Effect of Meloidogyne incognita on Reproduction of Pratylenchus penetrans in Red Clover and Alfalfa[1]

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Abstract: Roots of seedlings of red clover and alfalfa growing on 10^{-1} Hoagland and Arnon solution agar were inoculated with various combinations of *Meloidogyne incognita* and *Pratylenchus penetrans*. Egg-laying by *P. penetrans* decreased as the number of nematodes, the ratio of entrant *M. incognita* to entrant *P. penetrans*, and the priority of invasion of roots by *M. incognita* increased. Embryogeny and hatching of eggs of *P. penetrans*, and development of larvae of *M. incognita*, were not affected. In red clover, the greatest reduction occurred when there were 65 entrant nematodes, the ratio of *M. incognita:P. penetrans* was 4:1, and *M. incognita* was inoculated four days prior to *P. penetrans*. In alfalfa, the less-favorable host for both nematodes, the greatest reduction occurred when there were 45 entrant nematodes, the ratio of *M. incognita:P. penetrans* was 2:1, and *M. incognita* was inoculated 4 days prior to *P. penetrans*. *Key Words: Trifolium pratense, Medicago sativa,* concomitant nematodes.

When *Meloidogyne incognita* (Kofoid and White) Chitwood and *Pratylenchus penetrans* (Cobb) Filipjev and Schuurmans-Stekhoven are concomitant inhabitants of plants that are suitable hosts for both, *M. incognita* should dominate the population eventually because of its shorter generation time (20-30 days vs. 50-60 days for *P. penetrans*), greater fecundity (hundreds of eggs per female vs. tens of eggs per female for *P. penetrans*), and more efficient mode of reproduction (parthenogenesis vs. amphimixis for *P. penetrans*). This assumes that each nematode functions in the association as it does when alone. We reported (9) that invasion of roots of red clover (*Trifolium pratense* L.) and alfalfa (*Medicago sativa* L.) by larvae of *M. incognita* was reduced significantly when the ratio of entrant *P. penetrans* to *M. incognita* in inoculum was 2-3:1 and there were 150-200 nematodes per root. However, invasion of roots by adults of *P. penetrans* was not reduced in the reciprocal combination. These observations were made 72 h after inoculation and little was learned about development of the nematodes within roots.

Estores and Chen (4) reported that *M. incognita* repressed development of populations of *P. penetrans* in tomato (*Lycopersicon esculentum* Mill.) in 30 and 60 days, and presented evidence from split-root experiments that translocatable inhibitory substance(s) were involved. Their results indicate a deleterious effect of *M. incognita* on reproduction of *P. penetrans* during development of the first generation. In addition, they found that *P. penetrans* did not invade roots galled by *M. incognita* as readily as ungalled roots.

We investigated the influence of concomitant *M. incognita* on reproduction of *P. penetrans* in roots of red clover and alfalfa. The proportion of *P. penetrans* was kept low in order to avoid interference with invasion by *M. incognita*. An abstract which presents some of the results has been published (1).

MATERIALS AND METHODS

Larvae of *M. incognita* and females of *P. penetrans* were reared and collected for inoculum as described previously (9). Aseptic seedlings of 'Kenland' red clover and 'Buffalo'

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alfalfa, 24- to 48-h-old, were grown from seed treated with 2.1% NaOCl, and single seedlings were placed on 10^{-1} Hoagland and Arnon (6) solution solidified with 1.0% agar in 100-mm diam plastic petri dishes. The seedlings were placed near the sides of the dishes so that roots could grow across the agar. The dishes were stacked slanted at a 45° angle with root tips directed downward under 6,456 lx (600 ft-c) illumination (12-h light period) from fluorescent tubes for 18-24 h prior to inoculation. The elongating roots penetrated slightly or were appressed to the agar. Droplets of inoculum were placed in the line of root elongation approximately 0.5 cm from root tips. After inoculation, the closed dishes were left in the horizontal position until the droplets disappeared (1-2 h) and the nematodes were in the surface film of moisture on the agar. Then the dishes were taped shut and placed on edge, root tips directed downward, under the lights. The experiments were conducted at room temp, 25 ± 2 C.

Inoculum consisted of droplets containing the desired numbers and kinds of nematodes that had been collected in capillary pipettes from suspensions containing both species. Controls were inoculated with the suspending liquid minus nematodes. The inoculum was not aseptic, but there was no decay of roots during subsequent incubation. In some experiments, uninoculated controls were included and there were no obvious differences in appearance nor significant differences in growth of roots between these plants and those inoculated with the suspending liquid minus nematodes.

Plants were fixed and stained with acid fuchsin and examined as described previously (9). "Root" was defined as the region between the root tip and the proximal end of the piliferous region.

The amount of development of *M. incognita* was estimated by a visual rating system based on the fact that larvae of *Meloidogyne* enlarge by increasing girth without appreciably changing length (8). The developing larvae were considered to be cylinders of equal length and they were placed

TABLE 1. Eggs laid by *Pratylenchus penetrans* (Pp) in the presence of *Meloidogyne incognita* (Mi) in roots of 'Kenland' red clover seedlings growing on 10^{-1} Hoagland and Arnon solution solidified with 1% agar. Data are means of five replicates.

