

## Assessment of Guardian Peach Rootstock for Resistance to Two Isolates of *Pratylenchus vulnus*

A. P. NYCZEPIR<sup>1</sup> AND J. PINOCHET<sup>2</sup>

**Abstract:** Guardian, Lovell, and Nemaguard peach rootstocks were evaluated for their susceptibility and growth response to two isolates of *Pratylenchus vulnus*. One nematode isolate was obtained from peach in Georgia (*P. vulnus* [GA-isolate]) and the other from apple in Idaho (*P. vulnus* [ID-isolate]). Nematode reproduction and pathogenicity as related to rootstock were determined 29 months after inoculation in outdoor microplots. All rootstocks were susceptible to both nematode isolates. Guardian supported a greater number of nematodes per gram dry root weight than Lovell or Nemaguard rootstocks. All rootstocks supported greater numbers of *P. vulnus* (GA-isolate) than *P. vulnus* (ID-isolate). Tree growth among the three rootstocks was similar in the presence of either *P. vulnus* isolate, but growth suppression was greatest in *P. vulnus* (GA-isolate) plots, intermediate in *P. vulnus* (ID-isolate) plots, and least in the uninoculated plots.

**Key words:** host-parasitic relationship, nematode, pathogenicity, peach, *Pratylenchus vulnus*, *Prunus persica*, resistance, root-lesion nematode, rootstock.

Root-lesion nematodes (*Pratylenchus* spp.) are important pests of peach (*Prunus persica* (L.) Batsch) in the United States and other parts of the world (Nyczepir and Becker, 1998). At least eight species of root-lesion nematode (*P. penetrans* (Cobb) Chitwood & Oteifa, *P. pratensis* (de Man) Filipjev, *P. brachyurus* (Godfrey) Goodey, *P. zaei* Graham, *P. convallariae* Seinhorst, *P. neglectus* (Rensch) Filipjev & Schuurmans Stekhoven, *P. thornei* Sher & Allen, and *P. vulnus* Allen & Jensen) have been associated with peach throughout the world, but *P. vulnus* is the species of primary concern in California and other warm Mediterranean climates (Marull and Pinochet, 1991; Nyczepir and Becker, 1998). In California, damage by *P. vulnus* to plum on peach and plum rootstocks is estimated to be responsible for approximately 16% reduction in marketable fruit (reduction in fruit yield and size) (McKenry, 1989). In the southeastern United States, limited investigations have been conducted on *P. vulnus*, a migratory endoparasite that is not considered as important on peach as *Mesocriconema xenoplax* (Raski) Loof & de Grisse (= *Criconemella xenoplax* (Raski) Luc & Raski) or *Meloidogyne* spp. However, in Georgia *P. vulnus* was reported to be associated with reduced peach tree vigor and rapid deterioration and reduction of feeder roots (Fliegel, 1969), which are characteristic symptoms reported by others who have worked with this nematode (Marull and Pinochet, 1991; McKenry, 1989). This nematode destroys the root cortical parenchyma cells and may also create avenues for secondary infection by bacteria and fungi (Marull and Pinochet, 1991). Therefore, managing *P. vulnus* is essential for establishment and optimizing yield of an orchard.

Preplant chemical treatment with either 1,3-

dichloropropene (1,3-D) or methyl bromide (bromomethane) is recommended to provide effective control of *Pratylenchus* spp. in the Southeast. These fumigant nematicides lower nematode population density enough to prevent major root damage during the first years following tree establishment, thus allowing the tree to have a healthy start (Nyczepir, 1991; Nyczepir et al., 2000). In recent years nematode management research has focused on alternatives to conventional nematicide applications such as rootstock resistance (Alcaniz et al., 1996; Crossa-Raynaud and Audergon, 1987; Ledbetter, 1994). Emphasis on nonchemical control is partly due to apprehension about the environmental problems associated with soil fumigation with methyl bromide. As a result of its role in ozone depletion, a ban on the importation and manufacture of methyl bromide in the United States is scheduled for 2005 (Clean Air Act, 1990). Therefore, finding an alternative to chemical control of nematodes is warranted.

A potential multipurpose rootstock, Guardian, which provides greater peach-tree-short-life (PTSL) survival than the standard Lovell rootstock, was identified in Georgia and South Carolina trials with unbudded seedlings in 1983. In these trials, tree survival was greater for Guardian than for Lovell at the two different PTSL field sites through 8 years of evaluation (Okie et al., 1994). These results were substantiated in four follow-up budded-tree trials on PTSL sites in South Carolina (Reighard et al., 1997). Recently, Guardian was reported to exhibit resistance to certain populations of *M. incognita* (Kofoid & White) Chitwood and *M. javanica* (Treub) Chitwood (Nyczepir and Beckman, 2000; Nyczepir et al., 1999). Our objectives were to evaluate the susceptibility of Guardian peach rootstock to *P. vulnus* and determine the comparative reproduction and pathogenicity of two isolates of *P. vulnus* under field microplot conditions during the first 2 years of establishment.

