Dynamics of Concomitant Populations of Hoplolaimus columbus, Scutellonema brachyurum, **and** Meloidogyne incognita **on Cotton**¹

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Abstract: Cotton seedlings grown in a greenhouse and a growth chamber were inoculated with Scutellonema brachyurum, Hoplolaimus columbus, and Meloidogyne incognita, singly and in all possible combinations, at two initial population (Pi) levels (100 and 300/100 cm³). S. brachyurum alone was not pathogenic to cotton at these population levels. It fed primarily as an ectoparasite but matured and reproduced within the root when it penetrated. Populations of S. brachyurum increased in the presence of H. columbus but were suppressed by M. incognita. H. columbus suppressed dry shoot weights of cotton (P = 0.05) at a Pi of 300/100 cm³ soil. Simultaneous inoculation of H. columbus with either M. incognita or S. brachyurum increased H. columbus populations over treatments with H. columbus alone, both at 60 and 90 d after inoculation. M. incognita suppressed cotton shoot weights significantly (P = 0.05) at both Pi levels. Inoculation with S. brachyurum increased M. incognita populations 60 d after inoculation, while H. columbus suppressed populations of M. incognita. Most larvae of M. incognita did not develop to maturity in the presence of H. columbus. Giant cells aborted and were necrotic 20-25 d after inoculation. Since \hat{M} . incognita and H. columbus feed on different tissues, the inhibition of M. incognita may have resulted from a physiological effect of H. columbus on the host. Key words: Nematode interactions, population dynamics, cotton, lance nematode, spiral nematode, root-knot nematode.

Soils of agricultural lands rarely contain monospecific nematode populations. Instead, most soils contain numerous species of plant-parasitic nematodes with overlapping host ranges. In South Carolina, 25% of the samples collected in 1978 from cotton fields contained *Hoplolaimus columbus* Sher. Of these samples, 48% also contained *Scutellonema brachyurum* (Steiner) Andrássy and 7% *Meloidogyne* spp. (11). Recently, large populations of *S. brachyurum* (Steiner) Andrássy have been associated with poor growth of cotton in South Carolina (F. H. Smith, unpublished).

Population dynamics and pathogenicity of plant-parasitic nematodes in competition may differ from monospecific populations usually studied experimentally (6,7,14). Bird et al. (3) reported that H. columbus replaced M. incognita (Kofoid & White) Chitwood as the dominant species in a Georgia cotton field after four growing seasons. The nature of the competitiveness of H. columbus was not reported. Since H. columbus frequently occurs in combination with S. brachyurum and M. incognita in South Carolina, the potential exists for increased crop losses due to interactions among the three species. These interactions may require changes in nematode pest management programs.

The objectives of these studies were to 1) investigate interactions among H. columbus, M. incognita, and S. brachyurum on cotton, 2) establish the effects of con-

Received for publication 31 May 1979.

¹Contribution No. 1700 of the South Carolina Agricultural Experiment Station. Portion of a Ph.D. dissertation by the senior author, Clemson University.

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comitant populations of these nematodes on cotton in the greenhouse, and 3) examine possible interactions.

MATERIALS AND METHODS

Greenhouse studies: Pasteurized Varina sandy loam from Blackville, South Carolina, was amended with an equal portion of sterile sand and placed in 15-cm plastic pots. Cotton (Gossypium hirsutum L., 'Coker 310') seeds were germinated in sand and one seedling, selected for uniform growth, was transplanted into each pot after which nematodes were pipetted onto the root system.

H. columbus increased on cotton in the greenhouse and S. brachyurum from a peach (Prunus persica L.) orchard in Edgefield County, South Carolina, were extracted from soil with a semiautomatic elutriator (4) for inoculum. Nematodes were separated from the soil left on the sieves by flotationsieving (5) and Baermann funnel techniques (15). M. incognita was extracted from tomato (Lycopersicon esculentum Miller 'Marion') roots with a 1.25% sodium hypochlorite solution (8). Inocula were standardized to 1,000 nematodes or eggs/ml suspension. Treatments were H. columbus (L), S. brachyurum (S), and M. incognita (R), singly and in these combinations: (L+S), (L+R), (S+R), and (L+S+R). Initial population levels (Pi) were 1,500 and 4,500 nematodes/15-cm pot for each species (100 and 300 nematodes/100 cm³ soil, respectively). Controls received aliquots of a duplicate solution without nematodes. Fifteen replicates of each treatment were placed on a greenhouse bench in a randomized complete block design. Liquid fertilizer (HYPONEX, Hydroponic Chemical Co., Copley, Ohio, 7.4 g/l water) was added biweekly at 100 ml/pot.

