Growth Reduction of Apple Seedlings by *Pratylenchus penetrans* as Influenced by Seedling Age at Inoculation

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Abstract: Apple seedlings of different ages (1, 3, and 5 weeks) were inoculated with 6,900 *Pratylenchus penetrans* per seedling in 10-cm-diam pots in a growth chamber. Rate of growth suppression and total growth suppression of seedlings by *P. penetrans* were inversely proportional to seedling age at time of nematode inoculation. Younger seedlings were found to contain a higher number of nematodes per gram root. *Key Words:* root-lesion nematodes.

Pratylenchus penetrans (Cobb) Filipjev and Schuurmans Stekhoven, a pathogen of apples (7, 12, 18), significantly suppresses the growth and yield of apple trees in various parts of the world (1, 2, 4, 6, 8, 10, 13, 14, 15, 16, 17, 19). In New York, Mai et al. (15) hypothesized that protection of the tree during the first year in the orchard may be critical since established trees are apparently more tolerant of *P. penetrans* than are newly planted trees. The benefits of early protection have been reported for other nematode-crop systems (3, 5, 9, 20, 22). This investigation was undertaken to determine the relation between disease severity and the age of apple seedlings at the time of inoculation with *P. penetrans*.

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MATERIALS AND METHODS

General procedures: The soil used was a loamy sand collected from an apple orchard in Wayne County, New York. The sand fraction contained 9% very fine sand, 48% fine sand, 22% medium sand, and 21% coarse sand. Its pH ranged from 6.6 to 6.9. In all experiments, the soil had been autoclaved (121 C for 5 hr) at least 1 month before use.

Seeds from 'Northern Spy' apples (*Malus* *domestica,* Borkh.) were surface-disinfested in 0.6% sodium hypochlorite for 20 minutes. Germinated seeds were planted one per 10-cm-diam clay pot. Because the seeds vary genetically, 1.5–2 times as many seeds as needed were planted to permit selection of apparently uniform seedlings.

All experiments were conducted in a growth chamber maintained at 20 C, a 15-h photoperiod, and 21,000 lux. Humidity was not monitored. A soluble fertilizer (23-19-17) was applied to the seedlings every 2 weeks, starting at emergence and continuing until the experiment was terminated. Water was provided as needed.

The *P. penetrans,* obtained from alfalfa callus tissue (21), consisted of larvae and adults of both sexes. The callus tissue and associated agar were placed on a pie-pan modification of the Baermann funnel with tap water. Nematodes were collected daily, and the suspension was stored at 2 C for no more than 3 days before use. To inoculate, the suspension was added to the surface of the soil and to four holes formed in the soil. Controls received tap water.

P. penetrans was extracted from root samples as described by Mai and Abawi (13). Nematodes were extracted from the entire root system unless it weighed more than 2 g. In that case a random 2-g sample was selected. *P. penetrans* was extracted from soil by a pie-pan modification of the Baermann funnel. The suspension was collected and counted after 7 days.

Inoculum level: To select an inoculum level for experiments in which seedling age at inoculation would be a variable, 1-week-old seedlings were inoculated with 0, 100, 1,000, 5,000, or 10,000 *P. penetrans*/pot. There were 12 seedlings per treatment. Nine days after inoculation, four randomly selected seedlings from each treatment were harvested and the nematodes were extracted from the roots and soil to determine how many *P. penetrans* had survived inoculation. Five weeks after inoculation, the remaining eight seedlings per treatment were harvested. The experiment was repeated once. Except where noted, 6,900 *P. penetrans*/pot was the inoculum level used in the following experiments.

Effect of plant age at inoculation on disease severity: In two experiments (experiments 1 and 2), all seeds were planted on the same day. Groups of seedlings were inoculated 1, 3, and 5 weeks after emergence. At each of these times one group was left uninoculated. Within each group, eight seedlings were harvested every 2 weeks to obtain progressive measurements of root weight, shoot weight, and nematode numbers. The final harvest was made 9 weeks after emergence.

