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Interactions Between *Meloidogyne incognita* and *Pratylenchus brachyurus* on Soybean[1]

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Abstract: Interactions among *Meloidogyne incognita, Pratylenchus brachyurus,* and soybean genotype on plant growth and nematode reproduction were studied in a greenhouse. Coker 317 (susceptible to both nematodes) and Gordon (resistant to *M. incognita,* susceptible to *P. brachyurus*) were inoculated with increasing initial population densities (Pi) of both nematodes individually and combined. *M. incognita* and *P. brachyurus* individually usually suppressed shoot growth of both cultivars, but only root growth on Coker 317 was influenced by a *M. incognita* × *P. brachyurus* interaction. Reproduction of both nematodes, although dependent on Pi, was mutually suppressed on Coker 317. *P. brachyurus* reproduced better on Gordon than on Coker 317 but did not affect resistance to *M. incognita.* Root systems of Coker 317 were split and inoculated with *M. incognita* or *P. brachyurus* or both to determine the nature of the interaction. *M. incognita* suppressed reproduction of *P. brachyurus* either when co-inhabiting a half-root system or infecting opposing half-root systems; however, *P. brachyurus* affected *M. incognita* only if both nematodes infected the same half-root system.
Key words: Glycine max, antagonism, concomitant infection, population density, root-knot nematode, lesion nematode.

Concomitant infestations of soybean (*Glycine max* (L.) Merr.) fields with different species of plant-parasitic nematodes are common. In Georgia combinations of *Meloidogyne incognita* (Kofoid & White) Chitwood and *Pratylenchus brachyurus* (Godfrey) Filipjev & Schuurmans-Stekhoven occur with high frequency in soybean fields (T. L. Niblack and R. E. Motsinger, unpubl.). Although both nematode species are major pathogens on soybean (13), little is known about their interaction on this crop.

Interactions between migratory and sedentary endoparasitic nematodes vary, depending on the nematode species involved, initial nematode density, and susceptibility of the host to each species (3). Reproduction of *P. brachyurus* in the presence of *M. incognita* or *M. hapla* may be depressed on *M. incognita*-susceptible tobacco cultivars but not on *M. incognita*-resistant cultivars (8). Penetration of alfalfa and red clover by *M. incognita* is inhibited by *P. penetrans,* but only when the initial population density (Pi) of *P. penetrans* is greater than *M. incognita* and when *P. penetrans* infects the plant first (14). Although the reproduction of both *P. penetrans* and *M. incognita* is suppressed when they coinhabit tomato, *P. penetrans* is affected the most (4). In contrast, concomitant infection of cotton with *P. brachyurus* and *M. incognita* enhances the reproduction of *P. brachyurus,* compared with single infections, if the cultivar is a poor host for *M. incognita* (5).

The impact of combined infections of migratory and sedentary endoparasites on plant growth is usually additive but does not always result in more damage than single infections. Growth of tobacco is more severely suppressed following inoculation with *P. brachyurus* alone than when dually inoculated with *P. brachyurus* and *M. incognita* (11).

The objectives of our research were to determine the effect of interactions among *M. incognita* and *P. brachyurus* and soybean genotype on plant growth and nematode reproduction and to elucidate the nature of the interaction between the two nematode species.

Materials and Methods

Soybean cultivars Coker 317 (*M. incognita* susceptible, Maturity Group VII) and Gordon (*M. incognita* resistant, Maturity

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TABLE 1. Shoot and root weight (g) of Coker 317 and Gordon soybean grown in the greenhouse for 90 days following inoculation with *Meloidogyne incognita* (Mi) and *Pratylenchus brachyurus* (Pb) alone and combined.

Pi†		Coker 317		Gordon	
Mi	Pb	Shoot weight	Root weight	Shoot weight	Root weight
0	0	24.4	42.3	26.3	43.0
0	20	22.3	35.7	21.0	41.4
0	60	20.0	31.6	18.0	38.3
20	0	20.6	32.3	23.0	39.8
60	0	17.5	32.2	20.7	36.8
20	20	17.6	29.2	20.6	37.5
20	60	15.7	26.9	17.8	32.6
60	20	15.7	25.9	19.8	34.9
60	60	15.1	16.1	18.4	34.5
Significance for:					
Mi linear		**	**	ns	**
Mi quadratic		*	*	ns	*
Pb linear		**	**	*	**
Pb quadratic		ns	ns	ns	ns
Mi × Pb		ns	*	ns	ns

Data are averages of two experiments, each with six replications.
*,** = significant at $P = 0.05$ and $P = 0.01$ based on an F-test.
ns = nonsignificant at $P = 0.05$ based on an F-test.
† Pi = initial population densities of Mi second-stage juveniles or Pb juveniles and adults per 100 cm^3 soil.