Five replicates of each treatment were harvested for laboratory analysis 30, 60, and 90 d after inoculation. Roots were incubated 4 d in a mist extraction chamber (1), with zinc sulfate (ZnSO₄) added to the water at 100 μ g/l to maximize nematode extraction (2). Populations in 100 cm³ soil were extracted by semiautomatic elutriation (4) and centrifugal-flotation (9). Adult and juvenile nematodes in 1-ml portions were counted in an eelworm slide (Gelman Hawksley Ltd., Lancing, Sussex, England). Final populations (Pf) were calculated by adding nematodes from soil and roots. Shoots and roots were dried 4 d at 70 C to obtain dry weights. Duncan's multiple range procedure was used to separate treatment means.

Growth chamber studies: Sand was passed through a 20-mesh sieve, pasteurized at 80 C for 30 min, and placed in 50-ml vials (30 cm³ sand/vial). 'Coker 310' cotton seeds were treated for 10 min in 1.25% sodium hypochlorite and rinsed with distilled water. The procedures of McClure and Robertson (13) were used for seed germination. Three-day-old seedlings (one/ vial) were transplanted to the vials, and all nematode combinations were inoculated onto the roots.

H. columbus and S. brachyurum inocula were obtained as for the greenhouse study. M. incognita was propagated on Marion tomato in the greenhouse. Roots of 30-d-old cultures were placed in a mist chamber and larvae hatched on the 2nd and 3rd days were used as inoculum. Nematodes were treated in a 130 μ g/l solution of Aretan (Plant Protection Ltd., Yalding, Kent, England) under constant aeration for 24 h, followed by thorough rinsing with distilled water (12). Inocula were standardized to 30 nematodes/ml suspension. One-ml portions were pipetted onto the root at transplanting. Nematode species were added singly and in all possible combinations at 30 nematodes/species (equivalent to 100 nematodes/100 cm³ sand). Thirty-four replicates of each treatment were placed in a growth chamber under 14 h fluorescent light at 25 ± 2 C. The seedlings were watered with a solution containing NH4NO3, KNO3, Ca $(NO_3)_2$, and KCl each at 0.25 g/l, MgSO₄ at 0.50 g/l, and $KH_2PO_4 + EDTA$ at 0.10 and 0.025 g/l, respectively. Two seedlings of each treatment were taken for laboratory analysis at 24 h intervals.

The experiment was repeated with Pi 100 nematodes/vial for each species (equivalent to 330 nematodes/100 cm³ sand). Seedlings for laboratory analysis were taken at 48 h intervals. Root segments with nematodes were fixed and stained according to the modified McBryde method described by McClure and Robertson (13). Roots were

		Weight* in g (Pi = $100/100$ cm ³ soil) [†]					Weight* in g (Pi = $300/100 \text{ cm}^3 \text{ soil}$)‡					
Treatment	Shoot			Root		Shoot			Root			
	30	60	90	30	60	90		60	90	30	60	90
Control	1.2 a§	3.7 a	5.4 a	0.4 a	1.6 a	4.7 a	1. 2 a	3.7 a	5.4 a	0.4 ab	1.6 a	4.7 a
(L) H. columbus	1.0 abc	3.2 b	5.3 a	0.4 a	1.6 a	3.1 b	0.9 b	2.9 b	4.6 b	0.5 ab	1.7 a	3.3 b
(S) S. brachyurum	1.3 a	3.3 b	5.1 b	0.4 a	1.2 ab	2.6 b	1.1 a b	3.5 a	4.9 b	0.4 bc	1.3 abc	2.7 bc
(R) M. incognita	0.7 d	1.9 c	2.1 с	0.4 a	1.3 a b	3.0 b	0.4 c	1.6 c	1.8 c	0.2 с	1.2 bc	2.1 cd
L+S	1.1 ab	3.1 b	4.2 b	0.4 a	1.6 a	2.8 b	1.3 a	2.7 b	3.8 b	0.5 a	1.4 ab	2.7 bc
L+R	0.8 cd	1.9 c	2.6 c	0.4 a	1.4 ab	3.1 b	0.6 c	1.6 c	1.6 c	0.3 c	1.1 bc	1.7 d
S+R	0.9 bcd	1.6 c	2.1 c	0.5 a	1.2 b	2.0 b	0.5 c	1.2 c	1.8 c	0.2 c	1.1 bc	1.7 d
L+S+R	0.8 cd	1.6 c	1.4 c	0.3 a	1.1 b	1.8 b	0.6 c	1.5 c	1.4 c	0.3 c	0.9 c	1.5 d

