Competitive Interaction of Tylenchorhynchus claytoni and Pratylenchus penetrans in Tobacco Roots

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ABSTRACT

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The numbers of *Pratylenchus penetrans* in roots of tobacco cultivar Windsor Shade 117 were reduced by prior or simultaneous exposure of roots to *Tylenchorhynchus claytoni*. Protection occurred both in naturally-infested soils and in sterile soils to which the nematodes were added. Protection against *P. penetrans* was not observed in control soils or if soils were first frozen to kill *T. claytoni*. The degree of protection depended on the concentration of *T. claytoni*, and ≥68 *T. claytoni* per 100 grams of soil provided nearly complete protection against *P. penetrans*. With a split-root system, there was protection to *P. penetrans* when one of the halves was exposed to *T. claytoni* and the other half was exposed simultaneously or sequentially to *P. penetrans*. Protection was not due to reduction in numbers of viable *P. penetrans* in roots and soil.

Additional key words: nematode-nematode interactions, *Nicotiana tabacum*.

Invasion of a root by one species of nematode may influence penetration (17), reproduction (2, 3, 5, 6), or final density (13, 15) of another species of nematode. These effects were observed when populations of both the nematodes were initially present in the soil (2, 5, 13, 15, 17) and when they were added sequentially (2, 3, 5, 6).

We reported (8) that tobacco cultivar Windsor Shade 117 (WS 117) invaded by *Pratylenchus penetrans* (Cobb) does not develop black shank when subsequently inoculated with zoospores of *Phytophthora parasitica* (Dast.) var. *nicotianae* Breda de Haan (Tucker). This protection did not occur if plants were grown in soil containing a mixture of *P. penetrans* and *Tylenchorhynchus claytoni* Allen. This observation led us to study the interactions among these two nematodes and tobacco (12).

MATERIALS AND METHODS

Eight- to 10-week-old tobacco (cultivar WS 117) seedlings were used in these studies except for the split-root experiments, in which 14- to 16-week-old seedlings were used. The seedlings were grown in 237-ml styrofoam cups containing a sterilized mixture of equal volumes of soil, peat, and sand. Plants were exposed to nematodes either by transplanting into nematode-infested soil, or by pipetting one-half ml of a standardized nematode suspension [extracted by a combination of the centrifugation (10) and the tissue (9) methods] into each of two holes adjacent to the roots. The holes, which were made with a glass rod (5 mm in diameter), were filled with soil after the addition of the nematodes. Plants were maintained in a greenhouse for the duration of the experiments. Counts of *P. penetrans* in roots were made by removing plants from soil, washing roots free of soil, grinding them in a Waring Blendor, and counting the motile nematodes with a binocular scope at ×60 magnification. Each experiment contained five or 10 replicates and was repeated twice. Data, presented as averages for all observations, were subjected to an analysis of variance and Duncan's multiple range test of significance.

In initial experiments, plants were transplanted into a soil containing equal volumes of one soil infested with 40 *P. penetrans* per 100 grams and another infested with 52 *T. claytoni* per 100 grams (final nematode concentration = 20 *P. penetrans* and 26 *T. claytoni* per 100 grams of soil). Control plants were transplanted into sterilized soil, a mixture of *P. penetrans*-infested and sterilized soil, or into a mixture of *T. claytoni*-infested soil, which had been frozen at −15 C for 48 hours to kill the nematodes (11), and *P. penetrans*-infested soil. *Pratylenchus penetrans* in the roots were counted 4 days later.

In other experiments, plants were transplanted into soil containing zero or 56 *T. claytoni* per 100 grams of soil and after 1 or 4 days were removed from the soil, washed, and transplanted into soil containing zero or 37 *P. penetrans* per 100 grams. Numbers of *P. penetrans* in the roots were determined 1, 4, or 8 days after the second transplanting.

