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Competition of *Meloidogyne incognita* and *Rotylenchulus reniformis* on Cotton Following Separate and Concomitant Inoculations

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Abstract: It has been hypothesized *Rotylenchulus reniformis* (Rr) has a competitive advantage over *Meloidogyne incognita* (Mi) in the southeastern cotton production region of the United States. This study examines the reproduction and development of *Meloidogyne incognita* (Mi) and *Rotylenchulus reniformis* (Rr) in separate and concomitant infections on cotton. Under greenhouse conditions, cotton seedlings were inoculated simultaneously with juveniles (J2) of *M. incognita* and vermiform adults of *R. reniformis* in the following ratios (Mi:Rr): 0:0, 100:0, 75:25, 50:50, 25:75, and 0:100. Soil populations of *M. incognita* and *R. reniformis* were recorded at 3, 6, 9, 14, 19, 25, 35, 45, and 60 days after inoculations. At each date, samples were taken to determine the life stage of development, number of egg masses, eggs per egg mass, galls, and giant cells or syncytia produced by the nematodes. *Meloidogyne incognita* and *R. reniformis* were capable of initially inhibiting each other when the inoculum ratio of one species was higher than the other. In concomitant infections, *M. incognita* was susceptible to the antagonistic effect of *R. reniformis*. *Rotylenchulus reniformis* affected hatching of *M. incognita* eggs, delayed secondary infection of *M. incognita* J2, reduced the number of egg masses produced by *M. incognita,* and reduced J2 of *M. incognita* 60 days after inoculations. In contrast, *M. incognita* reduced *R. reniformis* soil populations only when its proportion in the inoculum ratio was higher than that of *R. reniformis*. *Meloidogyne incognita* reduced egg masses produced by *R. reniformis,* but not production of eggs and secondary infection.

Key words: antagonism, competition, concomitant infections, cotton, *Gossypium hirsutum, Meloidogyne incognita,* reniform nematode, root-knot nematode, *Rotylenchulus reniformis,* sequential infections.

Meloidogyne incognita and *Rotylenchulus reniformis* are two of the predominant plant-parasitic nematodes associated with Mississippi cotton production (McLean and Lawrence, 2000). Both nematode species depend on successful formation of feeding sites in roots that serve to nourish the nematodes. Researchers have stated that parasitism of roots by *Meloidogyne* spp. (Christie, 1936; Dropkin and Nelson, 1960) involves the successful induction of giant cells from provascular parenchyma. *Rotylenchulus reniformis* induces formation of syncytia, primarily from altered pericycle cells (Jones and Dropkin, 1975; Razak and Evans, 1976; Taha and Kassab, 1979). The type of relationship and extent of competition between nematode species depends upon many factors, including host crop, environmental suitability, initial population levels, nature of the infection and feeding process, and rate of reproduction (Gaur and Perry, 1991).

Interactions between *Meloidogyne* and *Rotylenchulus* can be suppressive for either or both species. In simultaneous inoculations on soybean (*Glycine max*) (Singh, 1976) and mung bean (*Vigna mungoi*) (Mishra and Gaur, 1981), *M. incognita* suppressed *R. reniformis*. In contrast, when *R. reniformis* was inoculated on tomato (*Lycopersicum esculentum*) with low numbers of *M. incognita,* the former inhibited *M. incognita* (Kheir and Osman, 1977). On cowpea (*Vigna sinensis*), *R. reniformis* initially inhibited *M. javanica* but was less competitive over time (Taha and Kassab, 1980). However, mutual antagonism of both nematode species is reported on grape (*Vitis vinifera*) (Ras and Seshadri, 1981). Host resistance was determined to be the key factor in determining the relationship between *M. incognita* race 2 and *R. reniformis* populations on *M. incognita* susceptible and resistant soybean (*Glycine max*) cultivars in the greenhouse (Stetina et al., 1997).

Interactions between *Meloidogyne* spp. and *R. reniformis* are also conditioned by population density of the nematodes and the time of infestation. In greenhouse experiments on sweet potato (*Ipomoea batatas*), both low and high levels of *M. incognita* reduced the reproduction rate of *R. reniformis,* but *R. reniformis* had no effect on the reproduction of *M. incognita* (Thomas and Clark, 1981). However, in field experiments, low levels of *R. reniformis* inhibited *M. incognita,* whereas *M. incognita* had no effect on *R. reniformis*. Thus each nematode species was capable of suppressing the other to become the dominant species (Thomas and Clark, 1983a; 1983b).

