Interaction of Three Plant-parasitic Nematodes on Corn and Soybean¹

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Abstract: Interaction of Belonolaimus longicaudatus, Meloidogyne incognita, and Pratylenchus brachyurus on corn and B. longicaudatus, M. incognita, and Heterodera glycines on soybean was investigated in microplots during two seasons for corn and one season for soybean. Changes in population densities and effects on plant growth of each nematode on corn or soybean alone and in mixed culture were compared. No interactions occurred on corn in 1987. In 1988, midseason population densities of B. longicaudatus were greater in corn plots infested with M. incognita ($P \leq 0.05$), but at harvest, population densities of B. longicaudatus were less in corn plots infested with P. brachyurus ($P \leq 0.05$). Except for stalk weight at harvest, plant growth was not affected by any treatment. In soybean, midseason densities of M. incognita were increased in combination with H. glycines ($P \leq 0.05$), but this trend reversed at harvest. Soybean yield was reduced in plots infested with H. glycines ($P \leq 0.05$), out mixed culture.

Key words: Belonolaimus longicaudatus, corn, Glycine max, Heterodera glycines, interaction, Meloidogyne incognita, Pratylenchus brachyurus, soybean, Zea mays.

Corn (Zea mays L.) and soybean (Glycine max (L.) Merr.), two of the most important crops grown in the United States, are hosts for several highly pathogenic nematode species (1,16). Production of these crops is interrelated with a wide range of other cultivated crops, particularly in the southeastern United States. Consequently they influence the population ecology of four important plant-parasitic nematodes—Meloidogyne incognita (Kofoid & White) Chitwood, Belonolaimus longicaudatus Rau, Pratylenchus brachyurus (Godfrey) Filipjev & Schuurmans-Stekhoven, and Heterodera glycines Ichinohe.

In most cases, the effects of these nematodes have been reported as individual species, but unfortunately a one-nematode-one-disease occurrence is an atypical situation. Damage by polyspecific communities of plant-parasitic nematodes is common, particularly on corn (12) and soybean (16), but only in a few instances have the combined affects of two or more species been investigated (5,8,9,14,15). Microplot methodology has been particularly useful in evaluating the effects of single and multiple nematodes on host crops (2). Our objectives were to determine the effects on corn and soybean of three nematode genera singly and in various combinations.

MATERIALS AND METHODS

Three separate tests, two on corn and one on soybean, were conducted in 76-cm-d microplots encircled with 60-cm-wide fiberglass sheets inserted 50 cm into the soil (7). The microplots were arranged in rows 1.5 m apart in an Arredondo fine sand (93% sand, 4% silt, 3% clay; pH 5.8; 1% organic matter) treated with 977 kg methyl bromide/ha (98% a.i.) applied broadcast under a 3-mil plastic covering 3-5 months before planting. No plant-parasitic nematodes were detected in the top 20 cm of soil when the microplots were sampled before planting. In each test, the experimental design was a $2 \times 2 \times 2$ factorial replicated six times.

Local populations of Belonolaimus longicaudatus, Meloidogyne incognita race 1, Pratylenchus brachyurus, and Heterodera glycines race 3 were reared in a greenhouse on bahiagrass (Paspalum notatum Flugge cv. Pensacola), tomato (Lycopersicon esculentum Mill. cv. Rutgers), snap bean (Phaseolus vulgaris L. cv. Harvester), and soybean (Glycines max

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cv. Davis), respectively. A selected initial population density was compared with zero of each nematode species (Tables 1-3). Nematode inocula (juveniles and adults of B. longicaudatus and P. brachyurus; eggs of M. incognita and H. glycines) were added and mixed into the top 9 cm of soil. The microplots were planted with Pioneer 304C corn on 29 April 1987 and 8 April 1988 and with Davis soybean on 3 June 1988. Three pairs of corn seed were planted 4 cm deep and 19 cm apart in a row and thinned to three plants per plot in 1987 and four plants per plot in 1988. Soybean seeds were planted 2.5 cm deep and 4 cm apart in a row and thinned to 10 or 11 plants per plot. Border rows (76 cm apart) of corn and soybean were maintained outside the microplots to simulate plant spacings typical of the field. Basic fertilizers, insecticides, and supplemental irrigation were provided as needed. Each soybean plot was infested with a mixture of Glomus intraradices Schenck & Smith and Gigaspora margarita Hall & Becker at the rate of 300 g soil and bahiagrass roots containing both fungi per plot on 24 May 1988. Soybean seeds were inoculated with a commercial source of Rhizobium japonicum (Kirchner) Buchanan.