Expt. No.	Days Mi before Pp	Number Inoculated		Entrant Nematodes			
				Mi		Pp	
		Mi	Pp	No.	G.I.[b]	No.	E/F[c]
I[a]	5	5	10	4.0	53.9	7.6	8.7
	5	10	10	6.8	52.4	7.0	10.3
	5	20	10	12.5	41.8	7.5	5.7
			10			7.6	8.2
II	4	20	5	8.0	57.5	4.0	6.8
	0	20	5	10.5	36.5	3.0	7.2
			5			4.0	8.4
III	6	5	5	4.5	61.6	4.0	6.3
	6	10	5	8.1	56.1	4.3	8.0
	6	20	5	13.2	46.2	4.3	5.7**
			5			4.4	9.7
IV	0	20	20	14.6	46.7	13.8	7.3*[d]
	0	100	20	49.6	27.8	13.8	6.4**[e]
			20			17.2	11.8
	4	20	20	15.6	47.9	14.6	6.1**
	4	100	20	52.4	26.2	12.6	4.0**
			20			13.2	9.5
		20		14.4	54.1		
		100		55.0	35.4		

[a]Duration of experiments: II, 17 days; I and III, 18 days; IV, 19 days.
[b]Growth Index (G.I.) is based upon body-diam.-increase classes 1, 2, 4, 6, and 8. It equals the sum of squares of the class designations divided by the number of nematodes in the group.
[c]Eggs/female.
[d]* indicates that the difference from Pp alone was significant at $P = 0.05$.
[e]** indicates that the difference from Pp alone was significant at $P = 0.01$.

TABLE 2. Length of roots of seedlings of red clover and alfalfa inoculated with 20 or 100 larvae of *Meloidogyne incognita* (Mi): (i) alone, (ii) simultaneously with 20 female *Pratylenchus penetrans* (Pp); or (iii) four days prior to inoculation with 20 female *P. penetrans*. Duration of experiment was 19 days and data are means of five replicates.

Inoculum and day applied			Day 19	
Day 1		Day 5	Root length (mm)[a]	
Mi	Pp	Pp	Red Clover	Alfalfa
			75 A	59 A
	20		80 A	72 A
		20	70 A	61 A
20			42 B	64 A
20		20	45 B	35 B
20	20		28 C	26 B
100			25 C	22 B
100		20	26 C	17 C
100	20		22 C	14 C

[a]Within each column numbers followed by the same capital letter do not differ significantly at $P = 0.05$.

in five classes based on body diam. These classes are: (i) having no increase in body diameter; and (ii), (iv), (vi) and (viii) wherein body diam increased 1-3, 3-5, 5-7, and 7-9 times, respectively. Thus, each class designation is the mean body-diam increase for that class, and the class designations squared, 1, 4, 16, 36, and 64, represent relative volumes. This scheme is appropriate for stages of development of larvae into mature pregravid females, which was the most-advanced stage encountered. The growth index (G.I.) for any group of larvae and/or females equals the sum of the squares of their individual class designations divided by the number of nematodes in the group.

Two types of treatments, each containing five replicates, were used. In the first, both nematodes were inoculated simultaneously; in the second, *M. incognita* was inoculated 4-6 days prior to inoculation with *P. penetrans*. Following inoculation with *P. penetrans* plants were incubated 11-15 days to allow embryogeny and/or hatching of eggs which require 11-13 days under these conditions. Corresponding inoculations with *P. penetrans* or *M. incognita* served as controls.

RESULTS

Red clover: Data on penetration by both nematodes into roots, growth of *M. incognita*, and egg-laying by *P. penetrans* are presented in Table 1. As expected (8, 9), neither nematode affected root penetration by the other, and many larvae of *M. incognita* became females when 20 or fewer were inoculated, but relatively few achieved this degree of maturity when 100 were applied.

When 15-25 nematodes were inoculated, the number of eggs laid by *P. penetrans* was not reduced significantly when the ratio of entrant *M. incognita:* entrant *P. penetrans* was less than 3:1 and the priority of inoculation of *M. incognita* was less than six days. At higher inoculum levels, 40 and 120 nematodes, the number of eggs laid by *P.*

TABLE 3. Eggs laid by *Pratylenchus penetrans* (Pp) in the presence of *Meloidogyne incognita* (Mi) in roots of 'Buffalo' alfalfa seedlings growing in 10^{-1} Hoagland and Arnon solution solidified with 1% agar. Data are means of five replicates.