Competition between nematodes is more severe between species with similar feeding habits, and the competitive advantage increases as the host-parasite relationship become more complex (Eisenback, 1985). The feeding sites of ectoparasitic and endoparasitic nematodes are different, and the two forms can coexist in the same host without any interaction (Haque and Mukhopadhyaya, 1979). Duncan and Ferris (1982, 1983) concluded that at given initial population levels, each nematode species in a concomitant infection may cause less root damage than in the absence of competing species, although total damage may be greater in the concomitant situation. The inhibition of growth of mung bean (Mishra and Gaur, 1981) and grape seedlings (Ras and Seshadri, 1981) was less in combined inoculations than in single inoculations of *M. incognita* and *R. reniformis*.

The objective of this study was to determine if *R.*

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reniformis has a competitive advantage over *M. incognita.* Life-stage development and reproduction of each nematode was followed after separate and concomitant inoculations at sequential time intervals on cotton.

Materials and Methods

Cotton (*Gossypium hirsutum*) cv. Delta and Pine Land 20 (DPL 20), which is susceptible to *M. incognita* race 3 and *R. reniformis,* was used in all tests. Seeds were germinated on 26- × 39-cm sterile germination paper for 48 hours. One seedling with a radical length of 1 to 2 cm was planted in each 11.5-cm-diam. clay pot filled with 500 cm^3 of a Freestone fine sandy loam (72.4, 12.0, 15.6, S-S-C, 0.6% OM, 14.9 CEC, and pH 6.1). Seedlings were inoculated 1 week after transplanting by pipeting the appropriate nematode suspension into three depressions (1-cm diam. × 3-cm deep) 2 to 3 cm away from the base of the stem. After inoculation, the depressions were filled with sterilized soil to prevent desiccation of the nematodes.

The *M. incognita* race 3 population was originally isolated from egg masses on cotton in Humphrey County, Mississippi, and increased on tomato in the greenhouse. The North Carolina differential host test confirmed the *M. incognita* population was race 3 (Myers, 1990). The *R. reniformis* population was isolated from a cotton field in Tallahatchie County, Mississippi, and was increased in the greenhouse on DPL 20.

Both nematode species were extracted from cultures in the greenhouse by gravity screening and centrifugal flotation (sucrose sp gr = 1.13) (Jenkins, 1964). *Rotylenchulus reniformis* vermiform adults were enumerated using a stereomicroscope and numbers adjusted to the pre-established inoculum levels. Eggs and J2 of *M. incognita* were extracted from tomato by immersing roots into 0.525% NaOCl for 4 minutes (Hussey and Barker, 1973). The solution was then poured through a 75-µm-pore sieve nested over a 28-µm-pore sieve. Eggs collected on the 28-µm-pore sieve were placed in water maintained at 28 ± 1 °C for 3 days. The hatched J2 were collected every 24 hours on the 28-µm-pore sieve for 3 days and maintained in water at 4 ± 1 °C until inoculation (Tang et al., 1994). Inoculum was enumerated in a grided petri dish using a stereomicroscope, and numbers were adjusted to the pre-established inoculum levels.

The experimental design consisted of 12 treatments including concomitant and single species of *M. incognita* (Mi) and *R. reniformis* (Rr) expressed as a percentage of the population Mi:Rr and an uninoculated control. The *M. incognita* inoculum ratio of 100% equaled 4,000 J2. The *R. reniformis* infective inoculum ratio of 100% consisted of 4,000 vermiform adult females and 4,000 adult males (only infective females parasitize the roots). Data were recorded at 3, 6, 9, 14, 19, 25, 35, 45, and 60 days after inoculation (DAI). Plants with concomitant ratios (Mi:Rr) 0:0, 100:0, 75:25, 50:50, 25:75, and 0:100 were harvested at each of the nine sampling dates. Single ratios (Mi:Rr) 75:0, 50:0, 25:0, 0:75, 0:50, and 0:25 were harvested at 60 DAI. All treatments were arranged in a completely randomized design and replicated four times for a total of 240 pots. The entire test was repeated three times.

Nematodes were extracted from the soil as previously described at each of the nine sampling dates. Populations of *M. incognita* and *R. reniformis* were enumerated and recorded. The life-stage development of *M. incognita* and *R. reniformis,* the number of galls, syncytia, egg masses, and eggs per egg mass were determined and recorded at each sampling date. To determine life-stage development, root samples were cleared and stained for detection of nematodes using a modified acid-fuchsin staining-destaining procedure (Byrd et al., 1983). *Meloidogyne incognita* life-stage development was described using a modification of Christie's method (Christie, 1946; Christie and Cobb, 1946; Tang et al., 1994). *Rotylenchulus reniformis* life-stage development was based on a revision of the genus *Rotylenchulus* by Dasgupta et al. (1968). The developmental stages and number of *M. incognita* and *R. reniformis* in each stage were recorded from 1 g of root tissue for all treatments. The number of galls induced by *M. incognita* was estimated at 14, 19, 25, 35, 45, and 60 DAI from 1 g of the root system in each treatment. Syncytia formation in response to *R. reniformis* infection was determined at all sampling dates by counting the number of feeding sites from 1 g of cotton roots from each treatment. Egg masses were counted at 14, 25, 35, 45, and 60 DAI by staining 1 g of fresh root from each treatment with phloxine B (Daykin and Hussey, 1985; Tang et al., 1994). Eggs per egg mass were determined from a sample of 10 egg masses from each root system.