Table 1. Dry weights of cotton (Gossypium hirsutum L.) shoots and roots 30, 60, and 90 d after inoculation with Hoplolaimus columbus (L), Scutellonema brachyurum (S), Meloidogyne incognita (R), and all possible combinations at two initial population levels (Pi).

•Average weight of five replicates. †Pi 1500 nematodes/15 cm pot = 100 nematodes/100 cm³. ‡Pi 4500 nematodes/15 cm pot = 300 nematodes/100 cm³.

\$Numbers followed by the same letter are not significantly different at the 95% confidence level.

not cleared in chloral hydrate, but were stored in lactophenol until they were mounted between two microscope slides. Percentage penetration, soil population, maturity stage, and location and position in the root were recorded for each species and treatment. *M. incognita* was classified as immature stages (I), swollen females (M), and egg-laying females (E).

RESULTS

Host responses-greenhouse: Host response to nematode treatments was more noticeable at Pi 4,500 than at Pi 1,500 (Table 1). Treatments containing M. incognita had significantly lower shoot weights than all other treatments, except for the S+R treatment at Pi 1,500 30 d after inoculation. Root weights were often suppressed in treatments with M. incognita at Pi 4,500 but not at Pi 1,500. Other nematodes did not ameliorate the influence of M. incognita on plant growth, and they alone did not suppress shoot growth to the same extent as M. incognita. Control plants usually had larger shoot weights than those in other treatments (Table 1). H. columbus appeared to suppress shoot weights (but not root weights) more than S. brachyurum, and, conversely, S. brachyurum suppressed root weights more than H. columbus. Differences were not significant, however. Combined species suppressed shoot weight more than either species alone. The combination of S+R generally reduced shoot and root weights more than L+R treatments. Shoot weights of plants with R alone were always significantly lower than those with L or S alone at both Pi levels.

Nematode responses-greenhouse: The presence of concomitant species affected the rate of root-knot nematode penetration, development, and reproduction. At the larger inoculum level, populations of M. incognita were largest when in combination with S. brachyurum 60 d after inoculation in the greenhouse (Fig. 1). Next largest populations were in the treatment with M. incognita alone. H. columbus inhibited development of M. incognita at both Pi levels in the greenhouse with the larger Pi having the greater effect. The M. incognita population was intermediate between S+R and L+R. For



Fig. 1. Population responses of *Meloidogyne* incognita (R) alone or in simultaneous inoculation with *Hoplolaimus* columbus (L), Scutellonema brachyurum (S), or both on cotton in the greenhouse 30, 60, and 90 d after inoculation (initial population = 1,500 or 4,500 nematodes/15-cm pot for each species, or 100 and 300/100 cm³, respectively).

all treatments, populations of M. incognita in the soil and roots were largest 60 d after inoculation, decreasing rapidly as the roots decayed. Largest populations were found in treatments with high Pi levels, except when H. columbus was present.

Populations of *H. columbus* in all treatments dropped below Pi levels 30 d after inoculation, then recovered and were highest after 90 d (Fig. 2). *H. columbus* reproduced better following simultaneous inoculations with *S. brachyurum*, *M. incognita*, or both. Inoculation with *H. columbus* alone resulted in smallest populations at both Pi levels, while L+S and L+S+Rresulted in largest populations.