Group VII) were used in these studies. Both cultivars are susceptible to *P. brachyurus.*

M. incognita (race 3) was cultured on tomato, *Lycopersicon esculentum* Mill. cv. Rutgers, and *P. brachyurus* was cultured on Lee soybean in a greenhouse. Nematode inoculum was obtained by extracting motile stages of *P. brachyurus* and second-stage juveniles (J2) of *M. incognita* from roots in a modified mist chamber (1) for 3 days.

Nematode reproduction was assessed by extracting nematodes from soil and roots. Nematodes were extracted from 250 cm^3 soil by a combination of elutriation (2) and centrifugal flotation (7). *M. incognita* eggs were collected from galled roots with 0.5% NaOCl (6), and *P. brachyurus* motile stages were collected daily for 5 days from infected roots incubated in the mist chamber. Roots of dually infected plants were treated with 0.5% NaOCl for recovery of *M. incognita* eggs prior to incubation in the mist chamber for extracting *P. brachyurus* (a preliminary experiment indicated that NaOCl did not affect recovery of *P. brachyurus*).

Cultivar experiment: Four seeds of Coker 317 or Gordon were planted in 15-cm-d plastic pots filled with 1,500 cm^3 soil (soil, sand, attapulgite clay, 3:1:1) previously fumigated with methyl bromide at a minimum rate of 1.36 kg/800 liters soil. After 7 days the seedlings were thinned to one per pot and inoculated with the nematodes. Plants were grown under 400-watt Multi-Vapor phosphor-coated lamps to provide a 16-hour photoperiod and fertilized biweekly with 123 mg of 20-20-20 (N = 20%, P = 8.7%, K = 16.6%) soluble fertilizer per pot. Nematode Pi densities were 0, 20, and 60 per 100 cm^3 soil for both species with factorial treatment combinations. The experiment was repeated once.

Quantitative effects were investigated by partitioning the sums of squares of *M. incognita* and *P. brachyurus* and the interaction of *M. incognita* × *P. brachyurus* into linear and quadratic single-degree-of-freedom comparisons. The experiment and replication effects were considered random, and *M. incognita* and *P. brachyurus* effects were considered fixed in the analysis of variance. Treatments were arranged in a randomized complete block design with six replications.

In a second experiment, nematode Pi were increased to 0, 90, 180, 270, and 540 per 100 cm^3 soil. Nine treatment combi-

TABLE 2. Population densities of *Meloidogyne incognita* (Mi) and *Pratylenchus brachyurus* (Pb) 90 days after inoculation alone and combined on Coker 317 and Gordon soybean grown in the greenhouse.

Pi†		Nematode numbers (in 1,000's)/plant			
		Coker 317		Gordon	
Mi	Pb	Mi‡	Pb‡	Mi‡	Pb‡
0	20		1.6		2.5
0	60		2.2		3.2
20	0	9.6		0.5	
60	0	17.1		0.7	
20	20	6.1	0.2	0.4	3.4
20	60	4.7	1.5	0.2	2.0
60	20	9.7	0.8	0.5	1.2
60	60	11.1	0.3	0.6	4.8
Significance for:					
Mi linear		**	**	**	*
Mi quadratic			**		ns
Pb linear		**	**	**	**
Pb quadratic		**		ns	
Mi × Pb		*	**	**	**

Data are averages of two experiments, each with six replications.
*,** = significant at $P = 0.05$ and $P = 0.01$ based on an F-test.
ns = nonsignificant at $P = 0.05$ based on an F-test.
† Pi = initial population densities of Mi second-stage juveniles or Pb juveniles and adults per 100 cm^3 soil.
‡ Number of Mi second-stage juveniles and eggs or Pb juveniles and adults in soil and roots.

nations were used in this experiment. The design was a randomized complete block with six replications; the experiment was repeated once. In the analysis of variance, experiment and replication effects were considered random and treatment effects were considered fixed.

Data for all experiments included dry shoot weight (80 C for 48 hours), fresh root weight, and nematode numbers in the soil and roots at 90 days after inoculation. Nematode data were transformed ($\log_{10}$) prior to analysis.

Split-root study: Seeds of Coker 317 were planted in vermiculite. After 10 days the tap roots of the seedlings were split in half up to the stem, and each half-root system was planted into separate sides of attached paper cups containing 200 cm^3 sterilized soil per side. After 7 days the plants were transplanted, one half-root system to a side, into double-compartment plastic containers filled with the sterilized soil (750 cm^3 per side) and inoculated with *P. brachyurus* or *M. incognita*. Plants were fertilized with 61.5 mg 20-20-20 fertilizer per compartment at 14-day intervals.