All data were analyzed by analysis of variance using the mixed model procedure of SAS (SAS Institute Inc., Cary, NC). Repeats of the experiment and replications were considered to be random effects. Treatment means were separated using Fisher's protected least significant difference ($P \leq 0.05$). All fixed effects, including linear and quadratic responses to nematode ratios, were modeled simultaneously.

Results

Post-infection development of Meloidogyne incognita and *Rotylenchulus reniformis:* Life-stage development of *M. incognita* was delayed in the presence of equal or higher numbers of *R. reniformis.* The highest percentage of *M. incognita* J2 (life stage A) were observed in cotton roots at 3 and 6 DAI (Table 1). At 6 DAI, life stage B (sausage-shaped juvenile with a conical tail) was recovered in all *M. incognita* and *R. reniformis* inoculum ratios. Fully grown *M. incognita* juveniles (life stage D) were recovered in all treatments at 9 DAI; however,

TABLE 1. Development of *Meloidogyne incognita* (Mi) and *Rotylenchulus reniformis* (Rr) on cv. DPL 20 cotton roots, inoculated with *M. incognita* (Mi) or *R. reniformis* (Rr) for population ratios of 100:0 Mi:Rr, 0:100 Mi:Rr, 75:25 Mi:Rr, 50:50 Mi:Rr, and 25:75 Mi:Rr per plant.

	100% *M. incognita*[a] developmental stages (%)							100% *R. reniformis*[b] developmental stages (%)				
DAI	Total Mi	A	B	C	D	E	F	Total Rr	A	B	C	D
3	599	100						618	100			
6	432	85.0	15.0					631	87.8	12.2		
9	555		9.2	68.7	22.1			665	41.8	58.2		
14	562		18.3	22.6	59.1			629		20.0	80.0	
19	589			4.6	12.1	58.7	24.6	673		19.3	80.7	
25	560				17.3	41.1	41.6	666			29.0	71.0
35	627	8.6			1.1	12.0	78.3	816	13.1		18.3	68.6
45	695	14.0	5.2	3.3		1.4	76.1	742	11.2		4.7	84.1
60	842	12.6	8.5	2.9	5.1	5.1	65.8	978	25.5	12.9	6.8	54.8
	75% *M. incognita* developmental stages (%)							25% *R. reniformis* developmental stages (%)				
DAI	Total Mi	A	B	C	D	E	F	Total Rr	A	B	C	D
3	457	100						211	100			
6	310	84.8	15.2					223	91.0	9.0		
9	427		16.6	58.1	25.3			229	29.3	70.7		
14	414		18.1	21.5	60.4			211		24.6	75.4	
19	363			4.1	10.5	63.1	22.3	238		16.0	84.0	
25	336				15.2	39.6	45.2	213			24.9	85.1
35	407				10.3	6.3	83.4	245	10.2		15.1	74.7
45	515	15.1	3.3	1.9		1.8	77.9	255	12.2		9.4	78.4
60	546	11.2	7.8	4.4	6.8	6.8	63.0	415	26.8	16.6	11.1	45.5
	50% *M. incognita* developmental stages (%)							50% *R. reniformis* developmental stages (%)				
DAI	Total Mi	A	B	C	D	E	F	Total Rr	A	B	C	D
3	249	100						306	100			
6	379	85.0	15.0					295	91.2	8.8		
9	269		14.5	58.7	26.8			302	35.8	64.2		
14	271		14.4	29.9	55.7			303		27.7	72.3	
19	223			5.4	9.4	55.6	29.6	312		17.9	82.1	
25	217				8.3	35.5	56.2	291			23.7	76.3
35	256				6.6	10.6	82.8	372	12.9		19.4	67.7
45	372	12.6	4.8	1.9		3.5	77.2	324	13.0		7.7	79.3
60	408	8.6	7.6	3.4	4.4	8.8	67.2	592	28.2	19.1	8.8	44.2
	25% *M. incognita* developmental stages (%)							75% *R. reniformis* developmental stages (%)				
DAI	Total Mi	A	B	C	D	E	F	Total Rr	A	B	C	D
3	203	100						517	100			
6	317	82.7	17.3					447	86.6	13.4		
9	168		14.3	58.3	27.4			480	42.0	58.0		
14	178		15.7	24.2	60.1			508		20.5	79.6	
19	144			7.6	11.1	57.7	23.6	506		23.3	76.7	
25	160				4.4	32.5	63.1	412			31.4	68.6
35	158	7.6				12.7	79.7	538	13.4		19.7	66.9
45	290	11.4	3.3	2.8		3.1	78.9	526	10.5		3.4	86.1
60	224	12.1	8.5	4.5	9.4	7.1	58.5	709	27.4	16.6	10.4	45.6

