Interactions of Pratylenchus penetrans and Meloidogyne incognita as Coinhabitants in Tomato[1]

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Abstract: Greenhouse experiments on the interactions of *Pratylenchus penetrans* and *Meloidogyne incognita* showed that the population densities of both nematode species were depressed when they coinhabited tomato roots. Fifty days after inoculation, the population level of a *P. penetrans* monoculture was about four times higher than when *M. incognita* was present. Conversely, *M. incognita* reproduced twice as fast alone as in combination. There were no significant differences in the numbers of *P. penetrans* when they were inoculated either 10 days prior to or after introduction of *M. incognita*. Root entry by *P. penetrans* was significantly inhibited by the presence of *M. incognita*. Split-root experiments showed that the inhibitory effects of *M. incognita* upon reproduction of *P. penetrans* involved factors other than the availability of feeding sites. On the other hand, the inhibitory effects of *P. penetrans* on *M. incognita* appeared to be primarily due to the quantity of available roots. *Key words:* population, penetration, inhibition.

Relatively few studies on the interactions between species of plant-parasitic nematodes coinhabiting a particular host have been reported (5, 6, 8, 9, 13, 16, 19, 20). Field surveys in New Jersey have shown that *Pratylenchus* sp. Filipjev and *Meloidogyne* sp. Goeldi are often found coinhabiting numerous host plants (7). This study was undertaken to investigate the population development of *P. penetrans* and *M. incognita* when both species coinhabited tomato roots.

MATERIALS AND METHODS

Two- to 3-week-old tomato (*Lycopersicon esculentum* Mill. 'Campbell 146') seedlings were used. Seeds were germinated in vermiculite, and the seedlings transplanted into 265-ml waxed paper cups containing steam-sterilized sandy loam soil. In experiments where final populations were to be determined 30 or more days after inoculation, the plants were repotted in 10-cm clay pots 20 days after treatment.

M. incognita larvae which were used in all experiments were reared on tomato in a greenhouse. Larvae were extracted from galled roots using a water mist system. By the discarding of the collection taken during the first 24 hr, most microphagous nematodes and oligochaetes were eliminated.

P. penetrans were obtained from monoxenic cultures in alfalfa (*Medicago sativa* L.) callus tissue. Nematodes were extracted from the callus by a modified Baermann funnel technique. Only adults collected within the first 12 hr were used. Prior to inoculation, the nematodes were washed three times in sterile water to remove any residual 2,4-D that might have been present (2,4-D was used in the culture substrate).

Plants were inoculated by pipetting the appropriate number of water-suspended nematodes into three holes, 7 mm wide and 20 mm deep, around the plant roots.

P. penetrans were extracted from tomato by submerging infected roots in water in 250-ml flasks. The flasks were placed on a shaker for 6 days. Every 24 hr, the nematodes were collected and the water changed to prevent bacterial build-up. Nematodes were stored in a refrigerator at 4 C until counted, and the number of nematodes was estimated by counting the number in five 1-ml aliquants in Scott counting slides.

Two substrates were used in the penetration studies: agar and sandy loam soil. With agar substrate, root segments 3.5 cm long taken from both healthy and *Meloidogyne*-infected (galled) plants were washed in sterile water. Each root segment was transferred to agar in petri dishes. Nematodes were introduced into each dish by pipetting onto two strips of filter paper located about 0.5 cm from each side of the root. With sandy loam soil substrate, 3-day-old seedlings were transplanted singly into waxed paper cups; into each cup, either 1000 *P. penetrans*, 2000 *M. incognita*, or a combination of both was introduced. In both experiments, 36 hr after inoculation, tomato roots were stained with acid fuchsin and the nematodes were teased out of the roots and counted.