Populations of S. brachyurum generally did not increase above Pi levels in the greenhouse. At the smaller Pi, only the L+Streatment had numbers of Scutellonema greater than Pi. Although Pf was larger at the higher Pi levels, Pf was always less than



Fig. 2. Population responses of Hoplolaimus columbus (L) alone or in simultaneous inoculation with Scutellonema brachyurum (S), Meloidogyne incognita (R), or both on cotton 30, 60, and 90 d after inoculation (initial population = 1,500 or 4,500 nematodes/15-cm pot for each species, or 100 and 300/100 cm³, respectively).

Pi (Fig. 3). The Pf of S. brachyurum was largest in the presence of H. columbus at both inoculum levels, while M. incognita had an inhibitory effect on the final population at the large Pi.

Nematode responses-growth chamber: M. incognita penetrated near the root tips and completed its life cycle in the stele. Penetration of roots (up to day 6) by M. incognita was generally suppressed when H. columbus was present (Table 2). Furthermore, development of M. incognita (after day 6) was impeded by the presence of H. columbus. Only a few of the M. incognita larvae that entered the root matured when H. columbus was present. Most giant cells were aborted and appeared necrotic 26-30 d after inoculation. During harvest days 11-14 at Pi 30, 8.2% of the M. incognita in treatment R and 4.0% of M. incognita in treatment R+L were mature (M) but had not produced eggs. No mature (M) M. incognita females were observed in treat-



Fig. 3. Population responses of Scutellonema brachyurum (S) alone or in simultaneous inoculation with Hoplolaimus columbus (L), Meloidogyne incognita (R), or both on cotton 30, 60, 90 d after inoculation (initial population = 1,500 or 4,500 nematodes/15-cm pot for each species, or 100 and 300/100 cm³, respectively).

ment R+L during harvests on days 8-12 at Pi 100, but they were present in treatment R. At the smaller inoculum level, 23% of the nematodes present in treatment R and 9% in treatment R+L were mature (M) after 15-17 days. Plants in treatment R at Pi 100 harvested during days 14-18 contained 38% mature females (M), while plants in treatment R+L contained 16% mature females. Furthermore, during days 20-24 at Pi 100, egg masses were evident in 31% of the nematodes present in treatment R and in only 3% of those in treatment R+L. This trend continued through termination of the experiment after 30 d with 70% of the nematodes in treatment R and 18% in treatment R+L having females with egg masses (E).

S. brachyurum did not suppress penetration of M. incognita up to day 6 at either inoculum level. There were fewer M. incognita in treatment R+S than in R in the harvests occurring on days 7-14 at Pi 30 and in the comparable 8-12 day harvests at Pi

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Table 2. Effects of Hoplola	imus columbus (L)	, Scutellonema	brachyurum	(S), or bo	oth on	the dev	elop-
ment of Meloidogyne incognita	(R) in a growth ch	amber at 25 \pm	2 C.				_

			% M. incognita in roots X days after infestation					
Treatment		1-3	4-6	7-10	11-14	15-17		
	I‡	6.7	47.8	95.0	90.5	74.4		
R*	М	0	0	0	8.3	22.2		
	E	0	0	0	0	0		
	I	7.8	41.1	70.8	78.3	77.8		
R+L*	М	0	0	0	3.3	7.8		
	E	0	0	0	0	0		
	I	7.8	47.8	62.5	72.5	71.1		
R+S*	М	0	0	0	6.7	24.4		
	E	0	0	0	0	1.1		
	I	17.8	38.9	60.0	66.7	67.8		
R+S+L*	М	0	0	0	1.7	21 .1		
	E	0	0	0	0	0		
		2-6	8-12	14-18	20-24	26-30		
	T	29.3	58.0	31.0	6.7	0.7		
R†	M	0	4.7	20.0	25.0	20.7		
	E	0	0	0.7	14.3	48.7		
	I	17.7	44.7	20.3	6.7	11.0		
R+L†	М	0	0	4.0	3.3	15.7		
	E	0	0	0	0.3	5.7		
	I	28.7	42.7	39.7	2.0	1.7		
R+S†	М	0	4.3	8.7	32.0	19.7		
	E	0	0	0	15.7	28.7		
	I	19.3	49.0	37.0	16.0	1.3		
R+S+L†	М	0	1.0	10.3	23.3	27.0		
	E	0	0	0	1.3	25.3		

*Initial population (Pi) = 30 nematodes/30 cm³. Two plants/treatment harvested every 24 h.

