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J.A. Gracia, R.D. Reeleder and G. Bélair

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Article abstract

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## Interactions between *Pythium tracheiphilum*, *Meloidogyne hapla* and *Pratylenchus penetrans* on lettuce

J.A. Gracia

Department of Plant Science, Macdonald College of McGill University,  
21 111 Lakeshore Rd., Sainte-Anne-de-Bellevue, Québec, Canada, H9X 1C0

R.D. Reeleder<sup>1</sup>

Agriculture Canada, Research Station, P.O. Box 186,  
Delhi, Ontario, Canada, N4B 2W9

<sup>1</sup> To whom correspondence should be addressed.

G. Bélair

Agriculture Canada, Research Station, 430 Gouin Blvd.,  
Saint-Jean-sur-Richelieu, Québec, Canada, J3B 3E6

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The growth of lettuce plants (*Lactuca sativa*) was reduced by *Pythium tracheiphilum*, *Meloidogyne hapla* and *Pratylenchus penetrans* under growth chamber conditions. A marked additive decrease in lettuce growth was observed when *P. tracheiphilum* and *M. hapla* were added to the soil simultaneously. Interactions between *P. penetrans* and *P. tracheiphilum*, however, were additive at low populations of *P. tracheiphilum*, but appeared to be negative at high populations of the fungus. The fungus had a negative effect on the populations of both species of nematode in roots.

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La croissance des plants de laitue (*Lactuca sativa*) en cabinet de croissance a été diminuée par la présence des *Pythium tracheiphilum*, *Meloidogyne hapla* et *Pratylenchus penetrans*. Une diminution additive marquée de la croissance de la laitue a été observée lorsque les *P. tracheiphilum* et *M. hapla* ont été ajoutés au sol simultanément. Une diminution additive a été observée lorsque le *P. penetrans* et une faible population du *P. tracheiphilum* ont été combinés. Par contre, lorsqu'une forte population du *P. tracheiphilum* était présente, les dommages observés n'ont pas augmenté significativement et une interaction négative a été observée. Le champignon a eu un effet négatif sur la population des deux espèces de nématode dans la racine.

### Introduction

A disease of lettuce (*Lactuca sativa* L.) causing a severe wilting and death of infected plants was observed in the muck soil region of Québec in 1983 and was consistently associated with the presence of the fungus *Pythium tracheiphilum* Matta (Reeleder *et al.* 1985). First described in 1965 in Italy (Matta 1965) and subsequently found in other parts of Europe, it also has caused considerable losses in the United States (Tortolero and Sequeira 1978). In Québec, losses vary from field to field and year to year with a range from 0 - 24% (Reeleder and Charbonneau 1987). Typical above-ground symptoms are stunting and wilting of the plant. A reddish-brown discoloration of the vascular tissue of the tap root results from colonization of the tissue by *P. tracheiphilum*. Infected plants

generally die before mid-season. The disease tends to be more severe in years of high precipitation and in areas with poor drainage. Weather conditions prevailing during the spring-seeded lettuce crop are more favorable for the disease than weather during summer plantings (Reeleder and Charbonneau 1987).

Presently there is little information available regarding the ecology of *P. tracheiphilum*. In Québec muck soils, plant parasitic nematodes are often associated with diseased vegetable crops. The northern root-knot nematode, *Meloidogyne hapla* Chitwood, and the lesion nematode, *Pratylenchus penetrans* (Cobb) Filipjev and Schuurmans-Stekhoven, are the most common species recovered from lettuce fields (Vrain and Dupré 1982). Populations of *M. hapla* in muck soil vegetable fields in southwestern Québec ranged from 1 to 550 per 100 cm<sup>3</sup> of soil. Populations of *Pratylenchus* spp. in these fields ranged from 2 to 235 per 100 cm<sup>3</sup> (Vrain and

Dupré 1982). These genera of nematodes have been reported as increasing or suppressing the activity of certain species of *Pythium* either by providing an entrance to the root system or by physiological effects on the host (Melendéz and Powell 1970a; Holtzmann and Santo 1971). Previous work with *P. tracheiphilum* suggests that wounding the root system increases the severity of the disease (Tortolero and Sequeira 1978; R.D. Reeleader, unpublished data). Wounding of roots by nematodes therefore may be a factor in root colonization by the fungus. The objective of this study was to investigate the nature of the interactions between *P. tracheiphilum* and two species of plant parasitic nematode: *M. hapla* and *P. penetrans*.

