Influence of Concomitant Pratylenchus brachyurus and Meloidogyne spp. on Root Penetration and Population Dynamics[1]

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Abstract: Populations of *Pratylenchus brachyurus* on cotton were increased significantly in the presence of either *Meloidogyne incognita* or *M. arenaria*. This occurred with either simultaneous inoculation or prior invasion by *M. incognita*. *P. brachyurus* penetrated cotton roots previously invaded by, or simultaneously inoculated with, *M. incognita*, as well as, or better than, in the absence of *M. incognita*. Prior invasion by *M. incognita*, however, suppressed *P. brachyurus* populations on tomato, while it had no effect on alfalfa and tobacco. Populations of *M. incognita* on cotton were generally inhibited by the presence of *P. brachyurus*. Simultaneous inoculation with, or previous invasion by, *P. brachyurus* also inhibited root penetration by *M. incognita*. These findings emphasize the importance of host susceptibility in the study of concomitant nematode populations. *Key Words:* cotton, *Gossypium hirsutum*; tomato, *Lycopersicon esculentum*; alfalfa, *Medicago sativa*; tobacco, *Nicotiana tabacum*; *Meloidogyne incognita, Meloidogyne arenaria*, coinhabitants.

Recently there has been increased interest in the influence of root invasion by concomitant species on nematode population dynamics. In most investigations, one species had a detrimental effect on root penetration, reproduction or equilibrium density of the other species, or no effect at all (1, 2, 3, 4, 5, 8, 11, 12, 14, 15). In some cases, the species were added simultaneously, while in others sequential invasion was involved. Johnson (10), however, reported that reproduction of *Pratylenchus brachyurus* increased in the presence of *Meloidogyne incognita* on a root-knot resistant variety of tobacco, but was suppressed on root-knot susceptible varieties. Reproduction of *P. brachyurus* was also suppressed in combination with *M. hapla* and *M. incognita* on a variety of tobacco resistant to *M. incognita*, but susceptible to *M. hapla*.

Ross (13) reported that concomitant populations of *M. incognita* and *Heterodera glycines* on soybean suppressed early season populations of *H. glycines*. Later in the same season, however, populations of *H. glycines* were greater in plots containing both nematodes than in plots with *H. glycines* alone. Johnson (9) found that populations of *Belonolaimus longicaudatus* were higher in combination with *Tylenchorhynchus martini*, than when alone on 'Tifdwarf' bermudagrass.

Jatala and Jensen (6) found that when *M. hapla* was introduced to sugar beets 10 days earlier than *H. schachtii*, a significant increase in cyst nematode development resulted. When *H. schachtii* was introduced 10 days before *M. hapla*, a significant reduction in the size of *M. hapla* galls occurred. With simultaneous inoculations, no significant population changes were noted for either parasite. Further studies indicated that, for maximum cyst formation, inoculation with *H. schachtii* must be made when the preinvading *M. hapla* are young females.

Meloidogyne incognita and *P. brachyurus* are two of the predominant nematodes in Georgia cotton fields, and they are frequently found in the same soil and root samples. The objective of our investigation was to study root penetration and population development of concomitant *M. incognita* and *P. brachyurus* on cotton. In addition, *Meloidogyne arenaria* was used in supporting experiments, along with alfalfa, tobacco, tomato and several other plants.

MATERIALS AND METHODS

Seven greenhouse experiments were conducted, all with *Pratylenchus brachyurus* (Godfrey) Filipjev & Schuurmans Stekhoven and *Meloidogyne incognita* (Kofoid & White) Chitwood, except for one experiment with *Meloidogyne arenaria* (Neal) Chitwood. Four cultivars of cotton, *Gossypium hirsutum* L., were used, in addition to a host range test, and a study with alfalfa, *Medicago sativa* L.; tobacco, *Nicotiana tabacum* L.; and tomato, *Lycopersicon esculentum* Mill. Inocula were juveniles of *Meloidogyne* and juveniles and

Received for publication 13 November 1972.

[1] Based on a M.S. Thesis by the senior author and supported by USDA, CSRS Grants No. 9161515 and No. 0161515(1) and the Georgia Cotton Commodity Commission.

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females of *P. brachyurus*, unless noted otherwise.