+Pi = 100 nematodes/30 cm³. Two plants/treatment harvested every 48 h.

 $\ddagger I = L_2 - L_3$ (immature); $M = L_4$; E = females with egg masses.

100. The end result at Pi 100 was a greater percentage of egg-laying females in R than in R+S (69% vs 57%). However, egg masses of *M. incognita* were larger and numbers of eggs were greater in R+S than in R.

The effect of both H. columbus and S. brachyurum on development (after day 6) of M. incognita differed with Pi. Greater numbers of M. incognita were found in roots inoculated with the three species together in harvests on days 1-3 than in the other treatments. However, the data for harvests on days 4-6 at Pi 30 and 2-6 at Pi 100 are similar in that suppression of M. incognita penetration occurred. Development of M. incognita was retarded through day 14 at Pi 30 and through day 30 at Pi 100, when 69% of the nematodes present in the roots of R and 47% of those in R+S+L had egg masses.

A smaller percentage of the initial *H.* columbus inoculum was found in roots compared with *M.* incognita (Table 3). *S.* brachyurum generally favored *H.* columbus penetration into roots of cotton plants at the larger Pi. Differences were not large, however. *M.* incognita slightly inhibited penetration of *H.* columbus. There was no effect on the ratios of adult and juvenile *H.* columbus in any treatment.

S. brachyurum was primarily an ectoparasite on cotton. It fed on the epidermis, primarily on the tap-root of the seedlings, and seldom penetrated root tissue. Occasionally, it was found beneath the epidermis in

Table 3. Effects of Scutellonema brachyurum (S), Meloidogyne incognita (R) or both on the development of Hoplolaimus columbus (L) in a growth chamber at 25 ± 2 C.

	% H. columbus in roots X days after infestation						
Theatment	1-3	4-6	7-10	11-14	15-17		
 L*	0	11.1	19.2	21.7	20.0		
L+S*	0	12.2	14.2	25.0	16.7		
L+R*	1.1	3.3	15.8	21.6	15.6		
L+S+R*	3.3	15.6	10.0	20.8	20.0		
	2-6	8-12	14-18	20-24	26-30		
L†	6.3	7.3	7.3	5.0	2.3		
L+S†	8.7	13.3	3.0	5.7	10.3		
$L+R^{\dagger}$	4.7	10.3	2.3	1.0	1.0		
L+S+R†	6.3	8.7	5.3	4.3	2.7		

•Initial population (Pi) = 30 nematodes/30 cm³. Two plants/treatment harvested every 24 h.

 $\dagger Pi = 100$ nematodes/30 cm³. Two plants/ treatment harvested every 48 h.

the exterior layers of the cortex. Only 1-2% of the initial inoculum was found inside the root. Of these, 85–100% had deposited eggs within the feeding area. Females formed a spiral under the epidermis and laid three to five eggs.

DISCUSSION

M. incognita completes its life cycle after establishing a permanent feeding site in the vascular parenchyma near root tips. H. columbus is also an endoparasite but feeds almost exclusively in the cortex (12). Although H. columbus may penetrate gall tissue induced by M. incognita, it stays within the swollen cortex of the gall. Since these nematodes feed on different root tissues, they probably interact via physiological changes in the root. The deleterious effect of H. columbus on development and reproduction of *M. incognita* does not seem to be a result simply of mechanical injury. Necrosis of giant cells and death of larvae could explain the observations in Georgia that H. columbus can replace M. incognita as the dominant species in a field (3). Necrosis of giant cells may result from phenolic compounds, common in cotton, released by tissue damaged by H. columbus. Split-root experiments would enable one to

determine if a translocatable factor were involved and if mechanical injury of feeding sites is important in the relationship of the two species. The detrimental effect of other nematode species on *M. incognita* reproduction has also been observed when *H. galeatus* (Cobb) Thorne (16) and *Pratylenchus brachyurus* Godfrey (7) were inoculated with *M. incognita*. The inhibitory effect of *H. columbus* may result from damage to the feeding site of *M. incognita* (16), but the lack of proximity of *H. columbus* to *M. incognita* feeding sites suggests that other factors are involved.