[a] *M. incognita*–nematode classification: Stage A: vermiform J2; Stage B: sausage-shaped J2 possessing a conical tail; Stage C: juvenile with hemispherical posterior end terminated by a spike; Stage D: fully grown juvenile, no spike; Stage E: females without eggs; Stage F: females with egg mass (modified from Tang et al., 1994).
[b] *R. reniformis*–nematode classification: Stage A: vermiform, non-swollen shape; Stage B: female body in open C, swollen shape in region of vulva; Stage C: typical reniform shape, without egg mass; Stage D: mature female with egg mass (modified from Dasgupta et al., 1968).

when *M. incognita* was inoculated alone, 68.7% were in life stage C compared with 58.7% in the concomitant ratios. Egg-laying *M. incognita* females were recovered at 19 DAI in all inoculum ratios. Between 63% and 55.6% of these *M. incognita* females had not developed eggs (stage E). The highest percentage of *M. incognita* females with egg masses (stage F) was observed at 35 DAI, and J2 were found reinfecting plants in the 100:0 and 75:25 Mi:Rr ratio treatments (Table 1). Second-stage juveniles were not observed reinfecting roots until 45 DAI in Mi:Rr ratios of 75:25 and 50:50 (Table 1). All developmental stages of *M. incognita* except stage D were observed in cotton roots at 45 DAI (Table 1).

The life stages attained by *R. reniformis* were similar with all inoculum ratios during 60 days of experimentation. Vermiform juveniles (stage A) were observed 3

DAI in all Mi:Rr inoculation ratios (Table 1). At 6 DAI, vermiform adult females (stage B) were recovered from all treatments. The reniform-shaped females (stage C) without egg masses were observed at 14 DAI, followed by mature egg-laying females (stage D) at 25 DAI in all treatments (Table 1). The highest percentage of *R. reniformis* stage D were observed at 25 DAI in the 75:25 and 50:50 Mi:Rr ratios. Secondary infections were observed in all inoculum ratios at 35 DAI.

Meloidogyne incognita (Mi) soil populations: Meloidogyne incognita soil populations related with DAI, ($Y_{Mi:Rr}$) best fit quadratic models (Fig. 1). The *M. incognita* soil populations from the single inoculum ratio of 100:0 Mi:Rr and concomitant inoculum ratios of 75:25, 50:50, and 25:75 Mi:Rr best fit positive quadratic models. The difference in population increases due to competition are evidenced by the differing slopes of the regression lines of the *M. incognita* populations. Although *M. incognita* populations increased on all inoculum ratios at 60 DAI (Table 2), the soil population densities of *M. incognita* in concomitant inoculations 75:25, 50:50, and 25:75 Mi:Rr were lower ($P \leq 0.05$) than the single species inoculations at the same level (75:0, 50:0, and 25:0 Mi:Rr).