Experiments were performed to determine the effect of the original numbers of *T. claytoni* on the subsequent entrance of *P. penetrans* into tobacco roots. Plants were grown either in *T. claytoni*-infested soil which was diluted with sterile soil to give concentrations of 68, 34, 17, 8, or 0 nematodes per 100 grams of soil, or 300, 150, 75, 40, or 0 *T. claytoni* were pipetted around roots of plants growing in sterilized potting mixture. After 5 days of exposure to *T. claytoni*, plants were removed and either transplanted into a soil containing 41 *P. penetrans* per 100 grams or they were transplanted into sterile potting mixture and 45 *P. penetrans* were pipetted around the roots. Five days

later the number of *P. penetrans* in the roots was determined as previously described. In the experiment using naturally-infested soil, a plug of soil around the roots of the plants was removed with a No. 6 cork borer, and the number of *P. penetrans* in this soil sample was determined.

To determine the rate of entry of *P. penetrans* into control and *T. claytoni*-treated roots, plants were grown for 2 days in sterilized soil or *T. claytoni*-infested soil (52 nematodes per 100 grams) and then transplanted into *P. penetrans*-infested soil (50 nematodes per 100 grams). The number of *P. penetrans* in the roots was determined 4, 8, 12, 24, 48, or 72 hours after transplanting.

To determine whether there was translocation of a factor or factors responsible for the reduced numbers of *P. penetrans* in tobacco roots previously exposed to *T. claytoni*, experiments were conducted using plants with split roots. One-half of each split root was placed either in a sterilized soil or soil containing 49 *T. claytoni* per 100 grams, but which had been frozen to kill the nematodes. The other half of each split root was placed into either sterilized soil or soil containing 40 *P. penetrans* per 100 grams. The number of *P. penetrans* per root system was determined 5 days later. In a similar experiment, one-half of each split root was placed either in sterilized soil, sand, or in soil containing 49 *T. claytoni* per 100 grams, and the other half was placed in sand or into soil which contained 40 *P. penetrans* per 100 grams. Some of the plants were left with half of their root systems in sand or *T. claytoni*-infested soil and the other half in sand. Five days later the half-root in sand was transferred to soil containing *P. penetrans*. After 5 days the number of motile *P. penetrans* in the half-root in *P. penetrans*-infested soil was determined.

TABLE 1. Reduced entry of *Pratylenchus penetrans* into roots of tobacco cultivar WS 117 after prior exposure to *Tylenchorhynchus claytoni*[x]

Length of exposure (days)		*P. penetrans* per root system (avg. no.)[y]
T. claytoni	*P. penetrans*	
0	1	15 c[z]
1	1	2 a
4	1	3 a
0	4	12 b
4	4	2 a
0	8	17 c
4	8	1 a

[x]Eight- to 10-week-old plants were transplanted into soil containing either zero or 56 *T. claytoni* per 100 grams of soil. After 0, 1, or 4 days the plants were transplanted into soil containing either zero or 37 *P. penetrans* per 100 grams of soil and roots were assayed 1, 4, or 8 days later for *P. penetrans*.

[y]Ten plants per treatment, repeated twice.

[z]Numbers followed by the same letter are not statistically different ($P = 0.01$) by Duncan's multiple range test.

RESULTS

The number of *P. penetrans* in tobacco roots was reduced by about 85% when plants were grown in a soil mixture containing 26 *T. claytoni* and 20 *P. penetrans* per 100 grams of soil. The number of *P. penetrans* entering roots was not reduced when *P. penetrans*-infested soil was mixed with the sterile soil mixture or with *T. claytoni*-infested soil which previously had been frozen to kill *T. claytoni*.

Very few *P. penetrans* were in roots of plants grown in *T. claytoni*-infested soil and then transplanted into *P. penetrans*-infested soil (Table 1). There was no significant difference ($P = 0.01$) in the number of *P. penetrans* in roots that were exposed to *T. claytoni* for 1 day and then to *P. penetrans* for 1 day or when exposed to *T. claytoni* for 4 days and then to *P. penetrans* for 1, 4, or 8 days. The number of *P. penetrans* in control roots was about the same regardless of exposure time.

