107	4,471	1.1	2.373
Isolate 2	3,358	231	99	3,688	0.9	2,332
Isolate 3	3,148	297	99	3,544	0.9	1,569
Contrast						
0 vs. 1 + 2 + 3	*	NS	NS	*	*	***
1 vs. 2	*	NS	NS	*	*	NS
2 vs. 3	NS	NS	NS	NS	NS	***
Source						
Fungus	**	NS	NS	**	*8	***

Data are means of five replicates.
† Pi = initial nematode infestation level (4,000/500 g soil). Pf = final nematode population density in soil.
*, **, *** significant at $P \leq 0.05$, 0.01, and 0.0001 based on F test, respectively; NS = nonsignificant.

0.0001). At 40 days after inoculation, *R. reniformis* did not affect either plant growth or disease indices (Table 2). The fungus reduced ($P \leq 0.0001$) shoot, root, and plant fresh weights. Each isolate reduced plant growth, but there were no differences between isolates.

Experiment 2: The only observed difference in reproduction ($P \leq 0.05$) between the two reniform nematode populations was in the numbers of juveniles recovered from soil (Table 3). The presence of *R. solani* resulted in an increase ($P \leq 0.05$) in juveniles, males, and total nematodes. Pf/Pi ratios, numbers of sessile females, and eggs/g root were also greater in the presence of *R. solani* ($P \leq 0.01$). The two *Rhizoctonia* isolates did not differ in their influence on reproduction by *R. reniformis*. Juveniles, total nematodes from soil, Pf/Pi ratios, numbers of sessile females, and eggs/g root were greater ($P \leq 0.05$) on DP 90 than on DP 41. The numbers of eggs/g root were affected by interactions between

TABLE 2. Effects of *Rotylenchulus reniformis* and *Rhizoctonia solani* isolates on fresh weights and disease indices of Deltapine 90 cotton at 40 days after inoculation.

Treatment	Inocula	Plant fresh weight (g)			Disease index‡
		Shoot	Root	Plant	
Nematode	0	4.8	4.8	8.8	1.3
	4,000†	4.8	4.2	9.0	1.3
Fungus	None	6.1	5.1	11.2	0.0
	Isolate 1	4.1	3.8	7.9	1.8
	Isolate 2	4.4	3.7	8.1	1.9
	Isolate 3	4.5	3.7	8.2	1.8
Contrast					
0 vs. 1 + 2 + 3	***	***	***	***	***
1 vs. 2	NS	NS	NS	NS	NS
2 vs. 3	NS	NS	NS	NS	NS
Source					
Nematode	NS	NS	NS	NS	NS
Fungus	***	***	***	***	***
N × F	NS	NS	NS	NS	NS

Data are means of five replicates.
† Vermiform stages/500 g soil.
‡ Disease index scale = 0–3 (0 = no hypocotyl necrosis or root discoloration; 1 = hypocotyl necrosis, slight root discoloration; 2 = hypocotyl necrosis, moderate root discoloration; 3 = hypocotyl necrosis, severe root discoloration).
*, **, *** = significant at $P \leq 0.05$, 0.01, and 0.0001 based on F test, respectively; NS = nonsignificant.

TABLE 3. Effects of *Rhizoctonia solani* isolates and the cotton cultivars Deltapine 90 (DP90) and Deltapine 41 (DP41) on reproduction of two populations of *Rotylenchulus reniformis* at 40 days after inoculation.

Treatment	Inocula/ cultivars	Nematodes/500 g soil						
		Juveniles	Males	Females	Total	Pf/Pi†	Sessile females‡	Eggs/g root
Nematode	Population 1§	1,723	150	37	1,909	0.5	1.4	423
	Population 2	1,848	139	34	2,021	0.5	1.8	442
Fungus	None	1,261	122	23	1,406	0.4	0.8	351
	Isolate 1	2,140	158	42	2,339	0.6	2.0	486
	Isolate 2	2,015	156	43	2,213	0.6	2.1	470
Contrast								
0 vs. 1 + 2		***	*	*	***	***	**	***
1 vs. 2		NS	NS	NS	NS	NS	NS	NS
Cultivar	DP90	2,045	151	42	2,238	0.6	1.9	535
	DP 41	1,526	138	28	1,692	0.4	13.	330
Source								
Nematode		*	NS	NS	NS	NS	NS	NS
Fungus		***	*	NS	***	***	**	***
Cultivar		***	NS	NS	***	***	*	***
N × F		NS	NS	NS	NS	NS	NS	*
N × C		NS	NS	NS	NS	NS	NS	NS
F × C		**	NS	NS	**	**	NS	*
N × F × C		NS	NS	NS	NS	NS	NS	NS