Experiment 1: Seeds of 'Carolina Queen' cotton were planted in 32 plastic pots (15-cm diam) of sandy loam soil treated with methyl bromide. Eight of the pots were immediately inoculated with tap water suspensions of *M. incognita*, P_i = 1250/pot; eight with *P. brachyurus*, P_i = 3875/pot; eight with *M. incognita*, P_i = 1250/pot and *P. brachyurus*, P_i = 3875/pot; and eight left as noninoculated controls. After 10 days, the roots were washed, stained with acid fuchsin in saturated chloral hydrate, cleared in lactophenol and the nematodes in each root system identified and counted.

Experiment 2: Seeds of 'Coker 310' cotton were soaked in water for 4 hr and planted in flats (20 × 30 cm) of soil infested with *P. brachyurus*, P_i = 10/100 g soil; *M. incognita*, P_i = 36/100 g soil; or soil treated with methyl bromide. Four plants from each treatment were removed 2, 4, 6, 8 and 10 days after seeding, and the roots treated as in Experiment 1, except that the acid fuchsin was in lactophenol. Ten days after planting the seeds, the remaining seedlings were individually transplanted into 7.5-cm pots of soil infested with *P. brachyurus*, P_i = 4/100 g soil; or soil infested with *M. incognita*, P_i = 20/100 g soil: so as to arrive at the four treatments listed in Table 3. Four plants of each treatment were removed every 2 days for the next 10 days, and the roots treated as described for the first part of the experiment.

Twenty days after seeding, the remaining plants were transplanted into 25-cm pots of soil treated with methyl bromide. Ten days later, six plants from each treatment were removed at random and processed as previously described. Forty-five days after seeding, the roots of the remaining six plants from each treatment were incubated for 72 hr on a gyratory shaker in a solution of 12 ppm ethoxyethylmercuric chloride and 50 ppm dihydrostreptomycin sulfate, and the nematodes counted.

Experiment 3: Seeds of 'Carolina Queen' cotton were soaked for 4 hr and two seeds were planted in each of 32 pots (15-cm diam) of soil treated with methyl bromide. Eight of the pots were inoculated 24 hr after seeding with 5-ml tap water suspensions of *M. incognita*, P_i = 660/pot; eight with *P. brachyurus*, P_i = 3518/pot; eight with *M. incognita* P_i = 660/pot and *P. brachyurus*, P_i = 3518/pot; and eight were left as noninoculated controls. One seedling from each pot was removed soon after emergence. The inoculum was poured into two 2.5-cm deep holes on either side of the planted seed, covered with soil and watered. The experiment was terminated after 96 days, random 5-g root samples were incubated as previously described and the nematodes counted. Soil samples were assayed using a modified sugar flotation-centrifugation technique (7).

Experiment 4: Three seeds of 'Coker 413' cotton were planted in each of 180 pots (8-cm diam) of soil treated with methyl bromide, and thinned to one seedling per pot soon after emergence. Twelve days after emergence, the seedlings and the soil around the roots were transplanted into a 4.5 × 4.4 × 1.0-m ground bed filled with a sandy loam soil treated with methyl bromide. The seedlings were planted in nine rows of 14 seedlings per row. The experiment consisted of four treatments, each treatment represented by a row, and each treatment row separated by a guard row of noninoculated seedlings. *M. incognita* for this test was obtained from infected tomato roots, and *P. brachyurus* from infected cotton roots. The roots were chopped finely with a razor blade, mixed thoroughly, and 1-g portions of the *M. incognita*-infected and 10 g of the *P. brachyurus*-infected roots placed around the roots of each appropriate transplanted seedling. The treatments included were *M. incognita*, P_i = 1629/seedling; *P. brachyurus*, P_i = 77/seedling; *M. incognita*, P_i = 1629/seedling and *P. brachyurus* P_i = 77/seedling; and a noninoculated control. Control plants received 10 g of chopped cotton roots grown in soil treated with methyl bromide. The experiment was terminated after 199 days. Random 5-g root samples were incubated on a gyratory shaker for 72 hr and the nematodes counted.

