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# Response of Peach Seedlings to Infection by the Root Lesion Nematode *Pratylenchus penetrans* under Controlled Conditions[1]

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*Abstract:* Twenty-one open pollinated populations of peach rootstock seedlings were evaluated for their response to infection by the root lesion nematode, *Pratylenchus penetrans,* over a period of 98 days. Nematode-infected peach seedling populations were shorter in plant height and had less shoot weight but more dry root weight than nematode-free controls. Rootstock differences were demonstrated for nematode increase over the 98-day period, and average total numbers of nematodes in soil and roots. Rootstocks were classified into three groups differing in total nematode population levels, ratio of nematode increase, and the number of nematodes per root. The heritable nature of rootstock response to nematodes was evident. Rootstocks showing the lowest response to nematode infection included Tzim Pee Tao, Rutgers Red Leaf, and two progenies of a cross of these two rootstocks.

*Key words: Prunus persica, Pratylenchus penetrans,* nematode populations, rootstock variability.

Longevity of peach, *Prunus persica* (L.), trees and orchards in southwestern Ontario and the northern United States has been considered primarily a problem of winter hardiness (6–8), and the choice of rootstock has been based on such agronomic characteristics as cold-hardiness, vigor, and drought tolerance (6).

The root lesion nematode, *Pratylenchus penetrans* Cobb, has been shown to affect peach longevity and productivity in the same region (10–12). Although no resistance to *P. penetrans* has been reported for peach rootstocks, quantitative differences in nematode populations have been asso-

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ciated with different rootstocks (3,5,6, 9,15). If rootstocks with tolerance could be identified, selection and breeding approaches might be used to combine cold-hardiness and nematode tolerance as an alternative to the present widely used practice of preplant soil fumigation.

This study was undertaken to evaluate the response of a number of rootstocks to nematode infection, with sample sizes adequate to allow for the expected genetic variability of rootstocks (1) as well as the variability associated with nematode infection and sampling, in comparison with non-infected seedling populations of the same material.

## Materials and Methods

Peach rootstock populations chosen for evaluation included several named cultivars of known cold-hardiness, two very cold-hardy selections of unknown parentage from China, four lines from California preselected for resistance to root-knot nematode (*Meloidogyne* spp.) (Dr. D. W. Ramming, pers. comm.), and eleven cold-hardy selections from the Harrow rootstock breeding program.

Seeds were soaked in an aqueous suspension of thiocarbamate (ferbam) fungicide for 24 hours at 21 C. After soaking, the seeds were placed on moistened paper towels in aluminum pans, covered with plastic film, and stored in the dark at 5.5 C for 74–83 days to stratify. When most seeds showed growth of the radicle, seeds were planted singly into 6- × 6-cm peat pots containing a 4:1 mixture of steam-sterilized Vineland silt loam and crushed montmorillonite and watered as required.

Root lesion nematodes were reared in greenhouse groundbeds in Vineland silt loam on sweet corn (*Zea mays* L.); population density was monitored by periodic sampling. Nematode-infested soil from the groundbeds was mixed 1:1 with steam-sterilized soil of the same type in a concrete mixer. Steam-sterilized Vineland silt loam was used as noninoculated check soil.

Seedling containers were 1.14-liter (7.5 × 7.5 × 25 cm) plasticized-cardboard milk cartons with a 2-cm drainage hole punched in the flat bottom. A piece of broken clay pot covered the drainage hole, and each carton was filled with 1,400 g either of nematode-infested soil mix or steam-sterilized mix and labelled. Before planting, a single soil core, 2 × 20 cm and weighing ca. 50 g, was removed from the long axis of each carton to determine nematode numbers by the Baermann pan method (16).

Twenty-five seedlings of each rootstock were transplanted in both nematode-infested soil and in noninfested (check) soil, except for the California selections where 20 seedlings were planted in nematode-infested soil and only 3–5 in check soil because of limited available seeds. At planting, seedlings were selected to be as nearly uniform in height as possible. The peat pot was removed completely, and the unbroken soil-root ball was set into the soil in the carton deep enough to cover the seedling stem to about the second leaf-node. Each plant received 100 ml of aqueous 10-52-10 starter fertilizer solution. Check and infected plants of the same rootstock were planted on the same day, placed in large boxes, and located at random on a greenhouse bench on wood strips to allow for aeration and drainage.