## Materials and methods

**Interactions between *M. hapla* and *P. tracheiphilum*.** Lettuce cv. Ithaca was seeded into plastic trays containing vermiculite which then were placed on a growth bench with a photoperiod of 16 h, a light intensity of approximately 250  $\mu\text{mol}/(\text{m}^2 \cdot \text{s})$ , and temperatures of 24°C (day) and 15°C (night). Trays were watered daily and fertilized weekly with Plant-Pro 20-20-20 (Plant Products Co. Ltd., Branalea, Ont.), at a rate of 2.5 g/L of water. Two-week-old seedlings were used in all experiments.

Nematode populations were obtained as follows. Tomatoes (*Lycopersicon esculentum* Mill) cv. Rutgers were grown in muck soil naturally infested with *M. hapla* and kept for 4 months in a greenhouse at  $24 \pm 4^\circ\text{C}$  with a photoperiod of 14 h. To estimate egg populations in tomato roots, 10 g of infected roots were chopped into 1-2 cm pieces and stirred for 3 min with a 0.5% sodium hypochlorite solution (Javex, Bristol-Myers Products Canada Inc. Toronto, Ont.) to dissolve egg mass matrices. The suspension then was passed through a series of sieves with openings of 1.00 mm, 710  $\mu\text{m}$ , 90  $\mu\text{m}$  and 25  $\mu\text{m}$ . Eggs collected on the 25- $\mu\text{m}$  sieve were rinsed in a stream of tap water and counted on a small plastic dish with a grid on the bottom.

A pasteurized soil mixture [4:1 muck:sand (v/v); steamed at 70-80°C for 30 min] was infested with sufficient pieces of nematode-infected tomato roots to reach densities of 0, 1 250, and 4 063 eggs/100  $\text{cm}^3$  of soil (0, 4 000 and 13 000 eggs/12.7-cm-diam. pot). The soil was kept in trays (35 cm  $\times$  30 cm

$\times$  14 cm) and water was added (100 mL/kg soil) to maintain moist conditions, allowing roots to decompose and eggs to hatch. Trays were enclosed within plastic bags and held on a growth bench at 24°C (day) and 15°C (night) with a photoperiod of 16 h. After 2 weeks, a sample of infested soil was taken and estimates were made of nematode populations, using the Baermann pan technique (Barker 1985). Populations at this time had reached the desired levels for inoculation of the lettuce seedlings.

Sporangia of *P. tracheiphilum* (isolate LW 7) were obtained from 10-day-old V8 agar cultures (Tortolero and Sequeira 1978). Contents of culture dishes were blended using tap water as a diluent. Preliminary observations indicated that hyphal fragments remaining in the resulting suspensions were non-viable and that oospores (rare) were non-germinable. An aliquot was drawn from the suspension and a hemacytometer was used to calculate the number of sporangia per mL (Tuite 1969). Sufficient sporangia were added to soil to provide populations of 1 000 or 10 000 sporangia/g of soil. Preliminary experiments showed that lettuce growth was affected significantly by such densities, although death of the plants was not observed commonly.

The sporangial suspension was added to nematode-infested soil and mixed by shaking in an inflated bag. Blended V8-juice agar was added to soils containing nematodes but no *Pythium*. Healthy tomato roots were added to soils containing *Pythium* but no nematodes. The control treatment consisted of soil mixed with healthy tomato roots and blended V8 agar. Plastic pots (12.7 cm diameter) were filled with these mixtures and 2-week-old lettuce seedlings were transplanted into the pots. Daily watering was made by adding water to saucers placed under each pot. Fertilization followed the same schedule as previously described.

Pots were arranged in a completely randomized design with 4 replicate-pots per treatment and one plant per pot. Five weeks after infestation of soil with sporangia, fresh and dry weights of leaves and roots, total leaf area, and numbers of nematodes in the root system were determined. The number of nematodes in roots was estimated by staining an aliquot of roots with acid fuchsin (Hussey 1985). Histological observations were made to assess the effect of

*M. hapla* on colonization of roots by *P. tracheiphilum*. Galls and secondary roots presumed to be infected with either the fungus or the nematode alone or by both pathogens were fixed in FAA (13 mL formalin, 5 mL glacial acetic acid, 200 mL ethyl alcohol 50%) embedded in paraffin, sectioned and then stained with fast green and safranin (Johansen 1940).

Plant responses to different treatments were detected using analysis of variance and orthogonal contrasts (Steel and Torrie 1980). Where main effect interactions were significant, linear and quadratic regressions of simple effects were examined. The experiment was carried out twice.