Data are means of five replicates.
† Pi = initial nematode infestation level. Pf = final nematode population density in soil.
‡ Females/10 root segments, each 2.5 cm long.
§ 4,000 vermiform stages/500 g soil.
*, **, *** = significant at $P \leq 0.05$, 0.01 and 0.0001 based on F test, respectively; NS = nonsignificant.

fungus and nematode ($P \leq 0.05$). Fungus isolate 1 had a more pronounced effect on egg production by population 1 than did isolate 2. In contrast, *R. solani* isolate 2 had a greater influence on egg production by population 2 than did isolate 1. Fungus × cultivar interaction affected ($P \leq 0.05$) the numbers of juveniles and total nematodes recovered from soil, Pf/Pi ratios, and eggs/g root. Treatment mean patterns for juveniles (Fig. 1) revealed that DP 90, which supports higher levels of *R. reniformis*, allowed a greater increase in soil juveniles in response to *R. solani* than did DP 41, which supports lower levels of *R. reniformis*. Similar trends were obtained for total nematodes present in soil, Pf/Pi ratios, and eggs/g root. Neither plant growth nor disease indices were influenced by *R. reniformis* in this 40-day-duration experiment (Table 4). The fungus caused reductions ($P \leq 0.0001$) in shoot, root, and plant fresh weights, but there were no differences between isolates. Shoot and plant fresh weights for DP 90 were lower than those for DP 41 ($P < 0.0001$). Fungus × cultivar interaction affected fresh shoot and plant weights ($P < 0.0001$). Reductions in shoot and plant weights caused by both fungus isolates were greater ($P \leq 0.05$) on DP 41 than on DP 90.

Experiment 3: Increasing the inoculum level of *R. reniformis* from 500 to 4,000 nematodes/pot resulted in an increase ($P \leq 0.05$) in all the life stages of the nematode

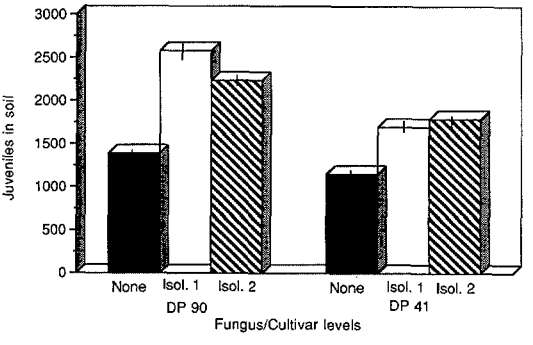

FIG. 1. Means for the juveniles of *Rotylenchulus reniformis* recovered from soil for the interaction between *Rhizoctonia solani* and the cotton cultivars Deltapine 90 and Deltapine 41. Vertical lines delimit standard errors of means. Isol. 1 = Isolate 1, Isol. 2 = Isolate 2.

TABLE 4. Effects of *Rotylenchulus reniformis* populations, *Rhizoctonia solani* isolates, and cultivars on cotton fresh weights and disease indices at 40 days after inoculation.

Treatment	Inocula/ cultivars	Plant fresh weight (g)			Disease index†
		Shoot	Root	Plant	
Nematode	None	4.4	3.1	7.5	1.2
	Population 1‡	4.3	3.2	7.5	1.3
	Population 2	4.2	3.2	7.4	1.2
Contrast					
0 vs. 1 + 2		NS	NS	NS	NS
1 vs. 2		NS	NS	NS	NS
Fungus	None	5.6	3.8	9.4	0.0
	Isolate 1	3.7	2.9	6.5	1.9
	Isolate 2	3.6	2.7	6.3	1.9
Contrast					
0 vs. 1 + 2		***	***	***	***
1 vs. 2		NS	NS	NS	NS
Cultivar	DP 90§	3.7	3.1	6.8	1.3
	DP 41	5.0	3.2	8.2	1.2
Source					
Nematode		NS	NS	NS	NS
Fungus		***	***	***	***
Cultivar		***	NS	***	NS
N × F		NS	NS	NS	NS
N × C		NS	NS	NS	NS
F × C		***	NS	***	NS
N × F × C		NS	NS	NS	NS

Data are means of five replicates.