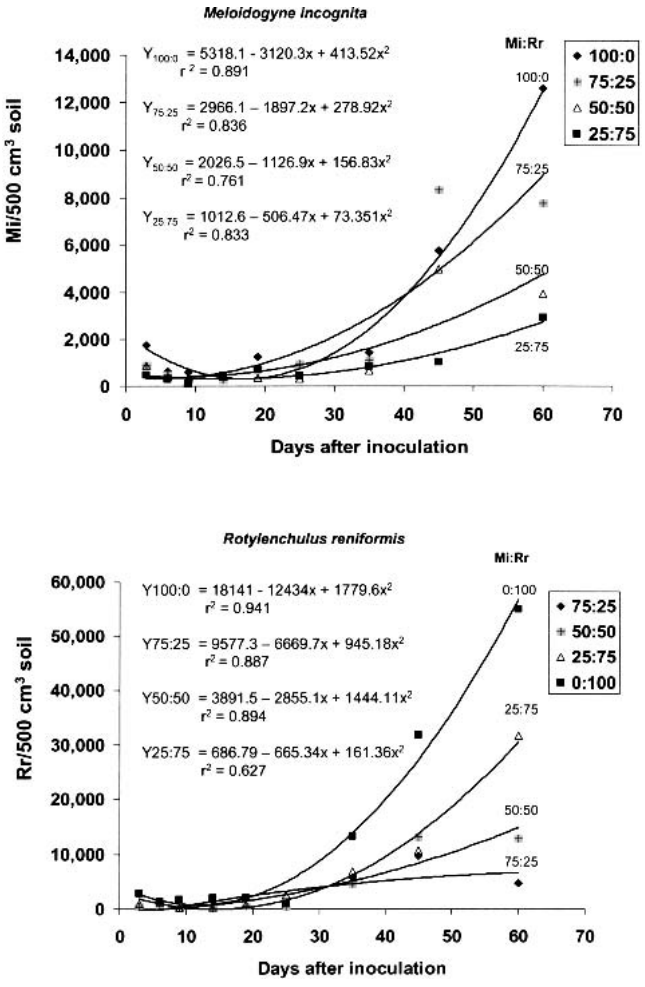

FIG. 1. *Meloidogyne incognita* (Mi) and *Rotylenchulus reniformis* (Rr) soil nematode populations recovered from cotton roots, cv. DPL 20, inoculated with Mi or Rr alone and in combinations. (Means are the averages of three tests with four replications each.)

TABLE 2. Soil population development of separate and concomitant inoculations of *Meloidogyne incognita* (Mi) and *Rotylenchulus reniformis* (Rr) 60 days after inoculations.

Inoculation ratio Mi:Rr	Population average/500 g soil	
	Mi	Rr
100:0	12,572 ab[1]	—
75:25	7,775 bc	4,680 de
50:50	3,922 d	12,808 cd
25:75	2,918 d	31,673 b
0:100	—	55,041 a
75:0	14,185 a	—
50:0	8,522 bc	—
25:0	9,779 ab	—
0:25	—	16,066 c
0:50	—	15,933 c
0:75	—	14,998 cd
FLSD ($P \leq 0.05$)	4,600.8	10,335.0

[1] Means in column with different letters differ ($P \leq 0.05$) (means are the average of three tests with 4 replications each).

Rotylenchulus reniformis (Rr) soil populations: Rotylenchulus reniformis soil population development, for all inoculum ratios (Mi:Rr), related with days after inoculations, ($Y_{Mi:Rr}$) best fit positive quadratic models (Fig. 1). The differences in population increases due to competition are evidenced by the differing slopes of the regression lines of the *R. reniformis* populations. *Rotylenchulus reniformis* populations increased on all inoculum ratios at 60 DAI (Table 2). In general, *R. reniformis* soil populations were lower in concomitant inoculations than in the single inoculation of 0:100 Mi:Rr. *Rotylenchulus reniformis* levels in the 25:75 Mi:Rr ratio were higher ($P \leq 0.05$) than in the single inoculation of 0:75 Mi:Rr. The 50:50 Mi:Rr was not different from the single inoculation of 0:50 Mi:Rr. However, in the inoculum ratio of 75:25 Mi:Rr, the soil population of *R. reniformis* was less ($P \leq 0.05$) than the single-species inoculations of 0:25 Mi:Rr.

Meloidogyne incognita and Rotylenchulus reniformis reproduction in cotton roots: The number of *M. incognita* egg masses in single and concomitant inoculum ratios, related with days after inoculations ($Y_{Mi:Rr}$), fit positive quadratic models for the 100:0, 50:50, and 25:75 Mi:Rr ratios (Fig. 2A). The 75:25 Mi:Rr ratio was not affected by time. The differences in number of egg masses produced by concomitant inoculations is reduced due to competition, and this is evidenced by the differing slopes of the regression lines of the number of *M. incognita* egg masses in 50:50 and 25:75 compared to 100:0 Mi:Rr ratios. Concomitant inoculations of 75:25, 50:50, and 25:75 Mi:Rr produced fewer *M. incognita* egg masses than inoculations of 75:0, 50:0, and 25:0 Mi:Rr. However, only the 75:25 was significant (Table 3). The number of egg masses produced by *R. reniformis* in