**Interactions between *P. penetrans* and *P. tracheiphilum*.** Lettuce plants were produced as described previously. Nematode populations were obtained using rye (*Secale cereale* L.) as a host. Soil containing *P. penetrans* (obtained from the Agriculture Canada Research Station, Vineland, Ontario) was mixed with pasteurized sandy soil. Rye seeds were spread onto the soil surface and covered with a light layer of sandy soil. Plants were kept in a greenhouse at  $21 \pm 4^\circ\text{C}$  with a 14-h photoperiod for 4 months.

For inoculation of lettuce, infected roots from 4-month-old rye plants were lightly washed with running tap water to remove soil. A 10-g subsample of roots was placed on a Baermann funnel in a mist chamber for a 10-day extraction period, during which the number of nematodes recovered was recorded every two days. Using the estimated number of nematodes per gram of root, appropriate amounts of infected rye roots were added to soil to obtain inoculum densities of 0, 937 and 4 687 nematodes/100 cm<sup>3</sup> (0, 3 000 and 15 000 nematodes/10.2-cm diam. pot). These populations produced significantly different effects on lettuce growth in preliminary studies.

As for *M. hapla*, *P. penetrans*-infested soils were left for 2 weeks in trays to allow for root decomposition prior to adding the fungus.

A suspension of sporangia of *P. tracheiphilum* was prepared as described previously and mixed with the nematode-infested soil prior to transplanting 2-week-old lettuce seedlings. Populations of the fungus were 0, 1 000 and 10 000 sporangia/g of soil. Results were recorded 4 weeks after infestation of soil with sporangia. The experimental design, environments, measurements and anal-

yses were similar to those used for experiments with *M. hapla*.

## Results and discussion

**Interactions between *M. hapla* and *P. tracheiphilum*.** Growth of above ground parts of lettuce was significantly reduced by either pathogen alone. Interaction between the two organisms was significant ( $P \leq 0.05$ ) for leaf dry weight, but was not significant for root dry weight. Leaf dry weight and leaf area data showed similar trends, therefore only leaf dry weight data are presented. The amount of reduction presented observed with either *P. tracheiphilum* or *M. hapla* alone generally was less than that observed with both organisms present.

When *P. tracheiphilum* was present at 1 000 sporangia/g, *M. hapla* at the 4 000 and 13 000 nematodes/pot densities caused successive significant reductions in leaf weight when compared to *Pythium* alone. These reductions followed a linear response (Table 1, Fig. 1). At 10 000 sporangia/g, both linear and quadratic effects were significant ( $P \leq 0.01$ ). The addition of 4 000 *M. hapla*/pot did not cause further significant reductions in leaf weight, however, the addition of 13 000 nematodes/pot did result in a marked reduction (64.6%) compared to *Pythium* alone (43.6%). This explains the quadratic response observed. These reductions seemed to be additive and were most likely due to the destruction of secondary roots by the fungus. Plants in soil with 10 000 sporangia/g had severely damaged root systems whether the nematode was present or not. The lack of significance of the interaction between these two organisms on root dry weight is probably due to the compensatory effect of the galls and an associated increase in root growth (Fig. 2). When alone, *M. hapla* did not have a significant effect on root growth (Fig. 2A). *P. tracheiphilum* significantly reduced root weights in the absence of *M. hapla*, although increasing the population of sporangia from 4 000 to 10 000 per gram of soil had no effect (Fig. 2B).

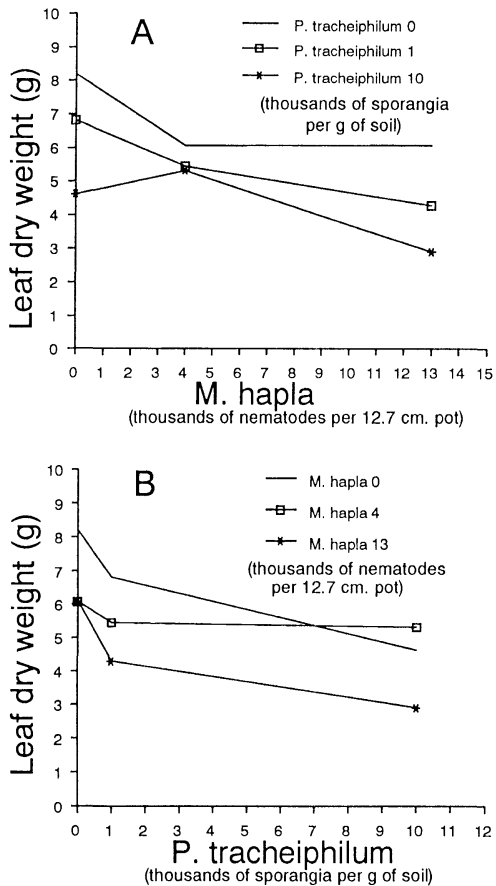
Most studies of interactions of these two plant pathogens have used sequential inoculations. In our tests a simultaneous inoculation was used because pre-inoculation of lettuce with *M. hapla* could not be justified from an epidemiological point of view. Melendéz and Powell (1970a, 1970b) observed that *P. ultimum* does not cause significant damage to roots