† Disease index scale = 0–3 (0 = no hypocotyl necrosis or root discoloration; 1 = hypocotyl necrosis, slight root discoloration; 2 = hypocotyl necrosis, moderate root discoloration; 3 = hypocotyl necrosis, severe root discoloration).

‡ Vermiform stages of 4,000 nematodes/500 g soil.

§ DP 90 = Deltapine 90, DP 41 = Deltapine 41.

*, **, *** = significant at $P \leq 0.05$, 0.01, and 0.0001 based on F test, respectively; NS = nonsignificant.

in soil and egg production on roots (Table 5). Pf/Pi ratios were inversely related to nematode inoculum levels. Presence of *R. solani* resulted in an increase ($P < 0.05$) in all the life stages of the nematode except sessile females. The two isolates of the fungus did not differ in their influence on reproduction of *R. reniformis*. Juveniles, total nematodes from soil, Pf/Pi ratio and eggs/g of root were greater ($P \leq 0.01$) on DP 90 than on DP 41. Pf/Pi ratio was affected by interactions between fungus and nematode ($P \leq 0.01$). Examination of individual treatment means revealed an inverse relationship between inoculum levels of the nematode and Pf/Pi ratios either in the absence or presence of the fungus. The interaction between nematode and cultivar influenced ($P \leq 0.01$) Pf/Pi ratio, and inspection of individual treatment means revealed an inverse relationship between nematode inoculum levels and Pf/Pi ratios on DP 41 and DP 90. The magnitude of difference in Pf/Pi ratio between DP 90 and DP 41 was greater at 500 than at 4,000 nematodes/pot. *Rotylenchulus reniformis* caused reductions in all plant growth parameters ($P \leq 0.0001$) (Table 6). *Rhizoctonia solani* reduced shoot, root, and plant fresh weights ($P \leq 0.0001$). Shoot, root, and plant fresh weights were higher for DP 41 than for DP 90.

Experiment 4: Increasing inoculum levels of *R. reniformis* resulted in stepwise increases ($P \leq 0.05$) in nematode life stages recovered from the soil, sessile females associated with roots, and eggs/g of root (Table 7). A linear relationship existed between nematode inoculum levels and reproduction ($P \leq 0.0001$). Pf/Pi ratios were inversely related to inoculum levels. The presence of *R. solani* in cotton increased (*P*

TABLE 5. Effects of nematode inoculum levels, *Rhizoctonia solani* isolates, and the cotton cultivars Deltapine 90 (DP90) and Deltapine 41 (DP41) on reproduction of *Rotylenchulus reniformis* at 90 days after inoculation.

Treatment	Inocula/ cultivars	Nematodes/kg soil				Pf/Pi†	Sessile females‡	Eggs/g root
		Juveniles	Males	Females	Total			
Nematode	500§	30,525	1,936	583	33,044	66.1	1.1	787
	4,000	54,905	2,435	819	58,160	14.5	2.0	1,406
Fungus	None	37,290	1,782	545	39,617	34.2	1.2	887
	Isolate 1	43,838	2,362	868	47,068	43.9	1.6	1,140
	Isolate 2	46,464	2,409	693	49,566	44.3	1.7	1,250
Contrast								
0 vs. 1 + 2		***	**	*	***	**	NS	***
1 vs. 2		NS	NS	NS	NS	NS	NS	NS
Cultivar	DP 90	44,880	2,253	751	47,884	45.2	1.7	1,281
	DP 41	40,216	2,112	649	42,977	36.4	1.4	909
Source								
Nematode		***	*	**	***	***	NS	***
Fungus		***	*	*	***	**	NS	***
Cultivar		**	NS	NS	**	**	NS	***
N × F		NS	NS	NS	NS	*	NS	NS
N × C		NS	NS	NS	NS	**	NS	NS
F × C		NS	NS	NS	NS	NS	NS	NS
N × F × C		NS	NS	NS	NS	NS	NS	NS

Data are means of five replicates.
† Pi = initial nematode infestation level. Pf = final nematode population density in soil.
‡ Females/10 root segments, each 2.5 cm long.
§ Vermiform stages/kg soil.
*, **, *** = significant at $P \leq 0.05$, 0.01, and 0.0001 based on F test, respectively; NS = nonsignificant.