### MATERIALS AND METHODS

**Inoculum source and production:** Two *P. vulnus* isolates originating from different geographic locations and

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<sup>1</sup> Research Nematologist, USDA ARS, Southeastern Fruit and Tree Nut Research Laboratory, 21 Dunbar Road, Byron, GA 31008.

<sup>2</sup> Nematologist, Agromillora Catalana, S.A., El Rebato s/n, 08739 T. M. Subirats, Barcelona, Spain.

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E-mail: anyczepir@saa.ars.usda.gov

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hosts were used in this experiment. One *P. vulnus* isolate was originally obtained from peach in Georgia (GA-isolate) and the other from apple (*Malus domestica* Borkh.) in Idaho (ID-isolate). Both isolates were reared monoxenically on carrot (*Daucus carota* L.) disk cultures (Moody et al., 1973) and incubated at 22 °C for several generations. Nematode inoculum was prepared by macerating the nematode-infested carrot disks in water in a commercial blender for 4 seconds at 5-second intervals. The nematode/carrot suspension was then concentrated using a 250- $\mu$ m sieve nested on a 38- $\mu$ m sieve (60 and 400-mesh, respectively). The carrot debris collected on the 250- $\mu$ m sieve was discarded, and the content on the 38- $\mu$ m sieve was placed on a Baermann funnel to obtain the nematode inoculum (Viglierchio and Schmitt, 1983). Nematode inoculum was adjusted to deliver 2,500 individuals per microplot.

*Microplot experiment:* Guardian, Lovell, and Nema-guard peach were evaluated for host susceptibility to *P. vulnus* in field microplots. The susceptible peach cultivar, Nema-guard (McKenry, 1989), was included to verify nematode infectivity. Approximately 4-week-old peach seedlings were planted singly in bucket microplots (25-cm diam.  $\times$  31 cm deep) (Barker, 1985) containing 15,000 cm<sup>3</sup> of steam-pasteurized loamy sand (86% sand, 10% silt, 4% clay; pH 6.1; 0.54% organic matter) soil in October 1996. Microplots were established in a shaded area (30% shade) in the field.

Approximately 15 days after planting, the soil in each microplot was infested with 2,500 *P. vulnus* (GA-isolate) or *P. vulnus* (ID-isolate) adults and juveniles in 80 ml total solution, which was added to two furrows (10 cm long  $\times$  3 cm wide  $\times$  7 cm deep) around each seedling. Ten replications of each peach rootstock cultivar were arranged in a randomized complete block with a split-plot design. Rootstock represented the main plot treatment and nematode the subplot treatment. Plants were watered as needed and fertilized with Osmocote [14-14-14 (N-P-K)]. Trunk diameters were measured 7.5 cm above the soil line on 10 February 1998 and 16 February 1999. Trees were pruned to a height of 25 cm above the soil line on 26 March 1998. Upon bud break following pruning in 1998, only a single bud was allowed to break dormancy and grow throughout the year. On 16 February 1999 fresh shoot weight, as determined on pruned new terminal growth only, was recorded (Nyczepir et al., 1987). Approximately 29 months (26 April 1999) after soil infestation, the study was terminated and fresh root weight and nematode population density in roots and soil were measured. Nematodes were extracted from a 100-cm<sup>3</sup> soil subsample with a semi-automatic elutriator (Byrd et al., 1976) and centrifugal-flotation (Jenkins, 1964). Nematodes in roots were extracted by randomly cutting an 8-gram (fresh weight) portion of the root system and placing it on a fine screen in a Seinhorst mistifier chamber (Hopper, 1970) for 9 days at 23 °C. After extracting the nematodes from

the roots, the dry root weight (dried at 70 °C in aluminum foil until no more weight loss occurred) of each tissue extraction sample was determined. The nematode reproduction factor (Rf = final population density [PF] of all life stages divided by initial population density [Pi]) was calculated as a measure of host susceptibility among the rootstocks. Rootstocks were considered to be a good host (susceptible) if Rf  $\geq$  1.

*Statistical analysis:* Nematode data were subjected to analysis of variance with the general linear models (GLM) procedure of SAS (SAS Institute, Cary, NC). Analysis of variance was also performed to determine treatment effect on trunk diameter, fresh root and shoot weights, and nematodes per gram dry root. Means were compared according to Fisher's protected least significant difference (LSD) test following a significant *F* test.

## RESULTS AND DISCUSSION

Because interaction between rootstock and nematode was not significant for all parameters, only main effect values are reported. Nematode soil population density and the reproduction factors (Rf) in soil and soil plus roots did not differ among the three rootstocks tested, regardless of nematode isolate (Table 1). However, nematode populations per gram dry root were greater ( $P \leq 0.05$ ) on Guardian than on Lovell or Nema-guard, indicating that Guardian is a better host for the nematode isolates tested in the study. No differences in number of nematodes per gram dry root were detected between Lovell and Nema-guard. Greater numbers of *P. vulnus* (GA-isolate) per gram dry root were recovered from all rootstocks than *P. vulnus* (ID-isolate), indicating that peach is a better host for the GA-isolate. Similar results were seen between the nematode isolates for the Rf in the soil plus roots, but not for Rf in the soil alone or the nematode soil population density. It appears that nematode populations in the root expressed on a per-gram-dry-root basis and the Rf in the soil plus root are a better measure of host resistance or susceptibility than soil population density and Rf in soil alone. These findings substantiate those of others working with different *Prunus* genotypes and *P. vulnus* (Culver et al., resistance 1989; Pinochet et al., 2000).