Experiment 5: Seeds of 'Coker 310' cotton were planted in plastic pots (15-cm diam) of soil treated with methyl bromide. Twenty-four hours later, nematodes in tap water suspensions were added to two holes on either side of the seed. The four treatments used were *M. arenaria*, P_i = 1600/pot; *P. brachyurus*, 530/pot; *M. arenaria*, P_i = 1600/pot and *P. brachyurus*, 530/pot; and a noninoculated control. Each treatment was replicated eight times. Seventy-five days after seeding, the nematodes were extracted from random 5-g root samples using the gyratory shaker

TABLE 1. Penetration of roots of cotton by *Meloidogyne incognita* and *Pratylenchus brachyurus* during the first 10 days after simultaneous inoculation with both nematodes.

Treatment	*M. incognita* per root system	*P. brachyurus* per root system
M. incognita	5 a[1]	0 a
P. brachyurus	0 c	342 b
M. incognita + *P. brachyurus*	1 b	228 b
Control	0 c	0 a

[1]Column means followed by the same letter are not significantly different (P = .05) according to Duncan's multiple range test.

technique and counted.

Experiment 6: Seeds of *N. tabacum* ('Hicks' tobacco), *L. esculentum* ('Rutgers' tomato) and *M. sativa* (alfalfa) were planted in flats (20 × 30 cm) of soil infested with *M. incognita*, P_i= 52/100 g soil, and soil treated with methyl bromide. Ten days after seeding, the plants were transplanted into plastic pots (15-cm diam) of soil treated with methyl bromide, so as to obtain six replicates of each plant inoculated with *M. incognita*, and six of each grown in noninfested soil. Immediately after transplanting, all pots received a 5-ml tap water suspension of *P. brachyurus*, P_i = 216/plant. Fifty days after seeding, the root systems were incubated using the gyratory shaker technique and the nematodes counted.

Experiment 7: Seeds of 13 species of plants (Table 7) were seeded individually in pots (8-cm diam) of soil treated with methyl bromide. Three-tenths of a gram of finely chopped tomato roots infected with *M. incognita*, P_i = 373/pot, or 0.7 g of cotton roots infected with *P. brachyurus*, P_i = 39/pot, were placed approximately 2 cm below each seed. Each treatment was replicated four times. The half of the experiment that received *M. incognita* was analyzed after 104 days, and the half that received *P. brachyurus* was analyzed after 107 days by counting the nematodes after extracting them by incubation of random 10-g root samples on a gyratory shaker.

RESULTS

Root penetration: In the first experiment, *P. brachyurus* penetrated cotton roots as well when alone as when simultaneous inoculations were made with *M. incognita* (Table 1). *M. incognita*, however, penetrated better when alone than when in combination with *P. brachyurus*.

Four days after the second experiment was planted, detectable numbers of both *P. brachyurus* and *M. incognita* had penetrated cotton roots. Penetration by *P. brachyurus* continued to increase rapidly through the 10th day, while *M. incognita* penetration failed to increase after the 6th day. Prior invasion by *M. incognita* seemed to enhance root penetration by *P. brachyurus*, although only data for the 30th day were significantly different (Table 2). While there were no significant differences in *M. incognita* penetration after 12, 14, 18 and 30 days, penetration was significantly inhibited after 16 and 20 days when the roots were previously invaded by *P. brachyurus* (Table 2).

Population dynamics: Numbers of *P. brachyurus* were significantly greater when cotton roots were previously (Expt. 2) or simultaneously (Expt. 3 & 4) invaded by *M.*

TABLE 2. Penetration of roots of cotton by *Meloidogyne incognita* and *Pratylenchus brachyurus* after prior invasion by the concomitant species.

Treatment[1]		*P. brachyurus*/g root[2]					
Initial inoculation	Final inoculation	12 days	14 days	16 days	18 days	20 days	30 days
M. incognita	*P. brachyurus*	123 a[3]	286 a	234 a	178 a	109 a	25 a
Check	*P. brachyurus*	49 a	226 a	195 a	245 a	48 a	4 b
		M. incognita/g root					
P. brachyurus	*M. incognita*	10 a	19 a	2 a	11 a	16 a	44 a
Check	*M. incognita*	11 a	15 a	10 b	5 a	48 b	38 a

[1]Initial inoculation made at seeding and final inoculation 10 days after seeding.
[2]Root population data reported from the date of seeding.
[3]Column means followed by the same letter are not significantly different (P = .05).

TABLE 3. The effect of prior invasion by the concomitant species on the population development of *Pratylenchus brachyurus* and *Meloidogyne incognita* after 45 days on cotton.