Soil nematode population densities were determined 3-4 weeks after planting, 12 weeks after planting, and at harvest. Five soil cores (2.5 cm d \times 20 cm deep) were collected per plot and bulked. The soil (100 cm³) was processed by a centrifugal-flotation technique (6). At harvest, three root systems were collected, chopped (5 g) in a blender, and incubated on Baermann trays for 48 hours.

Corn grain yield is reported as shelled corn at 15.5% moisture and soybean seed yield is reported at 10% moisture. Stalk weights and number of ears per plot were determined for each corn plot. In addition, plant growth measurements were taken twice during the growing season.

All nematode densities (number per 100 cm³ soil) were transformed by log_{10} (x + 1) before data analysis. Analyses revealed consistent significant relationships with the

transformed densities as well as with the raw nematode counts; thus the latter are reported. Data then were subjected to the appropriate analysis of variance for a factorial design (19).

RESULTS

Corn: In weeks 3 and 5 after planting the 1987 and 1988 test plots, respectively, no visual symptoms of nematode damage were apparent. Stalk diameter and extended plant height among plants in all plots were similar (Tables 1, 2). Except for stalk weight at harvest, no plant growth measurements were affected throughout the season by any of the three test nematodes. Parasitism by *P. brachyurus* resulted in a lower stalk weight at harvest in 1987 ($P \le$ 0.05), and in 1988 stalk weight at harvest was reduced by *M. incognita* parasitism ($P \le$ 0.05).

In 1987, 12 weeks after planting, population densities of B. longicaudatus were greater in plots infested with M. incognita $(P \le 0.05)$ (Table 1). In the same sampling period in 1988 population densities of B. longicaudatus were less in plots infested with *P. brachyurus* ($P \le 0.05$) (Table 2). In 1988, when higher inoculum levels of each of the three nematodes were used, M. incognita interacted with B. longicaudatus ($P \le 0.01$) at harvest causing a reduction in the population density of B. longicaudatus. In both years the population density of B. longicaudatus 0-15 cm deep in the soil was greatly reduced at harvest, relative to the 12-week sampling.

Soybean: By week 11 after planting, an interaction ($P \le 0.05$) of *B. longicaudatus* and *H. glycines* on plant height was observed (Table 3). Plant height was lower in plots infested with both nematodes than in plots without *H. glycines* ($P \le 0.05$), whereas *M. incognita* had no effect on plant growth either singly or in combination with *H. glycines*. No early season root abbreviation or stunted foliage growth was attributed to *B. longicaudatus*. Reductions in final soybean yield observed here were all attributed to *H. glycines* (Table 3).