unless *M. incognita* is added 3 or 4 weeks in advance. Activity of *P. tracheiphilum* appears to be greater in the spring when prevailing temperatures are relatively low. *M. hapla* has an optimal temperature for plant infection of about 24°C (Wong and Mai 1973) although infection can occur at 12°C (Vrain *et al.* 1978). It appears that, in Québec, conditions favoring infection by the nematode are not likely to occur in the field 3 or 4 weeks prior to conditions favoring attack by *P. tracheiphilum*. Thus, simultaneous inoculations were carried out in these experiments.

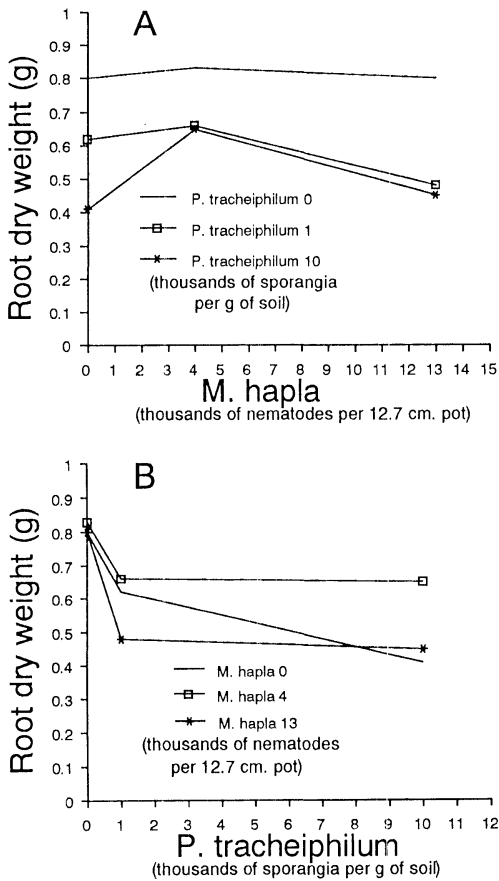
Plants inoculated with the fungus alone or in combination with *M. hapla* were stunted. Dis-

coloration of the vascular system of the tap root was observed only in a few plants. However, secondary roots consistently had the red-brown discoloration typically associated with *P. tracheiphilum*. When these roots were placed on agar media, the fungus was readily recovered. Plants may have been harvested before vascular discoloration became fully developed.

It also was observed that the fungus had a negative effect on populations of *M. hapla* inside the root system. Plants inoculated with only the nematode had 40 to 80% more nematodes than those inoculated with the nematode and the fungus (Table 2, Fig. 3). Several studies show that some species of soilborne fungi sup-



**Figure 1.** Leaf dry weight of lettuce plants inoculated with *Pythium tracheiphilum* and *Meloidogyne hapla*. **A.** Effects of *M. hapla* on dry weight for three populations of *P. tracheiphilum*. **B.** Effects of *P. tracheiphilum* on dry weight for three populations of *M. hapla*. Curves are lines drawn through mean values (n = 4) for each treatment.



**Figure 2.** Root dry weight of lettuce plants inoculated with *Pythium tracheiphilum* and *Meloidogyne hapla*. **A.** Effects of *M. hapla* on dry weight for three populations of *P. tracheiphilum*. **B.** Effects of *P. tracheiphilum* on dry weight for three populations of *M. hapla*. Curves are lines drawn through mean values (n = 4) for each treatment.