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To determine whether or not a transmissible substance was present in plants infected with *M. incognita* which might have an effect on the reproduction of *P. penetrans*, root systems of 1-week-old tomato seedlings were longitudinally split. Each half of a split root was transplanted into a separate waxed paper cup, two of which had been stapled together and filled with sandy loam soil. After 2-3 weeks, the nematodes were introduced. Treatments for each experiment, except penetration studies, are given in figures and tables in the RESULTS section. In penetration experiments, 3000 *M. incognita* were inoculated into a cup containing one-half of a split root, and 15 days later, 1250 *P. penetrans* were inoculated into the other half. One, 2, and 7 days after inoculation, the number of *P. penetrans* that entered the roots was determined.

Treatments in all experiments were replicated four-six times, and results were statistically analyzed using Tukey's (HSD) test (21).

RESULTS

Effect of coinhabitation on penetration. Three experiments were conducted.

Experiment 1. Fifty days after inoculation, the monoculture of *P. penetrans* yielded an average of 3000 nematodes/plant, whereas those that coinhabited with *M. incognita* averaged only 780. Likewise, *M. incognita* infesting alone yielded 64,100 larvae/plant, whereas those combined with *P. penetrans* yielded 31,500 larvae (initial inoculum used: 1000 *P. penetrans*; 2000 *M. incognita*).

Experiment 2. When *P. penetrans* was inoculated 10 days prior to or after the introduction of *M. incognita*, no significant differences in the population densities of *P. penetrans* were obtained 30 and 60 days after inoculation (Fig. 1). In the same period, however, population density of *P. penetrans* from monoculture was significantly higher than when coinhabiting with *M. incognita* regardless of the time of introduction of *P. penetrans*.

Experiment 3. When combined with two densities of *M. incognita*, the number of *P. penetrans* recovered was inversely related to the density of *M. incognita* (Fig. 2). Again, *P. penetrans* in monoculture reproduced at a higher rate than when coinhabiting with either density of *M. incognita*.

Effect of coinhabitation on penetration.

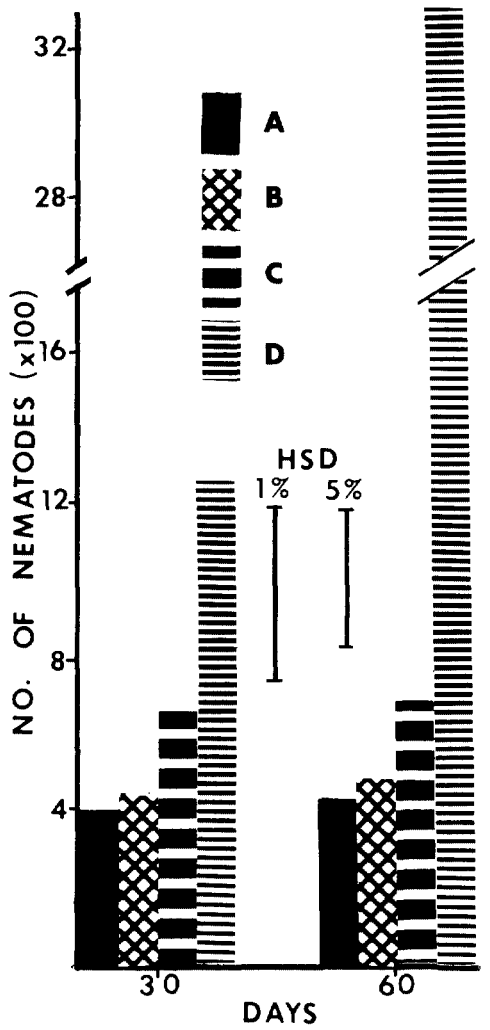

FIG. 1. *Pratylenchus penetrans* populations in tomato roots after 30 and 60 days when inoculated alone or with *Meloidogyne incognita*. Level of inoculum used: 1000 of each species. A = *P. penetrans* plus *M. incognita* simultaneously inoculated; B = *P. penetrans* plus *M. incognita* inoculated 10 days earlier; C = *P. penetrans* plus *M. incognita* inoculated 10 days later; D = *P. penetrans* inoculated alone.

Since previous results showed that lower numbers of *P. penetrans* were recovered from root systems parasitized by *M. incognita* and that modified penetration might account for this phenomenon, this factor was investigated. Both agar and soil substrates were used.