Expt. No.	Days Mi before Pp	Number Inoculated		Entrant Nematodes			
				Mi		Pp	
		Mi	Pp	No.	G.I.[b]	No.	E/F[c]
I[a]	5	50	20	17.8	9.4	16.5	4.0
			20			14.0	5.2
II	0	20	20	6.8	22.8	15.6	4.4
	0	100	20	32.4	9.1	14.8	3.9*
			20			17.0	6.8
	4	20	20	11.6	30.7	15.6	4.2
	4	100	20	29.4	5.8	16.0	2.5**
			20			19.0	6.4
		20		6.2	12.7		
		100		34.6	7.6		

[a]Duration of experiment I, 16 days; II, 19 days.
[b]Growth Index (G.I.) is based upon body-diam.-increase classes 1, 2, 4, 6, and 8. It equals the sum of squares of the class designations divided by the number of nematodes in the group.
[c]Eggs/female.
[d]* indicates that the difference from Pp alone was significant at $P = 0.05$.
[e]** indicates that the difference from Pp alone was significant at $P = 0.01$.

penetrans was reduced significantly when the ratio of entrant *M. incognita:* entrant *P. penetrans* was about 3:1 or when *M. incognita* was inoculated four or five days before *P. penetrans* and the number of entrant nematodes of both species was about the same. Reduction of egg-laying was most severe when *M. incognita* had both priority of time and superior numbers.

Eggs of *P. penetrans* developed normally and there were no significant differences among treatments in the percentage of eggs that developed larvae and/or hatched. Most of the newly hatched larvae had not migrated from areas in which eggs had been laid; single larvae were found occasionally at sites several millimeters from eggs or other nematodes. There was no consistent pattern of association or disassociation of the two species in roots, except that, females of *P. penetrans* were always in the cortex, even in galls where they were frequently only one or two cells away from the enlarged bodies of larvae or females of *M. incognita* that protruded from syncytia through the endodermis into the cortex. None was seen in any kind of association with the intrastelar syncytia.

Elongation of roots was determined to provide a measure of the overall effects of the nematodes on red clover (Table 2). *P. penetrans* alone had no significant effect on root elongation. *M. incognita* inhibited significantly root elongation when 20 larvae were inoculated (10-16 entrant) and root elongation ceased within 48 h after inoculation when 100 were inoculated (45-55 entrant). At the lower level, roots continued to grow slowly and the addition of 13-15 entrant *P. penetrans* simultaneously, but not 4 days later, further inhibited, but did not stop, root growth.

Alfalfa.—Results with alfalfa, the less favorable host for both nematodes, were similar to those with red clover (Table 3). Again, neither nematode affected root penetration by the other. *M. incognita* did not invade roots of alfalfa as well as those of red clover, nor did entrant larvae develop as well in roots of alfalfa as they did in red clover. *P. penetrans* invaded alfalfa as well as it did red clover, but it laid significantly fewer eggs in alfalfa than in red clover. When the ratio of entrant *M. incognita* to entrant *P. penetrans* was about 2:1, egg-laying by *P. penetrans* was reduced significantly, and when *M. incognita* had the additional advantage of 4-days prior inoculation, reduction in egg-laying was more pronounced.

As with red clover, *M. incognita* inhibited elongation of alfalfa roots when 20 larvae were inoculated (6-12 entrant) and the effect was greater when 100 (30-35 entrant) were inoculated. However, the effect on alfalfa was less than on red clover, and the addition of 15-17 entrant *P. penetrans* increased inhibition of elongation. Root-elongation ceased within 48 h when both combinations of 100 *M. incognita* plus 20 *P. penetrans* were used as inoculum.

DISCUSSION

P. penetrans lays eggs at the rate of 1-2 per day (5) and growth of entrant larvae of *M. incognita,* development of galled tissue, and egg-laying by *P. penetrans* were concomitant events in these experiments. Although females of *P. penetrans* invade roots more rapidly than do larvae of *M. incognita* (9), roots begin to respond to the presence of larvae of *M. incognita* even before they are invaded (2), and there was very little opportunity for *P. penetrans* to lay eggs in tissue unaffected by *M. incognita.* Tissue least affected by *M. incognita,* was in roots of plants inoculated simultaneously with low numbers of nematodes, and it was in these roots that egg-laying by *P. penetrans* was nearly the same as it was in roots devoid of *M. incognita.* Tissue most severely affected by *M. incognita* was in roots of plants preinoculated with a high proportion of *M. incognita,* and it was in these roots that egg-laying by *P. penetrans* suffered the greatest reduction. If the reduced number of eggs were the result of a decreasing rate of egg-laying as the amount of galled tissue increased, the percentage of eggs that developed larvae and/or hatched should have been greater in *M. incognita*-infected roots than in roots devoid of *M. incognita.* This was not the case. 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. Experiments including observations at shorter intervals over longer periods of time are needed to resolve this question.

Root-knot galled tissue is markedly different physiologically and morphologically from ungalled tissue (2, 3, 7). Most of the physiological differences have been determined by comparing several-weeks-old

galled tissue, including syncytia and cortex, with ungalled tissue. The events recorded here occurred soon after infection by the nematodes, and fruitful speculation about possible relationships between physiological and morphological characteristics of *M. incognita*-infected root tissue and egg-laying by *P. penetrans* will require the results of analyses made within 3 wk after infection.

Regardless of the mechanism of the effect, it is apparent that *M. incognita* represses reproduction by *P. penetrans*. This repression, coupled with its greater reproductive capacity, should assure complete dominance of populations derived from concomitant infections after two or three generations of *M. incognita* have developed.

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