Height of each seedling was measured from soil line to the apex at planting time. The plants were grown in the greenhouse for 14 weeks (22 March to 28 June 1979) and were hand-watered as uniformly as possible. At termination, each plant was severed at the soil line and height and fresh weight determined. Tops were oven-dried at 70 C for 72 hours and dry weight determined. Roots were separated from the soil by shaking, washed, and the fresh weight determined; check roots were immediately oven-dried as above and dry weights determined, whereas infected roots were put on Baermann pans for 2 weeks to extract nematodes and then oven-dried to determine dry weight. Soil from each infected carton was mixed and nematodes extracted from a 50-g sample by the Baermann pan method (16).

The nematode counts of soil and plant roots were the basis for the following variables for each plant infected by *P. penetrans*: 1) final nematode population in the soil, 2) final number of nematodes in the root, 3) nematodes per gram of root, 4) total nematodes at the end of experiment (total soil + total root nematodes), and 5) ratio of nematode increase (total final nematodes/initial nematode population).

Because a number of plants died before the termination of the experiment in both the infected and check series, the numbers of plants in each rootstock varied.

Rootstocks were classified for plant growth response using three categories: dead plants, weak plants (with less than 3 g shoot dry matter), and vigorous plants (3 g shoot dry matter or more) in both the infected and check series. The distribution of these vigor classes in the check and infected plants in each rootstock was compared, using a $\chi^2$ test for heterogeneity.

The nematode-infected and the noninfected rootstock populations were initially analyzed using all the available data, with rootstocks and error as the sources of variation in each population. A logarithmic transformation based on Proctor and Marks (13) was also used for the analysis of the nematode data. Variances within individual rootstock populations were calculated and compared for homogeneity.

Individual infected plants of each rootstock were classified by total nematode counts at the end of the 14-week period. Five classes of nematode response were used: fewer than 4,000 nematodes, 4–12,000, 12–20,000, 20–28,000, and more than 28,000.

On the basis of the distribution of these categories within individual rootstocks, the rootstocks were placed into three response groups, and $\chi^2$ tests were used to establish heterogeneity within and between these rootstock groups, labelled A, B, and C.

Group A included Tzim Pee Tao (TPT), Rutgers Red Leaf (RRL), the two RRL × TPT progenies H7154008 and H7154013, and the California selection 115-104.

Group B included the Siberian C × RRL selections H7141076 and H7141137, Bailey, Siberian C, Chui Lum Tao, and the remaining three California selections: 115-5, 115-95, and 115-102.

Group C included the five Bailey × Siberian C selections H7338010, H7338011, H7338013, H7338016 and H7338019, two Siberian C × RRL selections H7141064 and H7141128, plus Harrow Blood.

The possible bias introduced by variation in plant vigor within individual rootstocks was evaluated, using a two-way classification for the entire nematode-infected population: shoot vigor (less than 3 g dry weight, 3 g and over) and total nematodes (fewer than 12,000, 12–28,000, and 28,000 or more). A $\chi^2$ test for heterogeneity was used to determine if nematode response and plant vigor were independent. Covariance analysis based on the original data set was used to evaluate the bias in the nematode response of individual rootstocks associated with vigor differences and to adjust the rootstock means to the overall average rootstock vigor, using plant shoot weight as the independent variable. Since small, low-vigor plants would not likely be budded under nursery conditions, data are reported only for plants with 3 g of dry shoot weight or more. This data set was analyzed for rootstock effects. The rootstock sums of squares were separated into two components: 1) variance among the rootstock groups A, B, and C; and 2) variance within these groups.

Rankings of individual rootstock means based on the entire data set, means as adjusted by covariance analysis to a comparable vigor level, and the reduced data set of all plants with 3 g dry shoot or more were compared using Spearman's rank correlation.

Means were calculated for each rootstock group using the reduced data set. Comparisons among the group means were based on the individual error variances within each group.

## Results

Variation in the entire population of rootstock progenies for growth and survival response was largely associated with rootstock effects. Seedling vigor, as measured by plant shoot weight and survival, did not differ ($P > 0.05$) between check and inoculated plants in 18 of 21 rootstock progenies, or in the inoculated versus check populations as a whole. Tests of homogeneity of variances within individual rootstock populations for survival, vigor, and classification by nematode count indicated that comparisons could be made between the inoculated and check populations as a whole.