**Table 1.** Orthogonal comparisons of the effect on leaf dry weight of lettuce plants of *Pythium tracheiphilum* (Py) populations in the presence of *Meloidogyne hapla* (M) and of *M. hapla* populations in the presence of *P. tracheiphilum*

| Orthogonal comparison <sup>§</sup> | df  | Leaf dry weight <sup>†</sup> |                     |
|------------------------------------|-----|------------------------------|---------------------|
|                                    |     | Sum of squares               | <i>P</i> > <i>F</i> |
| M in Py 0                          | 2   | 0.4188                       | 0.0071              |
| M Linear in Py 0                   | (1) | 0.2325                       | 0.0158              |
| M Quadratic in Py 0                | (1) | 0.1863                       | 0.0291              |
| M in Py 1                          | 2   | 0.5941                       | 0.0014              |
| M Linear in Py 1                   | (1) | 0.5752                       | 0.0004              |
| M Quadratic in Py 1                | (1) | 0.0189                       | 0.4687              |
| M in Py 10                         | 2   | 0.8517                       | 0.0002              |
| M Linear in Py 10                  | (1) | 0.6072                       | 0.0003              |
| M Quadratic in Py 10               | (1) | 0.2445                       | 0.0136              |
| Error                              | 27  | 0.3646                       |                     |

| Orthogonal comparison | df  | Leaf dry weight |                     |
|-----------------------|-----|-----------------|---------------------|
|                       |     | Sum of squares  | <i>P</i> > <i>F</i> |
| Py in M 0             | 2   | 1.0349          | 0.0001              |
| Py in Linear in M 0   | (1) | 0.9605          | 0.0001              |
| Py Quadratic in M 0   | (1) | 0.0744          | 0.1567              |
| Py in M 4             | 2   | 0.0560          | 0.4602              |
| Py in M 13            | 2   | 1.2561          | 0.0001              |
| Py Linear in M 13     | (1) | 1.0196          | 0.0001              |
| Py Quadratic M 13     | (1) | 0.2368          | 0.0150              |
| Error                 | 27  | 0.3646          |                     |

§ Py: *Pythium tracheiphilum*; Py 0 = 0 sporangia per gram of soil; Py 1 = 1 000 sporangia per gram of soil; Py 10 = 10 000 sporangia per gram of soil. M: *Meloidogyne hapla*; M 0 = 0 nematode per 12.5 cm pot; M 4 = 4 000 nematodes per 12.5 cm pot; M 13 = 13 000 nematodes per 12.5 cm pot.

† All significant leaf dry weight interactions are presented for both analyses of variance. Root dry weight interactions were not significant.

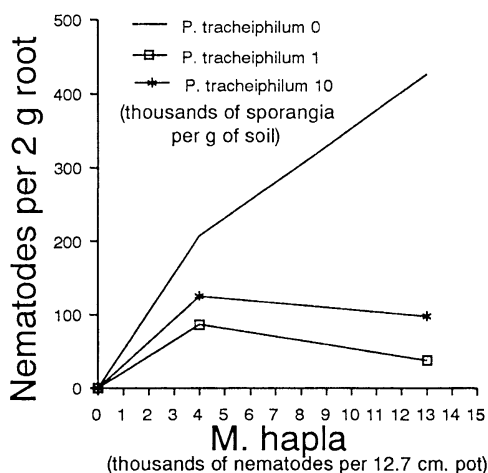
**Table 2.** Effect of *Pythium tracheiphilum* (Py) populations on numbers of *Meloidogyne hapla* (M) nematodes observed in lettuce roots where Py 0, Py 1 and Py 10 represent 1, 1 000 and 10 000 sporangia/g soil, respectively

| Orthogonal comparison | df  | Sum of squares | <i>P</i> > <i>F</i> |
|-----------------------|-----|----------------|---------------------|
| M in Py 0             | 2   | 16.5138        | 0.0001              |
| M Linear in Py 0      | (1) | 11.1755        | 0.0001              |
| M Quadratic in Py 0   | (1) | 5.3383         | 0.0001              |
| M in Py 1             | 2   | 8.4726         | 0.0001              |
| M Linear in Py 1      | (1) | 3.3603         | 0.0001              |
| M Quadratic in Py 1   | (1) | 5.1123         | 0.0001              |
| M in Py 10            | 2   | 11.1616        | 0.0001              |
| M Linear in Py 10     | (1) | 5.8725         | 0.0001              |
| M Quadratic in Py 10  | (1) | 5.2890         | 0.0001              |
| Error                 | 27  | 0.4250         |                     |

press the development of *Meloidogyne* spp. (Johnson and Littrell 1970; Ryder and Crittenden 1965).

Many *M. hapla* galls from plants co-inoculated with *P. tracheiphilum* did not contain nematodes. These galls were macerated and had a red-brown discoloration. Microscopic observations revealed the presence of high numbers of sporangia and oospores in gall tissue although in general the fungus also was present in the remainder of the root system. Tips of tertiary and secondary roots also were highly colonized by the fungus and commonly contained oospores and sporangia. It has been suggested that chemical changes and accumulation of various substances in the galls may enhance the reproduction of fungi (Brodie and Cooper 1964; Powell 1971). Histological observations revealed that nematodes were not colonized by the fungus. It was not clear if the galls or tissue surrounding the galls provided additional entry points into the root system for the fungus, however, pieces of mycelium were observed more commonly in vascular vessels of galled tissue than in non-galled tissue.