Determined in experiments 3, 4, and 5 were the growth rates of seedlings of different ages during a specified period following inoculation. In these tests, the seeds were planted at different times and inoculated on the same day, providing groups of seedlings which were 1, 3, and 5 weeks old at inoculation. For each group of inoculated seedlings there was also an equivalent number of uninoculated seedlings, planted at the same time. Eight uninoculated and eight inoculated seedlings in each age group were harvested at the time of inoculation and 2 and 4 weeks after inoculation. The growth statistics (increase in shoot and root fresh weight during a two-week period) of an inoculated group were divided by the growth statistics for an uninoculated group of the same age to obtain "growth expressed as a percent of the uninoculated control," or "relative growth rate." Data from all five experiments are included in the analysis.

Inoculation of different-aged seedlings with 6,900 *P. penetrans*/pot results in a higher inoculum level/g inoculated root in younger seedlings because younger seedlings have smaller root systems than older seedlings. In experiments 4 and 5, additional treatments were included in which inoculum levels/g inoculated root were equalized. To do that, the root weights of seedlings 1, 3, and 5 weeks old were determined at inoculation. If the roots of the 5-week-old seedlings, for example, were found to weigh n times those of the 1-week-old seedlings, the older seedlings received $n \times 6{,}900$ *P. penetrans*/pot.

RESULTS

Inoculum level: Inoculation of seedlings one week old with different levels of *P. penetrans* indicates that 5,000 and 10,000 *P. penetrans*/pot significantly suppresses shoot and root weight (Table 1). Only 20–30% of the nematodes added were recovered nine days after inoculation. At the

TABLE 1. Shoot length, shoot weight, and root weight of apple seedlings inoculated with different numbers of *Pratylenchus penetrans.*

No. of nematodes /pot	Shoot length (cm)	Shoot fresh wt (g)	Root fresh wt (g)
0	11.5 a	2.15 a	1.59 a
100	13.0 a	2.33 a	1.63 a
1,000	11.0 a	2.04 a	1.59 a
5,000	7.0 b	1.35 b	1.23 b
10,000	7.3 b	1.35 b	1.10 b

Means in a column followed by the same letter do not differ significantly at the 5% level as determined by Duncan's multiple-range test.

termination of the experiment, means of 508 and 1633 *P. penetrans*/g root were respectively recovered from seedlings inoculated with 5,000 and 10,000 *P. penetrans*/pot. These counts are within the range obtained from New York apple orchard samples.

Effect of plant age at inoculation on disease severity: Data from experiments 1 and 2 indicate that final shoot weight at week 9 was a function of seedling age at inoculation, i.e., seedlings inoculated at week 1 had smaller shoots than those inoculated at week 3 or week 5 (Fig. 1). Results were similar for root weight.

Comparison of relative shoot and root growth rates also indicates that seedlings 1 week old are more severely affected (P = 0.05) by inoculation with 6,900 *P. penetrans*/pot than are seedlings 5 weeks old (Table 2).

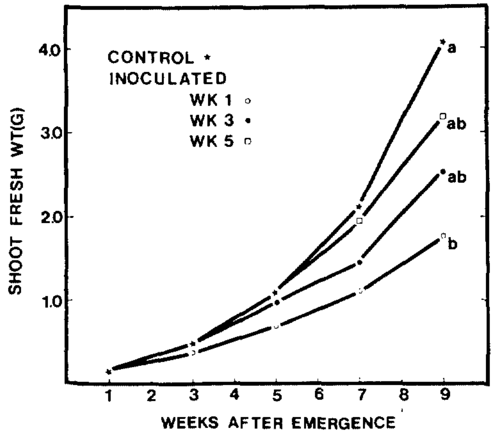

FIG. 1. Shoot fresh weight of apple seedlings inoculated at 1, 3, or 5 weeks with 6,900 *Pratylenchus penetrans*/pot. Means at week 9 followed by the same letter do not differ significantly at the 5% level as determined by Duncan's multiple-range test.

TABLE 2. Shoot and root growth of apple seedlings inoculated with 6,900 *Pratylenchus penetrans*/pot.