Differences between the check and nematode-infected populations as a whole were significant ($P = 0.05$) for plant measurements. The entire nematode-infected population of 21 rootstocks measured 6.2% less in average height increment than the check populations, 15.3% less in fresh top

TABLE 1. Weighted means of plant measurements and nematode counts by rootstock group, using only plants with 3 g dry shoot weight and over.*

| Variable | Group A† | Group B | Group C |
|---|---|---|---|
| Number of plants | 73 | 132 | 165 |
| Plant height increment, cm | 42 a | 42 a | 41 a |
| Fresh shoot weight, g | 15 b | 16 a | 16 a |
| Dry shoot weight, g | 5 a | 6 a | 5 a |
| Fresh root weight, g | 5 c | 7 b | 8 a |
| Dry root weight, g | 2 b | 3 a | 3 a |
| Total fresh plant weight, g | 20 b | 23 a | 23 a |
| Total dry plant weight, g | 7 b | 8 a | 8 a |
| Ratio of nema increase | 9 c | 10 b | 15 a |
| Soil nematodes | 17,000 c | 19,140 b | 22,770 a |
| Total root count | 4,440 c | 5,680 b | 6,530 a |
| Nematodes/g root | 2,210 a | 2,070 a | 2,300 a |
| Total nematode count | 21,610 c | 24,820 b | 29,210 a |
| Root count as % of total count | 21% a | 23% a | 22% a |

* Means in same rows followed by same letter not significantly different from each other at $P = 0.05$.
† For rootstocks included in Groups A, B, and C, see text.

weight, 21.5% less in fresh root weight, and 17.3% less in total fresh plant weight than the checks. The dry top weight of the infected population was 9.6% less than the check, but the average dry root weight of the nematode-infected plants was 16.7% higher than the checks.

Comparison of nematode response of individual rootstocks and rootstock groups A, B, and C was based on the analysis of data from all plants with at least 3 g dry shoot weight. Means of plant measurements and nematode counts as summarized by rootstock groups are shown in Table 1. Means of nematode counts by individual rootstocks are shown in Table 2.

Differences among rootstock means were significant ($P < 0.01$) for all the nematode response variables. When the rootstock sums of squares for the nematode measurement variables in the reduced data set were divided into two components, 1) among the rootstock groups A, B, and C (2 df) and 2) within the rootstock groups (18 df), the greater part of the rootstock sums of squares for total nematode count, soil nematode count, root count, and ratio of nematode increase was associated with the among rootstock groups component. However, numbers of nematodes per gram of dry root did not vary among groups A, B, and C. Absence of major differences in plant height increment and shoot and root weight associated with the nematode response groups (Table 1) indicated that the groups were homogenous for vigor.

The weighted group means in Table 1 indicate a clear progression in numbers of root and total nematodes from Group A to Group C, and this is reflected in a similar progression of ratio of nematode increase.

The means by rootstock of all the nematode count measures, ratio of increase, and ratio of root count to total count based on the reduced data set are shown in Table 2. The rootstock nematode count means for groups and for individual rootstocks indicate that variation in root and soil nematodes is largely associated with total dry root weight. The rootstock effect in the analysis of root count variation was almost entirely attributable to covariance with dry root weight.

The ratio of root nematodes to total nematodes in the system varied around 20–22%, and this was consistent for each group (Table 2) suggesting that, on an average, one-fifth of the total nematode population in any container appeared to be in the roots. Where the root nematode population was small, the total population of nematodes in the system was reduced accordingly.

The observed relationship between low nematode counts and low plant vigor in some plants was evaluated by a cross classification of all infected plants into three nematode response classes, and plants with less than 3 g or more than 3 g shoot weight. $\chi^2$ for heterogeneity was 240.72; 2 df, $P < 0.001$; the small plants tending to support fewer nematodes. Covariance analysis of the nematode population variables, using

TABLE 2. Nematode population means for soil and roots of 21 rootstocks based on plants with 3 g dry shoot weight and over.*