There were numerous interactions

Initia	al nematode popu density per plot	lation		Nematodes/ 100 cm³ soil (no	.)	Leaves/	Stalk	Plant beight	Nematod dry w	les/g root t. (no.)	Fresh stalk		Yield/plott
Bl	Mi	Pb	Bl	Mi	Pb	(no.)	(mm)	(cm)	Mi	Pb	(g)	(no.)	(g)
						19 N	Aay .						······
0	0.	0	0	0	0	6.1	6.9	42					
0	0	80	0	0.2	0	6.8	7.8	48					
0	5,250	0	0	2.8	0	6.1	6.3	38					
0	5,250	80	0	2.3	0	7.1	7.2	47					
525	、 0	0	0.3	0	0	7.1	7.3	44					
525	0	80	0	0	0	7.5	7.7	46					
525	5,250	0	0.5	4.3	0	7.0	7.7	46					
525	5,250	80	0.2	1.7	0.2	7.4	7.7	47					
Signi	ficant effects:		NS	Mi***	NS	B1*	NS	NS					
						21 J	uly						
0	0	0	0	0.5	0	0	25	260					
Ő	Ō	80	Ō	0	3.3		94	260					
0	5.250	0	1.0	175	0		24	250					
0	5,250	80	2.2	119	2.0		22	260					
525	0	0	7.6	0	0		24	260					
525	0	80	7.2	0.5	Õ		25	270					
525	5.250	0	11.8	220	0		24	260					
525	5.250	80	10.2	365	1.3		24	260					
Signi	ficant effects:		B1***	M;***	NS		NS	NS					
orgini	neam cirects.		Mi*	1411	110		110	110					
						23 Septe	ember						
0	0	0	. 0	0.2	0				0	0	896	4.6	352
0	0	80	0	3.7	44.5				1.6	413	756	4.5	449
0	5,250	0	0	1,436	0				19.3	0.75	841	4.3	367
0	5,250	80	0.7	1,472	17.7				17.4	199	718	4.7	408
525	0	0	2.3	0	0.8				0	0.96	907	5.2	316
525	0	80	2.0	0.2	18.2				0	90.7	775	5.3	422
525	5,250	0	0.8	1,021	0.2				10.2	106	931	4.3	422
525	5,250	80	2.7	1,354	6.8				5.5	169	789	5.3	380
Signi	ficant effects:		Bl***	Mi***	Pb***				Mi***	Pb*	Pb*	NS	NS

TABLE 1. Population changes of Belonolaimus longicaudatus (Bl), Meloidogyne incognita (Mi), and Pratylenchus brachyurus (Pb) and their effects on plant growth when inoculated singly or in combination on corn microplots, 1987.

Data are means of six replicates. *, **, *** denote significance at $P \leq 0.05$, 0.01, and 0.001, respectively NS = not significant. † Corn yield at 15.5% moisture.

Initial nematode population density per plot			Nematodes/ 100 cm³ soil (no.)			Stalk	Plant	Nematoo dry w	les/g root rt. (no.)	Fresh stalk	Fars (plat	Vield /plott
Bl	Mi	Pb	Bl	Mi	Pb	(mm)	(cm)	Mi	Pb	(g)	(no.)	(g)
					28	April						
0	0	0	0	0	0	12.5	72					
0	0	9,200	0	0	0.8	12.5	70					
0	58,300	0	0	3.0	0	12.4	68					
0	58,300	9,200	0	1.5	0.7	12.6	71					
7,120	0	0	1.7	0	0	14.0	72					
7,120	0	9,200	1.0	0	1.0	12.6	72					
7,120	58,300	0	1.3	6.2	0	12.3	69					
7,120	58,300	9,200	1.0	2.5	1.2	9.8	58					
Significant effects:		Bl***	Mi*	Pb***	NS	NS						
					30	June						
0	. 0	0	0.2	0	0	23	217					
0	0	9,200	0.5	0	1.7	24	225					
0	58,300	0	0.2	305	0	23	217					
0	58,300	9,200	0.3	748	5.2	23	217					
7,120	0	0	54.2	0	0	25	223					
7,120	0	9,200	24.7	0	2.2	25	230					
7,120	58,300	0	42.7	502	0.5	24	219					
7,120	58,300	9,200	20.7	297	3.3	24	222					
Signific	ant effects:		Bl***	Mi*	Pb***	NS	NS					
8			Pb* Bl × Pb*				-					

TABLE 2. Population changes of *Belonolaimus longicaudatus* (Bl), *Meloidogyne incognita* (Mi), and *Pratylenchus brachyurus* (Pb) and their effects on plant growth when inoculated singly or in combination on corn in microplots, 1988.

TABLE 2. Continued.