≤ 0.05) the numbers of juveniles, males, and total nematodes recovered from soil. Pf/Pi ratio as well as egg production was greater ($P \leq 0.01$) in the presence of the fungus. Juveniles and total nematodes in soil were affected ($P \leq 0.05$) by nematode × fungus interaction. The presence of *R. solani* at the 8,000 nematodes/pot inoculum level resulted in greater increase ($P \leq 0.05$) in juveniles than the 500 or 2,000 per pot levels. A similar trend was observed for total nematodes in soil. At 90 days after inoculation there were stepwise reductions ($P \leq 0.0001$) in fresh shoot, root, and plant weights that paralleled increases in nematode inoculum levels (Table 8). *Rotylenchulus reniformis* did not influence the disease severity. *Rhizoctonia solani* caused reductions ($P \leq 0.0001$) in plant growth. The interaction between nematode × fungus influenced shoot, root weights ($P \leq 0.01$), and effects on plant growth were antagonistic (Fig. 2); i.e., reductions in plant growth caused by both pathogens together were less than the sum of reductions caused by each alone.

DISCUSSION

Three general conclusions can be made on the basis of data presented herein: i) the presence of *R. solani* increased reproduction by *R. reniformis*; ii) *R. reniformis* had no detectable influence on severity of cotton seedling blight, and iii) with respect to plant growth, combined effects of the nematode and fungus were antagonistic.

Most investigations (1,4,6,8,25,30) of interrelationships between *R. solani* and either reniform or root-knot nematodes have focused mainly on effects of nematodes on the incidence or severity of disease. Few reports detail the influence of *R. solani* on reproduction by *R. reniformis* (17,18). In our studies, enhanced reproduction by *R. reniformis* in the presence of *R. solani* was detectable within 40 days of inoculation. This increased reproduction

TABLE 6. Effects of *Rotylenchulus reniformis* inoculum levels, *Rhizoctonia solani* isolates, and cultivars on cotton fresh weights and disease indices at 90 days after inoculation.

Treatment	Inocula/ cultivars	Plant fresh weight (g)			Disease index†
		Shoot	Root	Plant	
Nematode	0	27.8	20.9	48.6	0.9
	500‡	25.5	19.5	45.0	0.9
	4,000	24.4	18.5	42.9	1.0
Contrast					
Linear		***	***	***	NS
Fungus	None	29.9	22.7	52.6	0.0
	Isolate 1	23.8	18.3	42.1	1.5
	Isolate 2	23.7	17.7	41.4	1.4
Contrast					
0 vs. 1 + 2		***	***	***	***
1 vs. 2		NS	NS	NS	NS
Cultivar	DP 90§	20.5	14.5	35.0	1.0
	DP 41	31.3	24.8	56.1	0.9
Source					
Nematode		***	***	***	NS
Fungus		***	***	***	***
Cultivar		***	***	***	NS
N × F		NS	NS	NS	NS
N × C		NS	NS	NS	NS
F × C		NS	NS	NS	NS
N × F × C		NS	NS	NS	NS

Data are means of five replicates.
† Disease index scale = 0–3 (0 = no hypocotyl necrosis or root discoloration; 1 = hypocotyl necrosis, slight root discoloration; 2 = hypocotyl necrosis, moderate root discoloration; 3 = hypocotyl necrosis, severe root discoloration).
‡ Vermiform stages/kg soil.
§ DP 90 = Deltapine 90, DP 41 = Deltapine 41.
*, **, *** = significant at $P < 0.05$, 0.01 and 0.0001 based on F test, respectively; NS = nonsignificant.

occurred with three isolates of *R. solani*, two populations and four inoculum levels of *R. reniformis*, and two cultivars of cotton. Increases in population density of *R. reniformis* in soil were accounted for by the increased production of eggs. In an attempt

TABLE 7. Effects of nematode inoculum levels and *Rhizoctonia solani* on reproduction of *Rotylenchulus reniformis* on Deltapine 90 cotton at 90 days after *inoculation*.