Experiment 1: agar substrate. Thirty-six hours after inoculation, 20% of the *P. penetrans* in monoculture entered healthy root segments as compared to 15% when *M. incognita* was present. In the same period, not a single *P.*

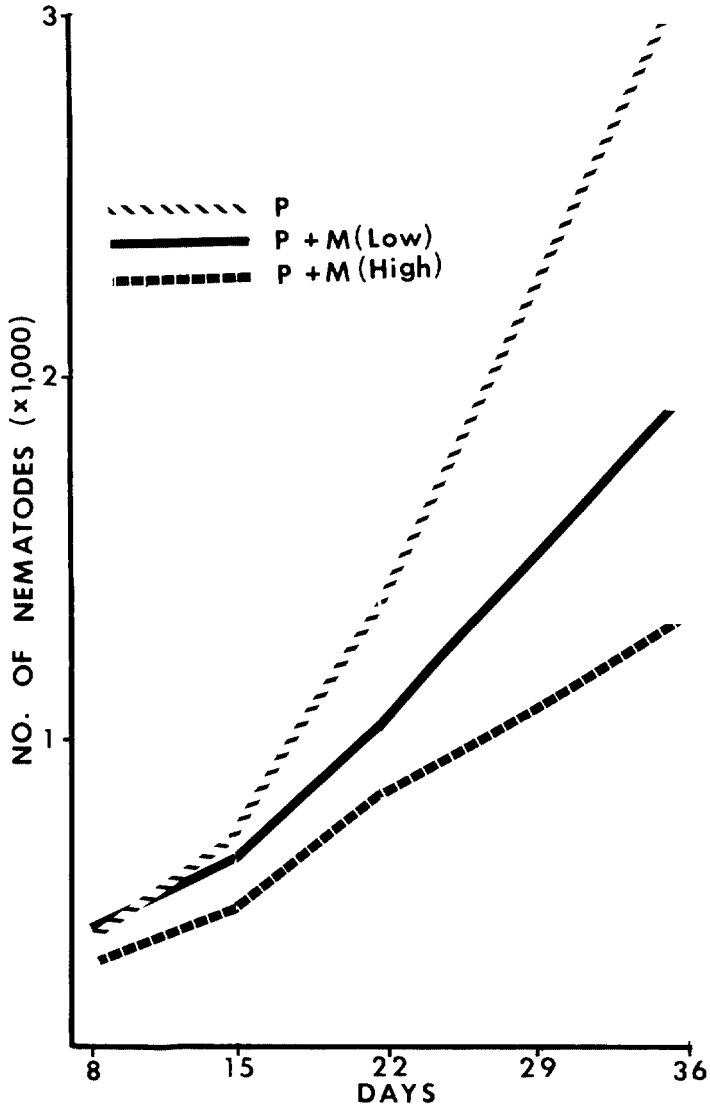

FIG. 2. Number of *Pratylenchus penetrans* in tomato roots after 8, 15, 22, and 36 days when inoculated alone or with two densities of *Meloidogyne incognita*. P = 1000 *P. penetrans* alone; P + M (Low) = 1000 *P. penetrans* plus 1000 *M. incognita*; P + M (High) = 1000 *P. penetrans* plus 3325 *M. incognita*.

penetrans entered galled roots (inoculum level used: 25 *P. penetrans*; 50 *M. incognita*).

Experiment 2: sandy loam soil substrate. Thirty-six hours after inoculation, 11% of *P. penetrans* in monoculture entered tomato roots as compared with 3% when *M. incognita* was present (statistically significant). No significant differences occurred in root entry by *M. incognita* whether in monoculture or in coinhabitation with *P. penetrans*.

Effect of possible transmissible substances on reproduction of P. penetrans. Assuming that the inhibitory effects of *M. incognita* on the reproduction of *P. penetrans* involved more than competition for feeding sites, these experiments were conducted.