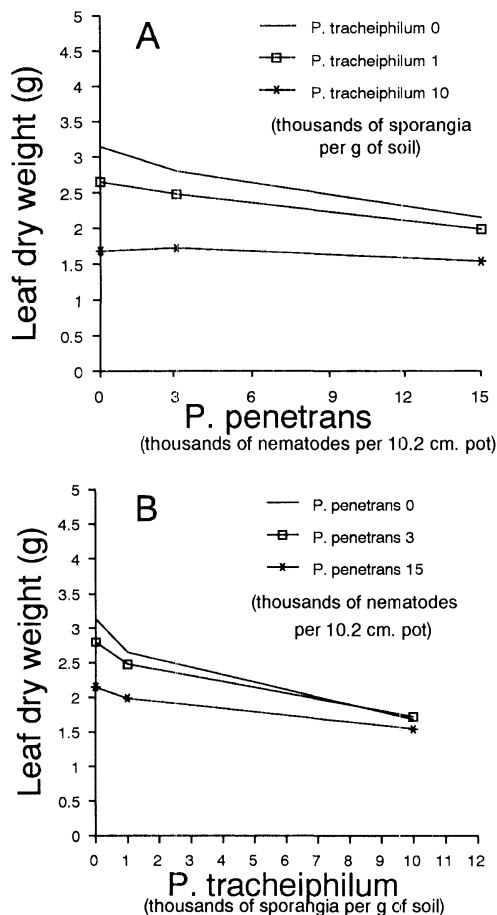
**Interactions between *P. penetrans* and *P. tracheiphilum*.** A linear response was observed for the effects of both pathogens when applied alone (Figs. 4 and 5). Interactions were not significant for leaf dry weight or leaf area.



**Figure 3.** Effect of the presence of *Pythium tracheiphilum* on the number of *Meloidogyne hapla* nematodes observed inside lettuce roots. Curves are lines drawn through mean values ( $n = 4$ ) for each treatment.

However, interactions for root dry weight were significant ( $P \leq 0.01$ ).

Reductions observed in leaf growth when *Pythium* was applied at a rate of 1 000 sporangia/g seemed to be partially additive. Decreases in leaf growth which occurred when nematodes were added (21.0% at 3 000 nematodes per pot and 36.6% at 15 000 nematodes per pot) were higher than those for the fungus alone (15.6%), but lower than the total of the reductions caused by each pathogen alone. When the two pathogens were acting alone, the effect on root dry weight was linear, but when the fungus popula-



**Figure 4.** Leaf dry weight of lettuce plants inoculated with *Pythium tracheiphilum* and *Pratylenchus penetrans*. **A.** Effects of *P. penetrans* on dry weight for three populations of *P. tracheiphilum*. **B.** Effects of *P. tracheiphilum* on dry weight for three populations of *P. penetrans*. Curves are lines drawn through mean values ( $n = 4$ ) for each treatment.

**Table 3.** Orthogonal comparisons of the effect on leaf dry weight of lettuce plants of *Pythium tracheiphilum* (Py) populations in the presence of *Pratylenchus penetrans* (Pr) and of *P. penetrans* populations in the presence of *P. tracheiphilum*

| Orthogonal comparison <sup>§</sup> | df  | Leaf dry weight <sup>†</sup> |                     |
|------------------------------------|-----|------------------------------|---------------------|
|                                    |     | Sum of squares               | <i>P</i> > <i>F</i> |
| Pr in Py 0                         | 2   | 0.0611                       | 0.0001              |
| Pr Linear in Py 0                  | (1) | 0.0611                       | 0.0001              |
| Pr Quadratic in Py 0               | (1) | 0.0000                       | 0.9829              |
| Pr in Py 1                         | 2   | 0.0487                       | 0.0001              |
| Pr Linear in Py 1                  | (1) | 0.0396                       | 0.0001              |
| Pr Quadratic in Py 1               | (1) | 0.0090                       | 0.0131              |
| Pr in Py 10                        | 2   | 0.0057                       | 0.1259              |
| Error                              | 27  | 0.0345                       |                     |