Treatment	Inocula	Nematodes/kg soil				Pf/Pi†	Sessile females‡	Eggs/g root
		Juveniles	Males	Females	Total			
Nematode	500§	46,420	3,593	953	50,967	101.9	0.7	980
	2,000	117,700	10,560	1,687	129,947	64.9	2.0	2,249
	8,000	215,952	18,282	2,904	237,138	29.6	2.6	3,088
Contrast								
Linear		***	***	***	***	***	*	***
Fungus	None	116,248	9,504	1,584	127,336	60.2	1.4	1,933
	Isolate 1	145,606	12,895	2,234	160,735	68.9	2.2	2,380
Source								
Nematode		***	***	**	***	***	*	***
Fungus		**	*	NS	***	**	NS	***
N × F		*	NS	NS	*	NS	NS	NS

Data are means of five replicates.
† Pi = initial nematode infestation level. Pf = final nematode population density in soil.
‡ Females/10 root segments, each 2.5 cm long.
§ Vermiform stages/kg soil.
*, **, *** = significant at $P \leq 0.05$, 0.01, and 0.0001 based on F test, respectively; NS = nonsignificant.

TABLE 8. Effects of *Rotylenchulus reniformis* inoculum levels and *Rhizoctonia solani* on fresh weights and disease indices of Deltapine 90 cotton at 90 days after inoculation.

Treatment	Inocula	Plant fresh weights (g)			Disease index†
		Shoot	Root	Plant	
Nematode	0	25.1	17.0	42.1	0.8
	500‡	22.6	15.8	38.4	0.7
	2,000	21.5	14.8	36.4	0.7
	8,000	20.3	13.7	34.0	0.6
Contrast					
Linear		***	***	***	NS
Fungus	None	25.3	17.0	42.4	0.0
	Isolate 1	19.2	13.4	32.6	1.4
Source					
Nematode		***	***	***	NS
Fungus		***	***	***	***
N × F		**	**	**	NS

Data are means of five replicates.

† Disease index scale = 0–3 (0 = no hypocotyl necrosis or root discoloration; 1 = hypocotyl necrosis, slight root discoloration; 2 = hypocotyl necrosis, moderate root discoloration; 3 = hypocotyl necrosis, severe root discoloration).

‡ Vermiform stages/kg soil.

*, **, *** = significant at $P \leq 0.05$, 0.01, and 0.0001 based on F test, respectively; NS = nonsignificant.

to include all life stages of the nematode in the population census, an effort was made to account for the numbers of females present on the root systems. In experiment 2, numbers of sessile females increased in the presence of *R. solani,* and in experiments 3 and 4 there were no significant increases in numbers of sessile females. This is attributed to the fact that the pattern of infection was not uniform across the plant root system. In view of the nonuniformity of infection, it was difficult to accurately account for the numbers of females present on cotton plants that had progressed past the seedling stage. The increase in total nematode population density in soil in the presence of *R. solani* resulted from the numbers of juveniles rather than from numbers of males or infective females.

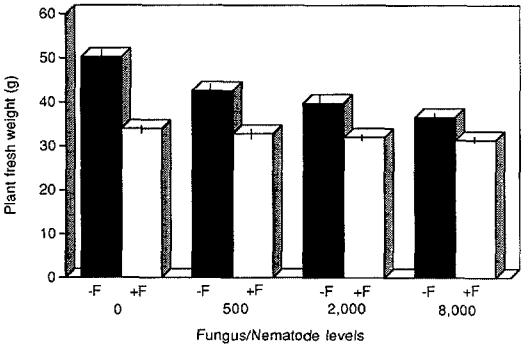

FIG. 2. Means for Deltapine 90 and Deltapine 41 plant fresh weights for the interaction between *Rhizoctonia solani* and *Rotylenchulus reniformis*. Vertical lines delimit standard errors of means. −F = absence of fungus. +F = presence of fungus. 0, 500, 2,000, and 8,000 nematodes/pot.