Initial nematode population density per plot			Nematodes/ 100 cm ³ soil (no.)			Stalk	Plant	Nematodes/g root dry wt. (no.)		Fresh stalk wt./plot	Fars/plot	Vield /plott
Bl	Mi	Pb	Bl	Mi	Pb	(mm)	(cm)	Mi	Pb	(g)	(no.)	(g)
					15 Se	ptember						
0	0	0	0.2	0.3	0	-		0	1.3	907	3.7	403
0	0	9.200	0.2	18.8	40.7			3.5	2,780	930	3.5	383
0	58,300	Ó 0	0	4,711	0			472	0.1	780	3.5	344
0	58,300	9,200	0	5,192	168			0	1,738	885	4.0	407
7,120	0	0	2.8	6.7	0.5			0.3	3.7	1,043	3.3	427
7,120	0	9,200	0.8	5.8	70.7			2.1	1,457	885	3.2	466
7,120	58,300	0	0	6,095	6.7			94.7	5.0	830	3.3	363
7,120	58,300	9,200	0	3,955	53.2			0	1,606	794	3.5	494
Signifi	cant effect:		Bl** Mi** Bl × Mi**	Mi***	NS			NS	Pb***	Mi*	NS	NS

Data are means of six replicates. *, **, *** denote significance at $P \le 0.05$, 0.01, and 0.001, respectively. NS = not significant. † Corn yield at 15.5% moisture.

Initial nematode population density per plot		Nematodes/ 100 cm³ soil (no.)			True leaves/ plant	Plant beight	Stem	Cysts/ 100 cm ³ soil	Nematode: dry wt.	s/g root (no.)	Seed vield	
Bl	Mi	Hg	Bl	Mi	Hg	(no.)	(cm)	(mm)	(no.)	Мі	Hg	(g/plot)†
					23 Ju	ıe						
0	0	0	0	0	0	1.8	15.3					
0	0	62,500	0	0	2.0	1.9	15.8					
0	97,500	0	0	6.0	0	1.9	16.1					
0	97,500	62,500	0.2	0.8	1.2	2.0	15.6					
3,750	0	0	0.8	0	0	1.9	16.0					
3,750	0	62,500	2.8	0	1.7	2.0	15.3					
3,750	97,500	0	1.5	1.7	0	2.0	15.8					
3,750	97,500	62,500	2.5	4.5	2.0	1.8	15.9					
Significant effects		Bl***	Mi***	Hg***	NS	NS						
8			Hg*** Bl × Hg*	Bl × Hg* Bl × Mi × Hg*	0							
					16 Aug	ust						
0	0	0	0.5	0	0		79	5.7				
Ő	0	62.500	0.5	0.2	994		81.2	6.2				
Ő	97.500	0	0	1.7	0		80.6	6.8				
0	97,500	62,500	3.2	27	572		83.3	6.6				
3,750	0	0	40.2	0	13.3		83.9	7.0				
3,750	0	62,500	29.2	0.8	486		78.1	6.4				
3,750	97,500	0	22.0	0.7	0		89.2	7.0				
3,750	97,500	62,500	28.8	40	647		83.1	6.5				
Signif	icant effect	s:	Bl***	Mi*	Hg***		Bl × Hg*	NS				
0				Hg* Mi × Hg*	-		-					

TABLE 3. Population changes of Belonolaimus longicaudatus (Bl), Meloidogyne incognita (Mi), and Heterodera glycins (Hg) and their effects on plant growth when inoculated singly or in combination on soybean in microplots, 1988.

TABLE J. Commucu.	TABLE	3.	Continued.
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Initial nematode population density per plot		Nematodes/ 100 cm ³ soil (no.)			True leaves/ plant	Plant height	Stem diameter	Cysts/ 100 cm³ soil	Nematodes/g root dry wt. (no.)		Seed vield	
Bl	Mi	Hg	Bl	Mi	Hg	(no.)	(cm)	(mm)	(no.)	Мі	Hg	(g/plot)†
					2 Nove	mber						
0	0	0	0.8	1.8	0.5				0.7	0.9	0	188.5
0	0	62,500	0.2	3.7	145				116	0	104	89.7
0	97,500	0	0	94.7	1.5				1.2	40.2	3.6	202.5
0	97.500	62,500	0.2	28.8	420				68.3	7.8	69.2	66
3,750	0	0	61.8	3.8	0.5				0.5	4.7	2.1	214.3
3,750	0	62,500	7.8	1.2	86.2				84	0.1	213	813
3,750	97,500	0	37.2	132	1.0				0	23.1	0.7	185.7
3,750	97,500	62,500	33.0	53.2	53.4				96.2	0.4	39.3	105.3
Significant effects:		Bl*** Hg*** Bl × Hg*** Mi × Hg*** Pl × Hg × Mi***	Mi*** Hg*	Hg*				Hg**	Mi** Hg* Mi × Hg*	Hg**	Hg***	