Effect on population. Fifteen and 35 days after inoculation, the number of *P. penetrans* recovered from one-half of a split root was significantly higher when the other half was uninoculated than when *M. incognita* was present in the other half (Table 1). Conversely, the number of *M. incognita* recovered from one-half of a split root was about the same

TABLE 1. Effect of coinhabitation on the populations of *Pratylenchus penetrans* and *Meloidogyne incognita* in split-root tomato plants.

Each half of split-root inoculated as follows:	No. days after inoculation		
	15	35	
	P.p.[a]	P.p.[a]	M.i.[a]
2000 *P. penetrans*; uninoculated	1830 a	3654 a	–
2000 *P. penetrans*; 6000 *M. incognita*	1306 b	2520 b	47,500 a
6000 *M. incognita*; uninoculated	–	–	51,750 a

[a]Average of six replicates; P.p. = *P. penetrans*; M.i. = *M. incognita*. Numbers followed by same letter are not significantly different at $P = .05$.

TABLE 2. Number of *Pratylenchus penetrans* per gram of roots and number of their larvae per plant in split-root tomato in which opposite halves were either severed, inoculated with *Meloidogyne incognita*, or uninfested 36 days after inoculation.

Each half of split-root inoculated as follows:	*P. penetrans*	
	No./gram roots[a]	No. larvae/plant[a]
2000 *P. penetrans*; 3000 *M. incognita*	1184 a	2297 a
2000 *P. penetrans*; severed	1792 ab	3200 b
2000 *P. penetrans*; uninoculated	2359 b	4297 c

[a]Average of five replicates. Numbers followed by the same letter are not significantly different at $P = .05$.

whether the other half was uninoculated or inoculated with *P. penetrans*.

Effect on penetration. Root entry by *P. penetrans* was inhibited when *M. incognita* was present in the opposite half of a split root, but the effect was not immediate. One-two days after inoculation, no statistical difference occurred whether the other half of a split root was uninoculated or inoculated with *M. incognita*. After 7 days, however, a significantly greater number of *P. penetrans* entered roots when the other half was uninoculated than when inoculated with *M. incognita*.

Effect of severing one-half of a split root on the reproduction of P. penetrans. No significant differences occurred in the number of *P. penetrans* per gram of roots in split-root plants whether the other half was uninoculated or severed, although actual numbers were higher in the former (Table 2). Similarly, the number of larvae per plant produced by *P. penetrans* was highest in monoculture, followed by that where the other half of a split root was severed; the lowest was obtained where *M. incognita* was present (Table 2).

DISCUSSION

P. penetrans and *M. incognita* mutually inhibited reproduction when they coinhabited tomato roots. The inhibitory effects of *P. penetrans* upon *M. incognita* appeared primarily to be competition for feeding sites. However, the inhibitory effects of *M. incognita* upon the reproduction of *P. penetrans* involved factors other than feeding sites. Split-root experiments indicated that transmissible substance(s) detrimental to the development of *P. penetrans* occurred in the host infected with *M. incognita*. This substance(s) may have been secreted by *M. incognita* or produced by the host in reaction to infection. Plant-growth regulators may be responsible, since indolebutyric acid was found in gall extracts of tomato and beet only after *M. incognita* infection (22). A substance similar to indoleacetic acid was found in roots of *Abelmoschus esculentus* infected by *M. javanica*, but not from healthy root extracts (1); and tryptophan, a precursor of IAA, was found in galled but not in healthy tissues (12). The above-mentioned plant-growth regulators may have been translocated to non-galled roots, since auxins synthesized in one tissue are frequently translocated to other organs of the plant (15). Azauracil, a growth inhibitor, inhibited RNA synthesis and consistently depressed the development of *Heterodera trifolii* beyond the early third stage (4).

The abnormally high amounts of amino acids and proteins (17, 18) as well as accumulation of certain mineral elements (3, 14, 17) in *Meloidogyne*-infected tomato plants could also be responsible for the inhibition of *P. penetrans*' reproduction. Reports (2, 10, 11) that high nutrition of sour cherry and Wando pea depressed population densities of *P. penetrans* somewhat support this hypothesis.

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