| Group | Rootstock | Nematode counts in thousands | | | | | |
|---|---|---|---|---|---|---|---|
| | | | Root nematodes | | | | |
| | | Soil nematodes | Total | Per g root | Total nematodes | Ratio of increase | Ratio root/total |
| A | Rutgers Red Leaf (RRL) | 17.1 | 5.3 | 1.8 | 22.4 | 10.6 | 0.24 |
| A | Tzim Pee Tao (TPT) | 14.3 | 4.5 | 2.4 | 18.8 | 8.1 | 0.24 |
| A | RRL × TPT H7154008 | 17.8 | 3.8 | 2.1 | 21.6 | 8.6 | 0.17 |
| A | RRL × TPT H7154013 | 18.2 | 5.3 | 2.7 | 23.5 | 10.6 | 0.23 |
| A | Cal. 115-104 | 17.6 | 3.3 | 1.9 | 20.9 | 6.4 | 0.16 |
| Group A mean (weighted) | | 17.0 | 4.5 | 2.2 | 21.5 | 8.9 | 0.21 |
| B | Bailey (B) | 20.1 | 4.7 | 1.8 | 24.8 | 10.6 | 0.19 |
| B | Siberian C (S) | 17.4 | 7.3 | 2.4 | 24.7 | 8.7 | 0.30 |
| B | Chui Lum Tao | 20.3 | 5.8 | 2.5 | 26.1 | 11.8 | 0.22 |
| B | S × RRL H7141076 | 17.9 | 5.5 | 1.8 | 23.4 | 9.6 | 0.24 |
| B | S × RRL H7141137 | 16.7 | 8.8 | 1.9 | 25.5 | 13.0 | 0.35 |
| B | Cal. 115-5 | 19.8 | 3.9 | 1.7 | 23.7 | 11.6 | 0.16 |
| B | Cal. 115-95 | 20.9 | 4.0 | 2.2 | 24.9 | 9.0 | 0.16 |
| B | Cal. 115-102 | 20.1 | 5.4 | 2.4 | 25.5 | 8.9 | 0.21 |
| Group B mean (weighted) | | 19.1 | 5.7 | 2.1 | 24.8 | 10.4 | 0.23 |
| C | Harrow Blood | 30.1 | 5.2 | 3.1 | 35.3 | 18.1 | 0.15 |
| C | S × RRL H7141064 | 20.4 | 6.5 | 2.2 | 26.9 | 11.1 | 0.24 |
| C | S × RRL H7141128 | 25.4 | 6.5 | 2.4 | 31.9 | 14.8 | 0.21 |
| C | B × S H7338010 | 21.0 | 7.6 | 2.5 | 28.6 | 16.8 | 0.27 |
| C | B × S H7338011 | 17.4 | 6.6 | 2.1 | 24.0 | 13.1 | 0.28 |
| C | B × S H7338013 | 19.7 | 7.6 | 2.7 | 27.3 | 17.0 | 0.28 |
| C | B × S H7338016 | 22.6 | 5.1 | 1.5 | 27.7 | 11.2 | 0.18 |
| C | B × S H7338019 | 25.7 | 7.2 | 2.0 | 32.9 | 20.3 | 0.22 |
| Group C mean (weighted) | | 22.8 | 6.5 | 2.3 | 29.3 | 15.3 | 0.23 |

* Group means differ from each other for numbers of soil nematodes, total root nematodes, and rate of increase, but not for nematodes per gram of root and root/total ratio.

shoot weight as an independent variable, indicated a statistically significant regression of nematode counts on shoot weight ($P < 0.001$). However, when covariance analysis was restricted to plants with 3 g shoot weight or greater, the effect of shoot weight was not significant ($P > 0.05$).

## DISCUSSION

The peach rootstock response to infection with *P. penetrans* as measured by total nematode counts was quantitative and appeared to be associated with differences in the amount of dry root tissue. Nematodes per gram of dry root appeared to be very similar for all rootstocks: approximately 2,000–2,200/g.

The most useful measure of plant response to nematode infection of peach seedlings appeared to be root counts of this parasite. The nematode densities in roots were apparently related to those in the soil by a ratio of 1:4. This ratio could reflect a relatively constant ratio of root penetrating to soil-inhabiting nematodes, or else the relative time of the nematode's life spent within root tissue.

Numbers of nematodes in roots depended on amount of available root tissue and plant growth. Root size and weight may themselves be a response to nematodes, as suggested by the increase in root dry matter associated with infection. The stimulation and proliferation of roots resulting from infection by *P. penetrans* has been described as the "mossy root" phenomenon (11).

When dry shoot growth was comparable, plants with the lowest weight of dry root tissue appeared to have the fewest nematodes. Group A rootstocks, with the lowest nematode populations, had less root weight, fresh or dry, than Group B or C rootstocks (Table 1). Comparisons of individual rootstock means within groups A, B, or C should be made with caution, although the effects

of differences in vigor among rootstocks and rootstock groups are negligible.

Comparisons among group means (Table 1) can be made with much greater confidence than those among individual rootstock means, since plant growth differences are largely eliminated as a source of variation among groups. The quantitative differences in total nematode response indicated that Group A rootstocks differed significantly from those of Group B, and the latter from those of Group C, in response to infection with *P. penetrans*. If minimizing the root-shoot ratio is advantageous for growth (14), Group A rootstocks would appear to have an inherent advantage over the other rootstocks tested, and this difference could be exploited.