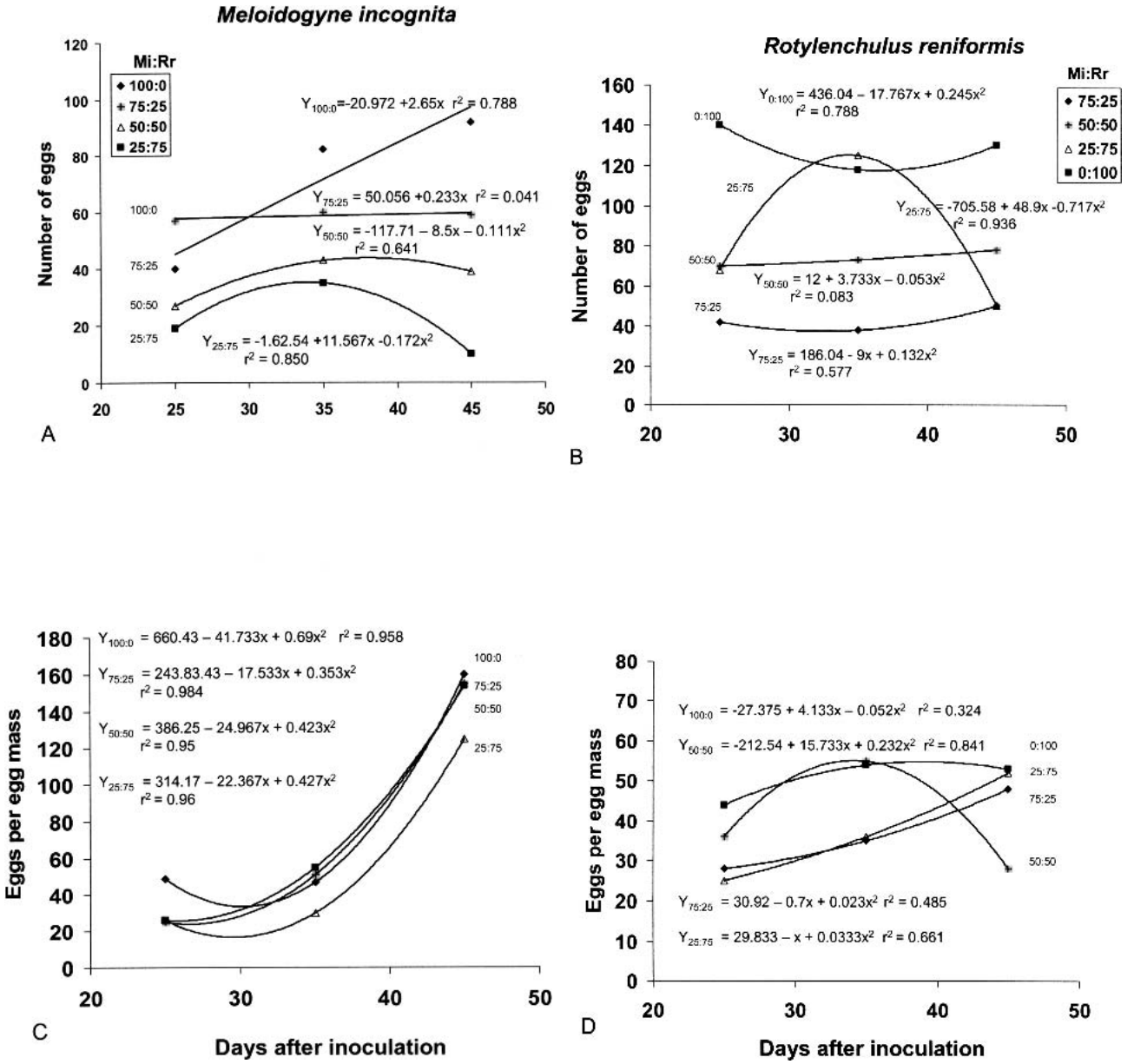

FIG. 2. Mean number of egg masses and eggs per egg mass produced by *Meloidogyne incognita* (Mi) (A and C) and *Rotylenchulus reniformis* (Rr) (B and D) on cotton roots, cv. DPL 20, inoculated with either nematode alone or in combination. (Means are the averages of three tests with four replications each.)

single and concomitant inoculum ratios over time ($Y_{Mi:Rr}$) fit a quadratic model with the inoculum ratio of 25:75 Mi:Rr (Fig. 2B). The number of egg masses in the 0:100, 50:50, and 75:25 Mi:Rr inoculum ratios remained constant and did not increase over time. Egg masses produced by *R. reniformis* were lower ($P \leq 0.05$) with concomitant inoculations than single inoculations (0:100 Mi:Rr) except at 35 DAI at the inoculum ratio of 25:75 Mi:Rr, where the number of egg masses was greater than the other concomitant inoculum ratios. Concomitant inoculations of 25:75, 50:50, and 75:25 Mi:Rr produced lower numbers ($P \leq 0.05$) of egg masses by *R. reniformis* than when inoculated alone at 0:75, 0:50, and 0:25 Mi:Rr (Table 3).

The number of eggs per egg mass produced by *M. incognita,* related with days after inoculations ($Y_{Mi:Rr}$), fit quadratic models with all inoculum ratios (Fig. 2C). The number of eggs per egg mass produced by *M. incognita* at 25 DAI was lower ($P \leq 0.05$) in the concomitant inoculations compared with the inoculation of *M. incognita* alone (100:0 Mi:Rr). Thereafter, the number of eggs per egg mass was not reduced by the concomitant inoculum ratios. At 60 DAI, no differences ($P \leq 0.05$) in number of eggs per egg mass were observed between single inoculation ratios and the corresponding concomitant ratio (Table 3). The number of eggs per egg mass produced by *R. reniformis,* related with days after inoculations ($Y_{Mi:Rr}$), fit quadratic models at