Treatment			
Initial inoculation	Final inoculation	*P. brachyurus*/g root	*M. incognita*/g root
M. incognita	*P. brachyurus*	88 a[1]	2 a
Check	*P. brachyurus*	18 b	—
P. brachyurus	*M. incognita*	1 b	3 a
Check	*M. incognita*	—	1 a

[1]Column means followed by the same letter are not significantly different ($P = .05$) according to Duncan's multiple range test.

TABLE 4. Population development of *Pratylenchus brachyurus* and *Meloidogyne incognita* associated with cotton roots following simultaneous inoculation with the concomitant species.

	Nematodes recovered after 96 days in 15-cm pots				Nematodes recovered after 199 days in ground beds	
	P. brachyurus/		*M. incognita*/		*P. brachyurus*	*M. incognita*
Treatment	g root	100 g soil	g root	100 g soil	/g root	/g root
Control	0 a[1]	0 a	0 a	0 a	0 a[1]	0 b
M. incognita	0 a	0 a	9 a	523 b	0 a	299 a
P. brachyurus	191 b	5 a	0 a	0 a	46 a	0 b
P. brachyurus + *M. incognita*	428 c	45 b	141 b	25 a	116 b	12 b

[1]Column means followed by the same letter are not significantly different ($P = .05$) according to Duncan's multiple range test.

incognita, than in the absence of the concomitant species (Tables 3 & 4). *P. brachyurus* populations were also significantly greater when cotton roots were simultaneously invaded by *M. arenaria* (Expt. 5), than in the absence of the concomitant species (Table 5). Invasion by *M. incognita* 10 days prior to inoculation with *P. brachyurus* (Expt. 6), however, suppressed populations of *P. brachyurus* in roots of tomato, and had no significant effect when on alfalfa or tobacco (Table 6).

Populations of *M. incognita* (Expt. 4) and *M. arenaria* (Expt. 5) were generally lower when cotton roots were simultaneously invaded by *P. brachyurus*, than in the absence of the concomitant species (Tables 4 & 5). Although more *M. incognita* per gram of cotton root (Expt. 3) were found in the concomitant treatment than with *M. incognita* alone, the reverse was found to be the case in the soil. When the soil and root populations were considered together, there were fewer *M. incognita* when the roots were simultaneously invaded by *P. brachyurus*, than in the absence of the concomitant species (Table 4). Although prior invasion (Expt. 2) by *P. brachyurus* had no significant influence on cotton root

TABLE 5. Population development of *Meloidogyne arenaria* and *Pratylenchus brachyurus* in roots of cotton, following simultaneous inoculation with the concomitant species.

Treatment	*P. brachyurus*/5 g root	*M. arenaria*/5 g root
M. arenaria	0 a[1]	226 a
P. brachyurus	57 b	0 b
M. arenaria + *P. brachyurus*	81 c	176 ab
Control	0 a	0 b

[1]Column means followed by the same letter are not significantly different ($P = .05$) according to Duncan's multiple range test.

TABLE 6. The effect of prior invasion by *Meloidogyne incognita* on population development of *Pratylenchus brachyurus* on tomato, tobacco and alfalfa.

Treatment		*P. brachyurus*/g root			*M. incognita*/g root		
Initial inoculation	Final inoculation	Tomato	Tobacco	Alfalfa	Tomato	Tobacco	Alfalfa
Check	*P. brachyurus*	71 a[1]	35 a	56 a	—	—	—
M. incognita	*P. brachyurus*	40 b	18 a	72 a	532	1469	27

[1]Column means followed by the same letter are not significantly different ($P = .05$).

populations of *M. incognita*, this experiment was considerably shorter, being less than two *M. incognita* generations in length.

Host suitability: Host suitability was evaluated using logarithmic groupings of reproductive potentials on 13 species of plants. Roots of all species tested contained *P. brachyurus*. Stringbean, cotton, clover and alfalfa were excellent hosts for *P. brachyurus* (Table 7). 'Contedia' stringbean was the best host and supported 2706 *P. brachyurus* per gram of root tissue, while cocklebur was found to be the poorest host and supported one *P. brachyurus* per gram of root tissue.