Nematode treatments (per 100 cm^3 soil) consisted of 1) 60 *P. brachyurus* on one half-root system, 2) 60 *M. incognita* on one half-root system, 3) 60 *P. brachyurus* and 60 *M. incognita* on same half-root system, and 4) 60 *P. brachyurus* and 60 *M. incognita* on opposite half-root systems. The experiment was repeated with inoculum levels of both nematodes increased to 180. A randomized complete block design with eight replications was used in both experiments. Plants were harvested after 80 days, and nematode numbers in the soil and roots were determined. Prior to statistical analyses, nematode numbers in soil and roots were summed and transformed ($\log_{10}$).

RESULTS

Cultivar experiments: In the first experiment shoot growth of Coker 317 was suppressed linearly but at a diminishing rate over increasing Pi for both nematodes, whereas Gordon's response was a linear decrease only for *P. brachyurus* (Table 1). No *M. incognita* × *P. brachyurus* interaction was detected for either cultivar. Root growth of Coker 317 and Gordon decreased linearly by Pi for both nematodes; in combined inoculations of *M. incognita* × *P.*

TABLE 3. Shoot and root weight (g) of Coker 317 and Gordon soybean grown in the greenhouse for 90 days following inoculation with *Meloidogyne incognita* (Mi) and *Pratylenchus brachyurus* (Pb) alone and combined.

Pi†		Coker 317		Gordon	
Mi	Pb	Shoot weight	Root weight	Shoot weight	Root weight
0	0	27.2	35.0	26.9	35.3
180	0	22.2	28.2	24.5	32.5
540	0	18.6	25.6	22.2	31.1
0	180	22.8	29.8	21.6	27.5
0	540	20.7	26.7	19.5	21.2
90	90	22.8	30.9	25.2	28.0
270	270	17.7	25.0	19.1	22.9
180	540	19.5	26.2	18.0	18.5
540	180	15.4	18.6	21.0	25.7
	LSD (0.05)	2.2	2.2	1.5	2.2

Data are average of two experiments each with six replications.

† Pi = initial population densities of Mi second-stage juveniles or Pb juveniles and adults per 100 cm^3 soil.

TABLE 4. Population densities of *Meloidogyne incognita* (Mi) and *Pratylenchus brachyurus* (Pb) 90 days after inoculation alone and combined on Coker 317 and Gordon soybean grown in the greenhouse.

Pi†		Nematode numbers (in 1,000s)/plant			
		Coker 317		Gordon	
Mi	Pb	Mi‡	Pb‡	Mi‡	Pb‡
180	0	20.0 b		1.4 c	
540	0	26.2 a		2.0 a	
0	180		3.6 ab		5.1 c
0	540		4.9 a		8.6 a
90	90	11.6 d	0.8 d	0.9 e	1.8 e
270	270	13.8 c	1.3 c	1.4 c	4.8 c
180	540	8.7 e	2.7 b	1.1 d	6.1 b
540	180	21.1 b	1.7 c	1.6 b	3.3 d

Data are averages of two experiments each with six replications.

Means followed by the same letter within a column are not different based on LSD ($P = 0.05$) performed on $\log_{10}$ transformed data.

† Pi = initial population densities Mi second-stage juveniles or Pb juveniles and adults per 100 cm^3 soil.

‡ Number of Mi second-stage juveniles and eggs or Pb juveniles and adults in soil and roots.

brachyurus at the highest densities, root growth was 62 and 20% less than the controls, respectively (Table 1).

Population densities of *P. brachyurus* were consistently greater on Gordon than on Coker 317, but were dependent on Pi on both cultivars (Tables 2, 4). Reproduction of *P. brachyurus* on Coker 317 was less in combined than in single inoculations (Table 2). *M. incognita* had the greatest adverse effect on *P. brachyurus* when the Pi for each nematode was equal and the least influence when the Pi ratio of *M. incognita* to *P. brachyurus* was 1:3. On root-knot resistant Gordon, reproduction of *P. brachyurus* was stimulated 39–49% when the Pi were equal but suppressed 37–51% with unbalanced Pi (Table 2). Reproduction of *M. incognita* was dependent on Pi for both cultivars and suppressed by 36–74% on Coker 317 by *P. brachyurus.* Low reproduction of *M. incognita* occurred on Gordon.

Nematode main effects and interactions between *M. incognita* and *P. brachyurus* on plant growth in the second experiment with higher Pi were similar to those observed in the first experiment. Shoot and root growth of Coker 317 was suppressed by all nematode treatments (Table 3). Shoot weight in single inoculations was similar for both nematode species at the same Pi and less than those of the control. The lowest shoot weight of plants dually inoculated with unbalanced ratios of *M. incognita* and *P. brachyurus* occurred on plants receiving the high rate of *M. incognita.* Root-growth responses were similar to shoot growth on Coker 317. Although all nematode treatments suppressed growth of Gordon, *P. brachyurus* caused more restriction of shoot and root growth than did *M. incognita* at comparable Pi (Table 3).