Rhizoctonia solani attacks the hypocotyl region of the cotton seedling near the soil line and causes postemergence damping-off, often called soreshin disease (27). During preliminary studies, it was determined that the isolates of *R. solani* used do not parasitize the root system. Reniform nematode remains confined to lateral roots and rarely, if ever, infects the tap root. Therefore, it is probable that any fungus-related effects on nematode reproduction were indirect via alterations in host physiology. This fungus does not have the same stimulatory effect on all populations or races of reniform nematode, since the work of Kumar and Sivakumar (18) with *R. solani* and *R. reniformis* on okra indicated that there was no fungus influence on nematode reproduction.

In experiment 2, reproduction of both populations of *R. reniformis* was increased

in the presence of *R. solani*. This uniformity of influence apparently does not apply across populations of nematode species, since Olthof (20) reported that in tobacco *Thielaviopsis basicola* promoted the development of one population of *Pratylenchus penetrans* but had no effect on another. In the second and fourth experiments, the two isolates of *R. solani* did not differ in their influence on reproduction of *R. reniformis*. Conversely, however, in tomato Overman and Jones (21) observed a 13-fold increase in the population density of *Tylenchorhynchus capitatus* in the presence of one isolate of *Verticillium albo-atrum* compared with a 9-fold increase with another isolate of the fungus.

Experiments 2 and 3, which were of 40 and 90 days duration, respectively, revealed the same relationship regarding the nematode host status of the two cotton cultivars. That is, in either the presence or absence of *R. solani,* DP 90 was a better host than DP 41 (15). Similar observations were made when two pepper cultivars were inoculated with *R. solani* and *M. incognita* (11).

In experiments 3 and 4, in which nematode levels ranged from 0.5–8/g of soil, Pf/Pi ratios were inversely related to initial inoculum densities. Our observations in experiments 3 and 4 indicated that, at all the nematode inoculum levels, the presence of *R. solani* enhanced reproduction of reniform nematode. In soybean, *Glycine max,* Overstreet et al. (22) observed that the presence of *Calonectria crotalariae* increased the reproduction of *Heterodera glycines* at high but not at low nematode inoculum levels.

Root injury caused by the nematode to the cotton root system did not influence the severity of seedling blight in our experiments in which nematode inoculum levels ranged from 0.5–8/g soil. Brodie and Cooper also (1) reported that at an inoculum level of eight nematodes/g soil, *R. reniformis* had no influence on postemergence damping-off of cotton caused by *R. solani.* This fungus, however, when combined with the root-knot nematode *M. incognita,* increased the disease severity or incidence of cotton seedling blight (4,6,8,30). Also, the presence of reniform nematode caused wilting symptoms in okra to appear earlier than those observed with *R. solani* alone (18).

All isolates of *R. solani* used in our studies were virulent. After many attempts to employ mycelial mat slurries or infested potato dextrose agar discs as inoculum units to establish sublethal infections of *R. solani,* the infested oat grain technique proved to be successful. The fungus reduced cotton growth in all experiments. The nematode alone caused plant damage only at 90 days. When combined with the fungus there was a consistent interaction that was antagonistic with respect to the growth of DP 90 cotton in experiment 4. In experiment 3, the interaction between nematode and fungus was nonsignificant in relation to plant growth across both cotton cultivars. However, when the data were sorted by cultivar and reanalyzed, there was interaction ($P < 0.01$), with respect to root weight of DP 90, and it was antagonistic. *Calonectria crotalariae* and *H. glycines* had a similar antagonistic effect on soybean growth, even though the presence of the fungus enhanced nematode reproduction (22). Tchatchoua and Sikora (29) found that *R. reniformis* alone caused reductions in shoot and root weights of cotton at population densities twice that employed in our studies, and combined inoculations with *Verticillium dahliae* resulted in synergistic reductions in plant growth. In our research the highest nematode inoculum level was 8/individuals g soil, since this represents the average density of *R. reniformis* encountered in cotton fields in Louisiana.

Our research demonstrates that there is an interrelationship between *R. solani* and reniform nematode. The fungus, therefore, impacts the cotton plant directly during the preemergence and postemergence growth stages and indirectly during later stages of growth through its stimulatory effect on reproduction of *R. reniformis.*

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