| Orthogonal comparison | df  | Root dry weight |                     |
|-----------------------|-----|-----------------|---------------------|
|                       |     | Sum of squares  | <i>P</i> > <i>F</i> |
| Py in Pr 0            | 2   | 0.0981          | 0.0001              |
| Py Linear in Pr 0     | (1) | 0.0979          | 0.0001              |
| Py Quadratic in Pr 0  | (1) | 0.0002          | 0.6965              |
| Py in Pr 3            | 2   | 0.0485          | 0.0001              |
| Py Linear in Pr 3     | (1) | 0.0363          | 0.0001              |
| Py Quadratic Pr 3     | (1) | 0.0122          | 0.0046              |
| Py in Pr 15           | 2   | 0.0121          | 0.0174              |
| Py Linear in Pr 15    | (1) | 0.0117          | 0.0053              |
| Py Quadratic Pr 15    | (1) | 0.0003          | 0.6081              |
| Error                 | 27  | 0.0345          |                     |

§ Pr: *Pratylenchus penetrans*; Pr 0 = 0 nematode per 12.5 cm pot; Pr 3 = 3 000 nematodes per 12.5 cm pot; Pr 15 = 15 000 nematodes per 12.5 cm pot. Py: *Pythium tracheiphilum*; Py 0 = 0 sporangia per gram of soil; Py 1 = 1 000 sporangia per gram of soil; Py 10 = 10 000 sporangia per gram of soil.

† All significant root dry weight interactions are presented for both analyses of variance. Leaf dry weight interactions were not significant.

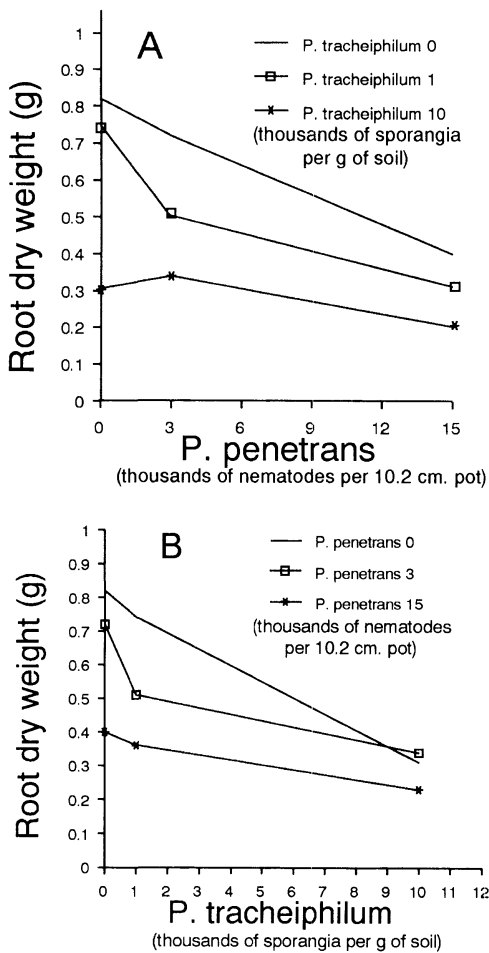
**Table 4.** Effect of *Pythium tracheiphilum* (Py) populations on the numbers of *Pratylenchus penetrans* (Pr) nematodes observed in lettuce roots where Py 0, Py 1 and Py 10 represent 0, 1 000 and 10 000 sporangia/g soil, respectively

| Orthogonal comparison | df  | Sum of squares | <i>P</i> > <i>F</i> |
|-----------------------|-----|----------------|---------------------|
| Pr in Py 0            | 2   | 19.6612        | 0.0001              |
| Pr Linear in Py 0     | (1) | 11.1696        | 0.0001              |
| Pr Quadratic in Py 0  | (1) | 8.4915         | 0.0001              |
| Pr in Py 1            | 2   | 16.5655        | 0.0001              |
| Pr Linear in Py 1     | (1) | 9.3155         | 0.0001              |
| Pr Quadratic in Py 1  | (1) | 7.2501         | 0.0001              |
| Pr in Py 10           | 2   | 15.9446        | 0.0001              |
| Pr Linear in Py 10    | (1) | 10.2686        | 0.0001              |
| Pr Quadratic in Py 10 | (1) | 5.6760         | 0.0001              |
| Error                 | 27  | 0.2010         |                     |