TABLE 2. Reduced entry of *Pratylenchus penetrans* into roots of tobacco cultivar WS 117 resulting from prior exposure to varying numbers of *Tylenchorhynchus claytoni*

Number of *T. claytoni*		Avg. number of *P. penetrans*[x]		
Per 100 grams of soil[y]	Pipetted around roots before transplanting[w]	Per root system	In soil[y]	Total *P. penetrans* (roots and soil)
0		25 g[z]	5 a	30 b
8		10 cde	16 b	26 ab
17		11 cde	15 b	26 ab
34		6 abc	20 c	26 ab
68		2 a	20 c	22 a
	0	17 f		
	40	8 bcd		
	75	4 ab		
	150	2 a		
	300	2 a		

[y]Eight- to 10-week-old plants were transplanted into soil containing *T. claytoni* which had been mixed with sterilized soil to give the desired concentrations of this nematode. After 5 days the plants were transplanted into soil containing 41 *P. penetrans* per 100 grams of soil and roots and soil were assayed for *P. penetrans* 5 days later.

[w]*Tylenchorhynchus claytoni* and *P. penetrans* were extracted from soil and the desired concentration of *T. claytoni* was pipetted around the roots of WS 117 growing in a sterile soil. Five days later the plants were transplanted into unused sterilized soil and 45 *P. penetrans* were pipetted around the roots of the plants. Roots were assayed for *P. penetrans* 5 days later.

[x]Five plants per treatment, repeated twice.

[y]A plug of soil around the roots of plants was removed with a No. 6 cork borer and the number of *P. penetrans* in this soil sample was determined.

[z]Numbers followed by the same letter are not statistically different ($P = 0.01$) by Duncan's multiple range test.

The reduction in the number of *P. penetrans* within tobacco roots was correlated with increase in numbers of *T. claytoni* in the soil (Table 2). The maximum reduction in the number of *P. penetrans* in roots occurred when the concentration of *T. claytoni* was ≥68 per 100 grams of soil and greater numbers did not result in additional reductions. Of the 40 plants which were exposed to 68 or more *T. claytoni* per 100 grams of soil prior to exposure to *P. penetrans*, 18 root systems contained no *P. penetrans* and 22 roots contained an average of four nematodes per root. The total number of *P. penetrans* isolated from both the roots and soil differed by no more than 26% in all treatments.

Tobacco roots previously exposed to *T. claytoni* contained low numbers of *P. penetrans* after exposure for 4 hours and remained low for 72 hours (Table 3). After 4 hours, the number of *P. penetrans* in roots of control plants was less than in *T. claytoni*-treated roots. The number of *P. penetrans* in control roots became maximum after 24 hours of exposure and remained constant during the next 48 hours.

There was a 72% reduction in root populations of *P. penetrans* in split-root experiments in which the two halves were exposed separately, but simultaneously, to *T. claytoni* and *P. penetrans* (Table 4). There was a greater but not significant ($P = 0.01$) reduction in the number of *P. penetrans* in roots that had been exposed to *T. claytoni* 5 days before exposure to *P. penetrans*.

In all experiments, tobacco roots exposed only to *P. penetrans* were stunted and necrotic. Roots exposed simultaneously to *T. claytoni* and *P. penetrans* or sequentially to *T. claytoni* and then to *P. penetrans* were normal in appearance, which gave further indication that they were protected from *P. penetrans*.

TABLE 3. Rate of *Pratylenchus penetrans* entry into roots of tobacco cultivar WS 117 after prior exposure to *Tylenchorhynchus claytoni*[x]

Initial treatment	Exposure to *P. penetrans* (hours)	Avg. number of *P. penetrans* per root system[y]
Sand	4	1 a[z]
T. claytoni	4	5 ab
Sand	8	6 b
T. claytoni	8	7 b
Sand	12	8 b
T. claytoni	12	6 b
Sand	24	28 c
T. claytoni	24	5 ab
Sand	48	28 c
T. claytoni	48	4 ab
Sand	72	28 c
T. claytoni	72	6 b

[x]Eight- to 10-week-old plants were transplanted into soil containing 52 *T. claytoni* per 100 grams of soil or into sand that contained no *T. claytoni*. Two days later plants were transplanted into soil containing 50 *P. penetrans* per 100 grams of soil. The roots were assayed for *P. penetrans* after the indicated hours of exposure.

[y]Ten plants per treatment, repeated twice.