Data are means of six replicates. *, **, *** denote significance at $P \le 0.05$, 0.01, and 0.001, respectively. NS = not significant. † Soybean yield at 10% moisture.

among the three nematode genera (Table 3). Twenty days after planting, the population density of *B. longicaudatus* was greater in combination with *H. glycines* and the population density of *M. incognita* was greater ($P \le 0.05$) in combination with *H. glycines* when *B. longicaudatus* was present, but decreased when *B. longicaudatus* was absent.

After 11 weeks, population densities of M. incognita in soil were greater in combination with H. glycines ($P \le 0.05$). At harvest, there were two-way and three-way interactions involving densities of B. longicaudatus in soil. Numbers of this nematode were reduced in combination with H. glycines or M. incognita ($P \le 0.001$). The reduction of B. longicaudatus in plots also inoculated with H. glycines was greater when M. incognita was absent, as indicated by the three-way interaction. Soil and root population densities of M. incognita were greater in the absence of H. glycines ($P \le 0.05$).

DISCUSSION

Two tests were run on corn in microplots in different years, but the pathogenic affects of B. longicaudatus, M. incognita, and P. brachyurus either singly or in various combinations were not evident. The yield of soybean, however, was readily reduced in plots infested with H. glycines, but not B. longicaudatus or M. incognita. Others also have failed to demonstrate corn yield reductions by plant-parasitic nematodes in microplots (K. R. Barker & J. R. Rich, pers. comm.). Once corn is beyond the seedling stage of growth, it rapidly produces an extensive root system because of the relatively high fertilization and watering used for corn production. These factors may result in a lack of measurable plant injury from nematodes in microplot tests.

The absence of plant damage to corn or soybean from *B. longicaudatus* was unexpected. This nematode normally attacks corn roots during the seedling stage of growth in the early spring causing abbreviated roots. Plant damage can be extreme and grain yields can be reduced by 73–80% (4). Much less is known about the pathogenicity of B. longicaudatus on soybean; however, it is reported as a pathogen on some soybean cultivars (13,18). In 1987-88, in nearby field plots of corn containing indigenous populations of B. longicaudatus, M. incognita, and P. brachyurus, plant growth-including stand count, grain yield, stalk weight, and size of young plants-was often inversely correlated with densities of B. longicaudatus and occasionally with P. brachyurus ($P \leq 0.05$), but not with densities of M. incognita (8). Also, in 1987 but not in 1988, growth of soybean in nearby field plots was inversely correlated with density of B. longicaudatus (9). The effect of B. longicaudatus on corn and soybean growth may be related to temperature and distribution within the soil profile. Root injury caused by B. longicaudatus on soybean planted in late May or early June is never as severe as that on corn planted in late February through mid-April (8,9). In field soil, 50% of the population densities of B. longicaudatus occurred in the upper 15-cm soil layer in May, but the species became more evenly distributed through greater soil depths as the season progressed (10,11). Our plots, which were planted during late April to early June, probably escaped severe root pruning by B. longicaudatus because the nematode moved deeper into the soil in response to increased soil temperature in the upper soil depths or other unknown factors (3, 10, 11).

Neither M. incognita nor P. brachyurus are known to be severe pathogens of corn, although both nematodes infect the roots and reproduce readily on corn (12). This study confirms that H. glycines is a highly virulent pathogen on susceptible soybean cultivars, whereas our population of M. incognita caused much less damage either alone or in combination with H. glycines or B. longicaudatus. An interaction between H. glycines and M. incognita on growth and yield of soybean has been shown (15), but whether the effect was additive depended on initial population densities of nematodes. In our tests M. incognita did not affect the soil or root population densities of H. glycines; however, there is one previous report that population densities of H. glycines were greater in plots containing both nematodes than in plots containing only H. glycines (16). The suppressive effect of H. glycines on the population densities of M. incognita and B. longicaudatus in this experiment is probably why H. glycines had the major impact on soybean yield.

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