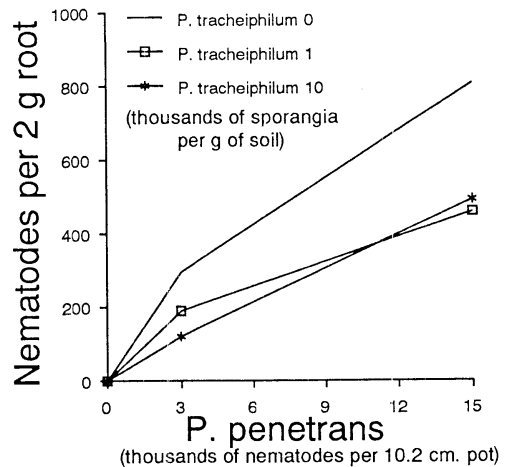


tion was 1 000 sporangia/g and nematodes were added to the soil, a quadratic effect was significant (Table 3, Fig. 5A). This reaction suggests that the detrimental effect of one of the pathogens is somehow inhibited by the presence of the other.

When the fungus was at the 10 000 sporangia/g density, the interaction for leaf dry weight appeared to be negative. The reduction in leaf dry weight from the fungus alone was 46.5% and, when combined with 3 000 or 15 000 nematodes/pot, the growth reductions were 45.2% and 51.0%, respectively. Addition of



**Figure 5.** Root dry weight of lettuce plants inoculated with *Pythium tracheiphilum* and *Pratylenchus penetrans*. **A.** Effects of *P. penetrans* on dry weight for three populations of *P. tracheiphilum*. **B.** Effects of *P. tracheiphilum* on dry weight for three populations of *P. penetrans*. Curves are lines drawn through mean values (n = 4) for each treatment.



**Figure 6.** Effect of the presence of *Pythium tracheiphilum* on the number of *Pratylenchus penetrans* nematodes observed inside lettuce roots. Curves are lines drawn through mean values (n = 4) for each treatment.

nematodes, at either density, did not increase significantly the reduction of leaf or root dry weight when *P. tracheiphilum* was at its highest density (Figs. 4 and 5).

It could not be determined whether the absence of an additive effect at 10 000 sporangia/g occurred because the pathogens were occupying the same space in the root system or because activity of one of them was being suppressed by the other. While most reports indicate that populations of migratory nematodes usually are increased by the presence of different soil fungi, it has been noted that populations of *Pratylenchus* in the presence of *Pythium* spp. tend to decline. Holtzmann and Santo (1971) noted that *Pratylenchus zeae* Graham increased by 220-fold when used to inoculate sugarcane, but increased only 8-fold when the plant was inoculated with both nematode and *Pythium graminicola* Subramanian.

*P. penetrans* populations in roots were significantly reduced in the presence of the fungus (Table 4, Fig. 6). This could explain the lack of additional leaf reduction when nematodes were combined with *Pythium*, especially when the latter was at a high density.

Differences observed in the reaction of the plants to the two species of nematode in the presence of *P. tracheiphilum* are likely due to differences in the way these species attack

plants. *M. hapla*-infected plants were harvested after five weeks while *P. penetrans*-infected plants were harvested after only four weeks, however, it seems unlikely that the additional week would have a major effect on differences between treatments.

Small feeder roots appeared to be destroyed from the combined action of the nematodes and the fungus. Mycelium, sporangia, and oospores of *P. tracheiphilum* were present in all discolored tissues of secondary roots.

## Conclusions

Plant parasitic nematodes did not appear to have a marked effect on penetration of roots by the fungus. However, large numbers of *Pythium* reproductive structures were observed in *M. hapla*-infected tissue. The fungus had negative effects on root populations of both nematodes.

An additive interaction was shown between *M. hapla* and *P. tracheiphilum* and it appears that when populations of *Pythium* are low, the presence of *M. hapla* can have a significant effect on lettuce growth. On the other hand, high inoculum densities of *M. hapla* are needed in order to cause significant further reductions in plant growth when populations of *Pythium* also are high. This latter observation may be related to the detrimental effect this fungus has on nematode populations.

Interactions between *P. penetrans* and *P. tracheiphilum* were less clear. No further reduction in lettuce growth was observed when both pathogens were present at the highest populations. When the fungus was at its lowest concentration, plants appeared to have a more marked reaction to the presence of both pathogens. This could be due to reduced inhibition of the nematode when low populations of *P. tracheiphilum* are present.

Our results suggest that plant parasitic nematodes, in particular *M. hapla*, may increase the severity of lettuce wilt caused by *P. tracheiphilum*. Further studies are required in order to determine how colonization by the fungus is affected in time and space by the presence of the nematode and whether or not decreases in nematode populations in the presence of *P. tracheiphilum* are due simply to competition for a specific site in the root system or to some physiological change occurring in the host.

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