[z]Numbers followed by the same letter are not statistically different ($P = 0.01$) by Duncan's multiple range test.

DISCUSSION

The reduction of numbers of *P. penetrans* in tobacco roots attacked by *T. claytoni* is not due to a direct interaction between the two nematodes. In soils where the two nematodes were mixed, all *P. penetrans* observed were alive, and the combined number of *P. penetrans* isolated from roots and soil differed by no more than 26% regardless of the number of *T. claytoni* to which the roots had been exposed. Therefore our results indicate that the host plant is involved with the reduced ability of *P. penetrans* to penetrate roots exposed simultaneously or sequentially to *T. claytoni*.

Other reports have suggested that, in concomitant or sequential exposures of two plant parasitic nematodes, reduction in the number of one of the associates may be due to competition for feeding sites within the roots (5). Our split-root experiments showed that the protection of roots against *P. penetrans* by *T. claytoni* involved factors other than the availability of feeding sites. Because we used short exposures (1-2 weeks) to the nematodes, our results should not be affected by the rates of nematode reproduction.

TABLE 4. Entry of *Pratylenchus penetrans* into half of a split-root of tobacco cultivar WS 117 when the other half of the root had been previously or was simultaneously exposed to *Tylenchorhynchus claytoni*[w]

Split-root treatments			Avg. number of *P. penetrans* per root system[x]
One-half	Other half		
Initial	Initial	Final	
Sand	*P. penetrans*		29 b[y]
T. claytoni	*P. penetrans*		8 a
T. claytoni (frozen)[z]	*P. penetrans*		29 b
Sand	Sand	*P. penetrans*	26 b
T. claytoni	Sand	*P. penetrans*	4 a
T. claytoni (frozen)	Sand	*P. penetrans*	28 b

[w]The roots of 14-week-old plants were split and one-half of the root was placed into soil containing 49 *T. claytoni* per 100 grams or into sand with no *T. claytoni*. The other half of the split roots were immediately put either into soil containing 40 *P. penetrans* per 100 grams or into sand with no *P. penetrans* and then from the sand into *P. penetrans* soil 5 days later. Roots were assayed for *P. penetrans* 5 days after being placed into *P. penetrans* soil.

[x]Ten plants per treatment, repeated twice.

[y]Numbers followed by the same letter are not statistically different ($P = 0.01$) by Duncan's multiple range test.

[z]Soil was frozen at −15 C for 48 hours to kill *T. claytoni* before the root half was placed in the soil containing *P. penetrans*.

Gay and Bird (6) suggested that the population of one species of nematode will be enhanced in the presence of another species if the host is relatively resistant to the second species, and conversely, that the population will be suppressed in the presence of another nematode if the host is relatively susceptible to the second nematode. The interaction we report does not support this hypothesis. Tobacco is susceptible to *T. claytoni* (4), and *P. penetrans* causes severe damage to tobacco roots (4). However, *P. penetrans* is unable to maintain its population on tobacco after several successive croppings (14), which suggests that the necrotic root lesions are a hypersensitive response.

Results from split-root experiments by Estores and Chen (5) suggested that a translocatable substance or substances detrimental to the development of *P. penetrans* occurred in tomato plants in which the other half of the split root was exposed to *Meloidogyne incognita*. They suggested that the substances (perhaps plant growth regulators) may have been secreted by *M. incognita* or produced by the host in reaction to attack by this nematode. The results from our split-root experiments suggest a similar pattern. Protection of half a split root system against *P. penetrans* when the other half is exposed at the same time to *T. claytoni* may be explained by the rapid movement of compounds through plants (1, 7, 16). We found that ^{32}P can move from half of a split root into the other half within at least 6 hours.

Other factors may be involved in the interaction that we report. Feeding by *T. claytoni* may reduce the suitability of the root as substrate for *P. penetrans* or lessen the attraction by the roots to *P. penetrans*. *Pratylenchus penetrans,* as it feeds on roots, may produce a pheromone(s) which attracts other *P. penetrans* to the roots. Feeding by *T. claytoni* may cause an alteration or inhibit the production of pheromone(s), thereby reducing the attraction of *P. penetrans* to the roots.

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