Pepper was by far the best host for our experimental isolate of *M. incognita* (Table 7). Alfalfa, wheat and tomato were also good hosts. Nematodes were recovered from roots of all plants except peanut and cocklebur. Cotton yielded five *M. incognita* per gram of root tissue, indicating that it is a relatively poor host for this isolate of *M. incognita*.

DISCUSSION

The presence of either *M. incognita* or *M. arenaria* resulted in significant increases in populations of *P. brachyurus* in roots of cotton. The presence of *M. incognita*, however, suppressed *P. brachyurus* reproduction in roots of tomato, and had no influence on numbers in roots of alfalfa or tobacco. These results were similar to those obtained by Estores and Chen (3) for tomato, and Turner and Chapman (15) for alfalfa. Johnson (10) reported that the influence of *M. incognita* on populations of *P. brachyurus* varied with different cultivars of tobacco. Populations were suppressed by a *M. incognita*-susceptible cultivar and enhanced by a resistant variety. In the present investigation, all cotton varieties used were relatively poor

TABLE 7. Population development of *Pratylenchus brachyurus* and *Meloidogyne incognita* on thirteen plant species.

Nematodes/g root	*P. brachyurus*	*M. incognita*
>1000	*Phaseolus vulgaris* L. 'Contedia' *Gossypium hirsutum* L. 'Carolina Queen' *Trifolium incarnatum* L. *Medicago sativa* L. 'Buffalo'	*Capsicum frutescens* L. 'Calif. Wonder'
100 - 1000	*Hordeum vulgare* L. 'Florida 102'	*Medicago sativa* L. 'Buffalo' *Triticum vulgare* Vill. 'Blueboy' *Lycopersicon esculentum* Mill. 'Rutgers'
10 - 100	*Citrullus vulgaris* Schrad. 'Dixie Queen' *Secale cereale* L. 'Gator' *Arachis hypogaea* Mill. 'Early Runner' *Lycopersicon esculentum* Mill. 'Rutgers' *Avena sativa* L. 'Florida 501' *Capsicum frutescens* 'Calif. Wonder'	*Hordeum vulgare* L. 'Florida 102' *Secale cereale* L. 'Gator' *Citrullus vulgaris* Schrad. 'Dixie Queen'
1 - 10	*Triticum vulgare* Vill. 'Blueboy' *Xanthium pensylvanicum* Wallr.	*Gossypium hirsutum* L. 'Carolina Queen' *Trifolium incarnatum* L. *Phaseolus vulgaris* L. 'Contedia' *Avena sativa* L. 'Florida 501'
0		*Arachis hypogaea* L. 'Early Runner' *Xanthium pensylvanicum* Wallr.

hosts for our isolates of *M. incognita* and *M. arenaria*, and populations of *P. brachyurus* were enhanced.

These studies emphasize the importance of host susceptibility in the study of concomitant nematode populations. They support the hypothesis that populations of one nematode species will be enhanced in the presence of another species if the host is relatively resistant to the second species. The hypothesis, however, may be limited to cases where the second species produces primary symptoms that are predominately hyperplastic. Conversely, populations of a species will be suppressed in the presence of another species if the host is relatively susceptible to the second species.

The presence of *P. brachyurus* suppressed populations of *M. incognita* and *M. arenaria*. These results resemble those reported in most of the previous studies of concomitant nematode populations. Since all of the cotton cultivars used in the present investigation were excellent hosts for *P. brachyurus*, the results appear to fit the preceding hypothesis. It should be expected, however, that any plant-parasitic nematode that produces primary symptoms that are predominately necrotic, would have a detrimental influence on the biological activities of a concomitant obligate parasite.

When root penetration of an endoparasitic nematode is inhibited or enhanced by the presence of a concomitant species, there will likely be a direct influence on the populations of the first species. Chapman and Turner (2) found that *P. penetrans* females deposit fewer eggs in red clover roots infected with *M. incognita*, than in the absence of that concomitant species. It may be possible to reverse such a phenomenon with the right combination of host and *M. incognita* isolate. Variation in egg viability, changes in the lengths of life cycles, alterations in the percent of progeny surviving a complete life cycle and nematode-induced changes in the number of a given species that can be supported by a given biomass of a specific host, are only a few of the many potential factors that may play important roles in the influence of one nematode on a concomitant species. Many ecosystems will have to be systematically reconstructed before we are likely to have an adequate understanding of the complex interactions that probably occur between hosts and concomitant nematode populations.

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