TABLE 3. Nematode post-infection development of separate and concomitant parasitism by *Meloidogyne incognita* (Mi) and *Rotylenchulus reniformis* (Rr), on cotton roots, cv. Delta and Pine Land 20, 60 days after inoculations.[1]

Inoculation ratio Mi:Rr	Egg masses/g root		Eggs/Egg mass		Galls	Syncytia
	Mi	Rr	Mi	Rr	Mi	Rr
100:0	53.7 a[2]		241.3 a		112.7 ab	
75:25	26.7 b	22.7 ef	205.3 ab	30.0 bc	107.0 ab	32.0 bc
50:50	37.0 ab	37.0 e	200.7 abc	24.3 bc	109.7 ab	35.3 bc
25:75	26.0 b	81.7 cd	149.0 c	28.7 bc	116.0 ab	56.3 ab
0:100		157.3 a		42.0 a		82.7 ab
75:0	55.3 a		156.3 bc		135.7 a	
50:0	44.3 ab		151.0 c		98.7 b	
25:0	33.3 b		193.7 abc		101.3 b	
0:25		74.3 d		37.0 ab		32.7 bc
0:50		111.0 bc		32.0 ab		53.0 abc
0:75		132.3 ab		38.3 ab		97.7 a
FLSD ($P \leq 0.05$)	17.0	35.3	52.5	10.0	29.9	47.0

[1] Average per gram of cotton root.
[2] Means in column with different letters differ ($P \leq 0.05$) (means are the averages of three tests with 4 replications each).

the inoculum ratios of 25:75 and 50:50 Mi:Rr (Fig. 2D). However, the inoculum ratios of 0:100 and 75:25 Mi:Rr did not fit linear or quadratic models; thus, no relationship between eggs per egg mass over time and inoculum level was evident. At 25 DAI the number of eggs per egg mass produced by *R. reniformis* was lower in concomitant inoculations compared with inoculations of *R. reniformis* alone. However, at 35 DAI the number of eggs per egg mass was significantly lower at inoculum ratios of 75:25 and 25:75 Mi:Rr ($P \leq 0.05$) compared with the 50:50 and 0:100 Mi:Rr inoculum ratios. At 45 DAI, the 50:50 Mi:Rr inoculum ratio ($P \leq 0.05$) produced fewer eggs per egg mass than all other treatment ratios. Inoculations of 50:50 Mi:Rr produced lower numbers ($P \leq 0.05$) of eggs per egg mass by *R. reniformis* compared with the number of eggs produced by *R. reniformis* inoculated alone (Table 3).

The number of root galls produced by *M. incognita* related with DAI ($Y_{Mi:Rr}$) indicated no relationship over time existed for any of the inoculum ratios. At 14, 19, 25, 35, and 45 DAI, more galls developed ($P \leq 0.05$) in inoculum ratios 100:0, 75:25, and 50:50 Mi:Rr than with 25:75 Mi:Rr (Table 3). However, the number of galls produced was not different ($P \leq 0.05$) for any inoculum ratios by 60 DAI. Syncytia numbers over time ($Y_{Mi:Rr}$), fit a quadratic model for the ratios of 0:100, 50:50, and 25:75 Mi:Rr; however, the R^2 values were low. The 75:25 Mi:Rr ratio did not fit a linear or quadratic model. Numbers of syncytia associated with the presence of *R. reniformis* on cotton roots were observed at 3 DAI. At 14, 19, and 45 DAI, syncytia were fewer in all concomitant inoculations of *M. incognita* and *R. reniformis* compared with *R. reniformis* alone (0:100 Mi:Rr) (Table 3). However, at 25, 35, and 60 DAI, the numbers of syncytia were consistent in all single and concomitant inoculations. As seen with *M. incognita,* the number of syncytia produced were not different ($P \leq 0.05$) for any inoculum ratios by 60 DAI.

DISCUSSION

The effects of nematode species interactions are related to their nature of parasitism, and competition tends to be the greatest between species with similar feeding habits (Eisenback, 1985). This study showed that *M. incognita* was more susceptible to antagonism by *R. reniformis* than the reverse.

In greenhouse experiments, *M. incognita* and *R. reniformis* inoculated simultaneously were capable of initially inhibiting each other when the amount of inoculum of one species was higher than that of the other. *Meloidogyne incognita* and *R. reniformis* concomitantly inoculated on cotton roots at a 3:1 ratio reduced the number of egg masses produced by the other species. This competitive interaction between the two nematodes may be associated with their feeding activities: more nematodes of one species may reduce the available feeding sites of the other species, subsequently suppressing the development of the other species (Eisenback, 1985). Similar competition was reported between *M. incognita* and *R. reniformis* by Thomas and Clark (1983a, 1983b) on sweet potato.