Reproduction of both nematodes was generally more responsive to the other species on Coker 317 than on Gordon. Reproduction of *P. brachyurus* on Coker 317 decreased 46–52% in the presence of unbalanced ratios of *M. incognita,* whereas *M. incognita* reproduction was inhibited more when its Pi was lower than that of *P. brachyurus* (Table 4).

Split-root experiments: In combined inoculations of *M. incognita* and *P. brachyurus* on the same half-root system and also in single inoculations of opposite half-root systems, *M. incognita* suppressed the reproduction of *P. brachyurus* by 80–85%, compared with the plants inoculated only with *P. brachyurus,* at both the two initial densities used (Table 5). In contrast, reproduction of *M. incognita* was suppressed by *P. brachyurus* only when both nematodes infected the same half-root system (Table 5). Compared with *M. incognita* alone, *M. incognita* population densities declined 30% when *P. brachyurus* was on the same half-root system and 12–18% when the two species infected opposing half-root systems.

TABLE 5. Population densities of *Meloidogyne incognita* (Mi) and *Pratylenchus brachyurus* (Pb) 80 days after inoculated alone and combined on split-root systems of Coker 317 soybean grown in the greenhouse.

Pi†		Nematode numbers (in 1,000s)	
Half-root A	Half-root B	Mi juveniles and eggs	Pb juveniles and adults
Experiment 1			
60 Pb	0		1.7 a
60 Mi	0	10.3 a	
60 Mi	0	6.8 b	
60 Pb	0		0.2 b
60 Mi	60 Pb	8.8 a	0.2 b
Experiment 2			
180 Pb	0		2.1 a
180 Mi	0	14.8 a	
180 Mi	0	10.3 b	
180 Pb	0		0.4 b
180 Mi	180 Pb	12.1 ab	0.3 b

Data are averages of eight replications for each experiment. Means followed by the same letter within a column and experiments are not different based on LSD ($P = 0.05$) performed on $\log_{10}$ transformed data.

† Pi = initial population densities of Mi second-stage juveniles or Pb juveniles and adults 100 cm^3 soil.

DISCUSSION

In our greenhouse studies, growth of both cultivars was suppressed by all Pi of *M. incognita* and *P. brachyurus*. These results differ from microplot experiments where growth of root-knot resistant soybean cultivars was commonly stimulated by low *M. incognita* Pi (9) and *P. brachyurus* caused very little damage to soybean (12). Growth suppression of dually infected Coker 317 and Gordon usually increased in an additive manner but was generally greater on Coker 317. Only root growth of Coker 317 was affected by a *M. incognita* × *P. brachyurus* interaction and only at the high Pi, indicating this to be density dependent.

Gordon was as effective in limiting the reproduction of *M. incognita* in these greenhouse studies as previously reported in microplot tests (9), and the resistant response was not altered by the presence of *P. brachyurus*. The ability of one nematode species to alter the plant resistance to another nematode species (3) apparently is restricted to specific intraspecies interactions. Soybean resistance to either *M. incognita* or *H. glycines* is unaffected also in concomitant infections with these nematodes (10).

The mutual antagonism of *M. incognita* and *P. brachyurus* on soybean is similar to interactions reported on other crops between these species and with other species within these genera (3). Although *M. incognita* suppresses reproduction of *P. brachyurus* on soybean more than *P. brachyurus* affects *M. incognita*, the Pi ratio of these nematode species influences the outcome of the interaction.

The nature of the interaction between *Meloidogyne* and *Pratylenchus* species is related to the feeding habits of these parasites. *Meloidogyne* species are highly specialized parasites and frequently have a systemic effect on other nematodes feeding on the same plant (4). *Meloidogyne* species were equally effective in suppressing *Pratylenchus* species when infecting either the same root system or opposite half-root systems.

The antagonism of *M. incognita* by *P. brachyurus* was not as severe as the *M. incognita* effect on *P. brachyurus*, and it was observed only when the two species cohabited soybean roots. *P. penetrans* has been shown to depress *M. incognita* reproduction on tomato roots (4).

Interactions between nematodes have been shown to be influenced by the suitability of the plant as a host for the parasites. Reproduction of one nematode species may be stimulated on a host resistant to the other nematode species in concomitant infections (5,8). Although our data tend to support this conclusion, the effect of host suitability on nematode interactions may be population density dependent.

Since plant-parasitic species usually occur in polyspecific communities in nature and frequently infect the same plant, multiple-species interactions undoubtedly influence the severity of the disease induced and related population dynamics. Even though the interaction of *M. incognita* and *P. brachyurus* had little effect on soybean growth, reproduction of both species was mutually suppressed. Nevertheless, *M. incognita,* with its more complex host–parasite relationship and higher reproductive rate, became the dominant species in this interaction.

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