The decline in prominence of M. incog*nita* in a cotton field and the increase of H. columbus is probably also related to the suppressing of penetration by M. incognita. Whether these two factors are of such a magnitude that they can totally account for the decline of *M. incognita* cannot be determined. The relationship does not appear to be density dependent, but the effect of various ratios of H. columbus to M. incognita has not been tested. Races of M. incognita may differ in their reaction to H_{a} columbus (3) such that field-to-field results may differ. However, a situation similar to that reported in Georgia (3) has developed in a South Carolina field (H. L. Musen, unpublished).

The data on the effect of S. brachyurum on M. incognita is not conclusive. S. brachyurum has been considered a relatively unimportant parasite in agricultural soils (10), but the apparent increase in numbers of M. incognita eggs and the suppression of shoot weight at 60 d and root weights at 60 and 90 d in combined inoculations should be further quantified. This relationship, if established, also has implications in the peach industry in South Carolina, where many orchards have infestations of these species.

The evidence that *H. columbus* and *S. brachyurum* mutually benefit from their association gives further impetus to the possibility of a stimulatory effect of *Hoplolaimus* on ectoparasites (16).

LITERATURE CITED

l. Barker, K. R. 1978. Determining nematode population responses to control agents. In E. I. Zehr, ed. Methods for evaluating plant fungicides,

48 Journal of Nematology, Volume 13, No. 1, January 1981

nematicides and bactericides. Am. Phytopathol. Soc. and the Soc. of Nematol.

2. Bird, G. W. 1971. Influence of incubation solution on the rate of recovery of Pratylenchus brachyurus from cotton roots. J. Nematol. 3:378-385.

3. Bird, G. W., O. L. Brooks, and C. E. Perry. 1974. Dynamics of concomitant field populations of Hoplolaimus columbus and Meloidogyne incognita. J. Nematol. 6:190-194.

4. Byrd, D. W., Jr., K. R. Barker, H. Ferris, C. J. Nusbaum, W. E. Griffin, R. H. Small, and Connie A. Stone. 1976. Two semi-automatic elutriators for extracting nematodes and certain fungi from soil. J. Nematol. 8:206-212.

5. Byrd, D. W., Jr., C. J. Nusbaum, and K. R. Barker. 1966. A rapid flotation-sieving technique for extracting nematodes from soil. Plant Dis. Reptr. 50:954-957.

6. Chapman, R. A., and D. R. Turner. 1975. Effect of Meloidogyne incognita on reproduction of Pratylenchus penetrans in red clover and alfalfa. J. Nematol. 7:6-10.

7. Gay, C. M., and G. W. Bird. 1973. Influence of concomitant Pratylenchus brachyurus and Meloidogyne spp. on root penetration and population dynamics. J. Nematol. 5:212-217.

8. Hussey, R. S., and K. R. Barker. 1973. A comparison of methods of collecting inocula of

Meloidogyne sp. including a new technique. Plant Dis. Reptr. 57:1025-1028.

9. Jenkins, W. R. 1964. A rapid centrifugation flotation technique for separating nematodes from soil. Plant Dis. Reptr. 48:692.

10. Kraus-Schmidt, H., and S. A. Lewis, 1979, Scutellonema brachyurum: host plants and pathogenicity on cotton. Plant Dis. Reptr. 63:688-691,

11. Lewis, S. A., and F. H. Smith. 1976. Host plants, distribution and ecological association of Hoplolaimus columbus. J. Nematol. 8:264-270.

12. Lewis, S. A., F. H. Smith, and W. M. Powell. 1976. Host-parasite relationships of Hoplolaimus columbus on cotton and soybean. J. Nematol. 8:141-145.

13. McClure, M. A., and J. Robertson. 1973. Infection of cotton seedlings by Meloidogyne incognita and a method of producing uniformly infected root segments. Nematologica 19:428-434.

14. Ross, J. P. 1964. Interaction of Heterodera glycines and Meloidogyne incognita on soybeans. Phytopathology 54:304-307.

15. Thorne, G. 1961. Principles of Nematology. McGraw-Hill Book Co., New York.

16. Yang, H., N. T. Powell, and K. R. Barker. 1976. Interactions of concomitant species of nematodes and Fusarium oxysporum f. sp. vasinfectum on cotton. J. Nematol. 8:74-80.