Growth period and seedling age at inoculation	Growth expressed as % of uninoculated control: Shoot	Root
0–2 wk after inoculation		
1 week	66[a]	67[a]
3 week	81	78
5 week	89	88
2–4 wk after inoculation		
1 week	51[a]	63[a]
3 week	72	84
5 week	101	99

[a]Differs (P = 0.05) from week 5 using the *t*-test.

At an inoculum level of 6,900 *P. penetrans*/pot, more *P. penetrans*/g root were recovered from seedlings inoculated at a younger than an older age (Table 3).

Equalization of inoculum/g root did not result in equal numbers of *P. Penetrans*/g root recovered 2 weeks after inoculation (Table 4). When older seedlings with roots weighing *n* times those of 1-week-old seedlings were inoculated with *n* times 6,900 *P. penetrans*/pot, the numbers of nematodes/g root were higher in the older seedlings. Although the relative growth rates were not statistically different, the results suggest that the growth rates of the older seedlings may be higher. Results were similar in the period 2 to 4 weeks following inoculation.

DISCUSSION

Investigators have reported that control

TABLE 3. Number of *Pratylenchus penetrans*/g root recovered 2 and 4 weeks after inoculation with 6,900 *Pratylenchus*/pot.

Seedling age at inoculation (wk)	2 wks after inoculation	4 wks after inoculation
1	1989[a]	1196[a]
3	1169	1151
5	441	456

[a]Differs (P = 0.05) from the average for seedlings inoculated when 5 weeks old (using the *t*-test).

TABLE 4. Growth of apple seedlings and numbers of *Pratylenchus penetrans* 2 weeks after inoculation.

Age at inoculation (wk)	No. of nematodes added /pot[a]	Final no. of *P. penetrans* /g root	Two weeks' growth expressed as % of uninoculated control: Shoot	Root
1	6,900	1,555	68	69
3	19,250	2,411	81	81
5	59,200	3,760	82	75
LSD (P = 0.05)		835	17	19

[a]Inoculum/pot was adjusted to achieve the same numbers of *P. penetrans* infecting, per g of root.

of nematodes early in the development of certain crops gave substantial increases in yield. There are several possible explanations for this phenomenon. First, a reduction in the length of time that a suscept is exposed to a pathogen will likely reduce disease severity. Second, it is possible that established crops are in some way more resistant or tolerant than newly planted crops. This increased resistance or tolerance may be physiological or anatomical, or it may result from some other phenomenon, such as the development of deep roots, which, in an older plant, may remain undamaged and may compensate for damaged shallow roots. The data obtained from these experiments with apple seedlings and *P. penetrans* again suggest the importance of protecting the newly planted crop. When measured at 9 weeks, seedlings that had been inoculated at 1 week were smaller than seedlings inoculated at 3 or 5 weeks. The fact that the relative growth rates of infected younger seedlings were less than the relative growth rates of infected older seedlings indicates that the differences in growth measured at 9 weeks were not simply the result of differences in exposure time to the pathogen.

A possible reason that the younger seedlings were more severely affected was that they contained more *P. penetrans*/g root than did older seedlings. If the apple root acts as a sink for *P. penetrans* (11), one might propose that as root quantity increases relative to inoculum level, the number of nematode penetrations/g root decreases. Part of the benefit gained by protecting newly planted crops may result from this apparently simple relation between root quantity and nematode numbers.

Experiments designed to determine the effect of inoculation with equal numbers of *P. penetrans*/g inoculated root indicate that seedlings inoculated at an older age may have a higher relative growth rate than seedlings inoculated at a younger age, even though more *P. penetrans*/g root were recovered from older seedlings. Since that phenomenon was examined in only two experiments and the data were highly variable, the results should be considered preliminary.