The reduction of egg masses produced by *M. incognita* in concomitant compared to single species inoculations may indicate a density-dependent ability of *R. reniformis* to inhibit *M. incognita* egg mass production. Kinloch and Allen (1972) reported that the invasion by *M. incognita* was more density dependent than that of *M. hapla* in concomitant inoculations of both nematodes on tomato. The negative effect of *R. reniformis* on *M. incognita* egg mass formation also may be associated with a competition between the two nematodes for available feeding sites.

The number of eggs per egg mass by *M. incognita* may indicate that the lower population levels of *M. incognita* have intraspecific competition between developing juveniles and available feeding sites. Chapman and

Turner (1975) found that *Pratylenchus penetrans* females deposit fewer eggs in *Trifolium pratense* roots infected with *M. incognita* than in the absence of that concomitant species. They reported that whether the smaller number of eggs was the result of an overall lower rate or early cessation of egg laying in *M. incognita*-infected tissue could not be determined.

The number of egg masses produced by *R. reniformis* was lower in concomitant inoculations than in single inoculations. *Meloidogyne incognita* may have an inhibitory effect on *R. reniformis* egg mass production due to crowding; however, it did not affect the production of eggs per egg mass. This may be due to interspecific competition from crowding by *M. incognita.* Nevertheless, once *R. reniformis* infested the root it was capable of normal development.

In general, galls produced by *M. incognita* and syncytia produced by *R. reniformis* were fewer in concomitant inoculations than single inoculations. Interspecific competition exists between *M. incognita* and *R. reniformis* for feeding sites when the population level of one nematode is higher than the other species. This competition thus appears to be based on crowding. This is similar to the results of Thomas and Clark (1983a) with *M. incognita* and *R. reniformis* on sweet potato. In greenhouse studies, they reported that when *M. incognita* increases, it eventually will become self-limiting as the sweet potato roots begin to die and subsequent available feeding sites decline. As fewer root tips were available for penetration by *M. incognita, R. reniformis* continued to increase in number because they are capable of entering at any location on the root.

Studies on cotton have reported that the total period of time from root penetration to egg production, at 28 °C, was between 21 and 28 days (Starr and Veech, 1986). On *Lycopersicum esculentum,* at 29 °C, the first egg-laying females were found 19 to 21 days after penetration (Triantaphyllou and Hirschmann, 1964). In the current studies, life-stage development of *M. incognita* was altered in the presence of *R. reniformis.* Mature egg-laying females were recovered at 19 DAI in both single and concomitant inoculations. The second generation of *M. incognita,* however, was delayed in concomitant inoculation with *R. reniformis.* This indicates that *R. reniformis* negatively affected the life cycle of *M. incognita* when in concomitant inoculations. This phenomenon was probably due to infection by *R. reniformis,* which diminished the potential feeding sites that could be infected by *M. incognita.*

The rate of development of *R. reniformis* was not affected by the presence of *M. incognita* in this study. Mature egg-laying females (stage D) were recorded at the same sample date in both single and concomitant treatments. Additionally, *M. incognita* did not affect the period of time between egg production or secondary reinfection of *R. reniformis.* The *R. reniformis* life cycle, under the conditions in this study, was completed in 25 DAI, which coincides with data obtained by Birchfield (1962), who found that *R. reniformis* on cotton completed its life cycle between a period of 17 to 23 days.

In our study, as the inoculum level of *M. incognita* decreased and was replaced with *R. reniformis,* the number of *M. incognita* continued to decrease. This decrease appears to be a direct response to the presence of *R. reniformis.* These results are coincident with that of Kheir and Osman (1977), who found that the development and growth rate of *M. incognita* in *L. esculentum* roots were retarded by *R. reniformis* because each adult female laid fewer eggs.

In summary, we found that concomitant infection of cotton with *M. incognita* and *R. reniformis* was capable of inhibiting the soil population density of each other when the amount of primary inoculum of one species was higher than that of the other. After plant infection, *M. incognita* was susceptible to the antagonistic effect of *R. reniformis. Rotylenchulus reniformis* affected the life-stage development and secondary infection of *M. incognita.* Comparatively, *M. incognita* reduced *R. reniformis* soil populations only when the inoculum ratio was in higher proportion than *R. reniformis* and the number of egg masses produced by *R. reniformis* was reduced, but not the production of eggs.

It is reported that *R. reniformis* has steadily been increasing in incidence and frequency of recovery on cotton throughout the cotton production region in the southern United States (McLean and Lawrence, 2000). Our study indicates *R. reniform* is antagonistic to *M. incognita* under controlled greenhouse conditions. Physiological and biochemical studies of the host infected with both nematodes may provide an answer to the population changes of one species in the presence of the other species and clarify aspects related to the interspecific competition between *M. incognita* and *R. reniformis* on cotton roots.

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