The close association of rootstocks related genetically, such as the two Rutgers Red Leaf × Tzim Pee Tao progenies and their parents in Group A, or all five Bailey × Siberian progenies in Group C, suggests that there are genetic differences among rootstocks, which, directly or indirectly, affected variation in nematode increase and indicates that plant breeding may be effective in increasing tolerance of peach rootstocks to *P. penetrans*.

The indicated quantitative genetic approach to the improvement of peach rootstocks for greater resistance or tolerance to this root lesion nematode will require accurate and careful appraisal of test plants. A selection experiment to establish estimates of heritability of nematode response in peach rootstocks might furnish further useful information.

The apparent relation of plant vigor to nematode numbers in roots and surrounding soil, as shown by the bias introduced by weak plants and in covariance analysis, may be attributed to the availability of food resources, as suggested by Johnson (4). Elimination of the weak plants from the data base analyzed apparently largely eliminated the effect of plant vigor on nematode numbers.

The association of variation in seedling vigor with nematode numbers may explain some of the conflicting results reported for rootstocks (2,5). Where seedling vigor is superimposed on genetic variation, and distributed binomially, small sample experiments could lead to very variable results.

## Literature Cited

1. Allard, R. W., S. K. Jain, and P. L. Workman. 1968. The genetics of inbreeding populations. Advances in Genetics 14:55–131.

2. Allen, W. R., and C. F. Marks. 1977. Chemical control and population studies of *Pratylenchus penetrans* on fruit tree understocks. Plant Disease Reporter 61:84–87.

3. Hesse, C. O. 1975. Peaches. Pp. 285–335 *in* J. Janick and J. N. Moore, eds. Advances in fruit breeding. West Lafayette: Purdue University Press.

4. Johnson, P. W. 1975. Effects of rate and depth of application of nematicide on nematode vertical distribution and tomato production in a sandy loam greenhouse soil. Canadian Journal of Plant Science 55:1017–1021.

5. Johnson, P. W., V. A. Dirks, and R. E. C. Layne. 1978. Population studies of *Pratylenchus penetrans* and its effects on rootstocks. Journal of the American Society of Horticultural Science 103:169–172.

6. Layne, R. E. C. 1974. Breeding peach rootstocks for Canada and the northern United States. HortScience 9:364–366.

7. Layne, R. E. C. 1976. Peach rootstock breeding, rootstock hardiness and rootstock influence on scion cultivars. Rivista dell'Ortoflorofrutticoltura Italiana 60:113–119.

8. Layne, R. E. C. 1980. Prospects of new hardy peach rootstocks and cultivars for the 1980's. Compact Fruit Tree 13:117–122.

9. Manzo, P. 1974. Prove comparativetra diversi portinnesti di pesco in terreno "Stanco" fumigato e non fumigate. Instituto Spermitale per la Frutticoltura—Annali 5:35–50.

10. Mountain, W. B., and H. R. Boyce. 1958. The peach replant problem in Ontario. V. The relation of parasitic nematodes to regional differences in severity of peach replant failure. Canadian Journal of Botany 36:125–134.

11. Mountain, W. B., and H. R. Boyce. 1958. The peach replant problem in Ontario. VI. The relation of *Pratylenchus penetrans* to the growth of young peach trees. Canadian Journal of Botany 36:135–151.

12. Mountain, W. B., and Z. A. Patrick. 1959. The peach replant problem in Ontario. VII. The pathogenicity of *Pratylenchus penetrans* (Cobb, 1917) Filip. + Stek. 1941. Canadian Journal of Botany 37:459–470.

13. Proctor, J. R., and C. F. Marks. 1974. The determination of normalizing transformations for nematode count data from soil samples and of efficient sampling schemes. Nematologica 20:395–406.

14. Ryle, G. J. A., R. A. Arnott, and C. E. Powell. 1981. Distribution of dry weight between root and shoot in white clover dependent on $N_2$ fixation or utilizing abundant nitrate nitrogen. Plant and Soil 60: 29–39.

15. Sharpe, R. H. 1974. Breeding peach rootstocks for the southern United States. HortScience 9:362–363.

16. Townshend, J. L. 1963. A modification and evaluation of the apparatus for the Oostenbrink direct cottonwool filter extraction method. Nematologica 9:106–110.