REFERENCES

1. ARK, P. A., and H. E. THOMAS. 1936. Anguillulina pratensis in relation to root injury of apple and other fruit trees. Phytopathology 26:1128-1134.
2. ARNESON, P. A., and W. F. Mai. 1976. Root diseases of fruit trees in New York State. VII. Costs and returns of preplant soil fumigation in a replanted apple orchard. Plant Dis. Rep. 60:1054-1057.
3. BERGESON, G. B. 1968. Evaluation of factors contributing to pathogenicity of Meloidogyne incognita. Phytopathology 58:49-53.
4. BOSHER, J. E., and W. NEWTON. 1957. Pratylenchus penetrans in clonal apple stocks. (Abstr.) Phytopathology 47:4.
5. BRODIE, B. B., and P. D. DUKES. 1972. The relationship between tobacco yield and time of infection with Meloidogyne javanica. J. Nematol. 4:80-82.
6. COLBRAN, R. C. 1953. Problems in tree replacement. I. The root lesion nematode Pratylenchus coffeae Zimmerman as a factor in the growth of replanted trees in apple orchards. Austral. J. Agr. Res. 4:384-389.
7. DECKER, H. 1960. Pratylenchus penetrans als Ursache von 'Mudigkeitserscheinungen' in Baumschulen der DDR. Nematologica Supplement No. 2:68-75.
8. DUNN, R. A., and W. F. MAI. 1972. Growth of apple trees in response to preplant treatment with nematicides and broad-spectrum fumigant. Plant Dis. Rep. 56:577-580.
9. GRIFFIN, G. D., and O. J. HUNT. 1972. Effect of plant age on resistance of alfalfa to Meloidogyne hapla. J. Nematol. 4:87-90.
10. HOESTRA, H., and M. OOSTENBRINK. 1962. Nematodes in relation to plant growth. IV. Pratylenchus penetrans on orchard trees. Neth. J. Agric. Sci. 10:286-296.
11. LAVALLEE, W. H., and R. A. ROHDE. 1962. Attractiveness of plant roots to Pratylenchus penetrans (Cobb). Nematologica 8:252-260.
12. MAI, W. F. 1960. Growth of apple seedlings in

relation to soil temperature and inoculation with Pratylenchus penetrans. Phytopathology 50:237-238.

13. MAI, W. F., and G. S. ABAWI. 1978. Determining the cause and extent of apple, cherry, and pear replant diseases under controlled conditions. Phytopathology 68:1540-1544.
14. MAI, W. F., G. S. ABAWI, and R. A. DUNN. 1976. Root diseases of fruit trees in New York State. VIII. The influence of application method on the effectiveness of methyl bromide as a preplant treatment for increasing growth of apple trees. Plant Dis. Rep. 60:1057-1060.
15. MAI, W. F., K. G. PARKER, and K. D. HICKEY. 1970. Populations of Pratylenchus penetrans and growth of apple in response to soil treatment with nematicides. Plant Dis. Rep. 54:792-795.
16. OOSTENBRINK, M. 1961. Nematodes in relation to plant growth. III. Pratylenchus penetrans (Cobb) in tree crops, potatoes, and red clover. Neth. J. Agric. Sci. 9:188-209.
17. PARKER, K. G., and W. F. MAI. 1974. Damage caused by Pratylenchus penetrans to apple trees in the orchard growing on different rootstocks. Plant Dis. Rep. 58:1007-1011.
18. PITCHER, R. S., Z. A. PATRICK, and W. B. MOUNTAIN. 1960. Studies on the host-parasite relations of Pratylenchus penetrans to apple seedlings. Nematologica 5:309-314.
19. RACKHAM, R., J. D. RADEWALD, C. L. HEMSTREET, and F. SHIBUYA. 1975. Lesion nematode control in apples. Calif. Agr. 29(4):14-15.
20. RIEDEL, R. M. 1969. Influence of onion seedling age on the development of symptoms caused by Ditylenchus dipsaci. J. Nematol. 1:24-25.
21. RIEDEL, R. M., and J. G. FOSTER. 1970. Monoxenic culture of Ditylenchus dipsaci and Pratylenchus penetrans with modified Krusberg's and White's media. Plant Dis. Rep. 54:251-254.
22. SEINHORST, J. W., and J. KOZLOWSKA. 1977. Damage to carrots by Rotylenchus uniformis, with a discussion on the cause of increased tolerance during development of the plant. Nematologica 23:1-23.