Our results indicate that all rootstocks were hosts (Rf  $\geq$  1) for both isolates but were more susceptible to *P. vulnus* (GA-isolate) than *P. vulnus* (ID-isolate). Two explanations for the differences observed between nematode isolates with regard to host susceptibility could be: (i) the existence of root-lesion nematode races and (or) (ii) geographic origin of the isolates. In our study, the GA-isolate was originally obtained from peach and the ID-isolate from apple. Different races of *P. vulnus* have been reported with different damage potentials (Pinochet et al., 1993), and distinct races among *P.*

TABLE 1. Populations and reproduction of two *Pratylenchus vulnus* isolates on peach rootstocks in microplots 29 months after inoculation in Byron, Georgia.<sup>a</sup>

| Treatment              | <i>P. vulnus</i> per     |            | Rf <sup>b</sup> |            | Trunk diam. (mm) |             | Fresh weight (kg) |         |
|------------------------|--------------------------|------------|-----------------|------------|------------------|-------------|-------------------|---------|
|                        | 100 cm <sup>3</sup> soil | g dry root | soil            | soil+roots | 10 Feb 1998      | 16 Feb 1999 | root              | shoot   |
| Rootstock <sup>c</sup> |                          |            |                 |            |                  |             |                   |         |
| Guardian               | 1,141 a                  | 158 a      | 67.1 a          | 148.8 a    | 6.82 a           | 12.6 a      | 0.100 a           | 0.086 a |
| Lovell                 | 1,165 a                  | 92 b       | 68.5 a          | 137.1 a    | 6.00 a           | 12.0 a      | 0.102 a           | 0.069 a |
| Nemaguard              | 1,209 a                  | 36 b       | 71.1 a          | 109.1a     | 6.60 a           | 12.4 a      | 0.100 a           | 0.075 a |
| Nematode <sup>c</sup>  |                          |            |                 |            |                  |             |                   |         |
| GA-isolate             | 1,187 a                  | 166 a      | 69.8 a          | 172.8 a    | 5.6 b            | 9.4 c       | 0.048 c           | 0.031 c |
| ID-isolate             | 1,157 a                  | 27 b       | 68.1 a          | 91.8 b     | 5.2 b            | 11.7 b      | 0.086 b           | 0.068 b |
| Check                  | — <sup>d</sup>           | —          | —               | —          | 8.6 a            | 15.8 a      | 0.175 a           | 0.130 a |

<sup>a</sup> Data are means of 10 replicates.

<sup>b</sup> Rf = reproductive factor (Pf/Pi), where Pi = initial population density of 17 *P. vulnus* per 100 cm<sup>3</sup> soil.

<sup>c</sup> Means within a main effect and column followed by the same letter are not different ( $P \leq 0.05$ ) according to Fisher's least significant difference.

<sup>d</sup> — = not included.

*vulnus* isolates have been identified such that an apple and rose isolate of *P. vulnus* did not attack citrus, but a walnut isolate did (Pinochet et al., 1994). Furthermore, in California, Nemaguard peach is considered to be susceptible to *P. vulnus*; whereas when Nemaguard was challenged with *P. vulnus* (Spain-isolate) from rose, it was rated a poor host (McKenry, 1989; Pinochet et al., 1992). Soil temperature regimes between the two geographical locations also could explain why the GA-isolate was more pathogenic than the ID-isolate. Maybe the ID-isolate is less pathogenic in warmer Georgia soils than in the cooler Idaho soils.

Differences in tree growth, as measured by trunk diameter and fresh root and shoot weight, did not differ among the three rootstocks but were influenced by nematode treatment (Table 1). Fifteen months (February 1998) after nematode inoculation, trunk diameter was lower ( $P \leq 0.05$ ) with both *P. vulnus* isolates than in the untreated check. No differences were detected between the two *P. vulnus* isolates at this time. At 27 months (February 1999), trunk diameter as well as fresh root and shoot weight was greatest in the untreated, intermediate in the *P. vulnus* (ID-isolate), and lowest in *P. vulnus* (GA-isolate) plots. These data indicate that *P. vulnus* (GA-isolate) is more pathogenic to the peach rootstocks tested than *P. vulnus* (ID-isolate). *Pratylenchus vulnus* is an economic pest to the California and Mediterranean peach industries (Marull and Pinochet, 1991; McKenry, 1989), but its distribution throughout the southeastern United States peach-growing regions is uncertain. *Pratylenchus* spp. have been reported to occur in more than 60% of PTSL and non-short-life orchards surveyed in Georgia and South Carolina, but nematode species were not determined (Nyczepir et al., 1985). *Pratylenchus vulnus* is known to occur in Georgia peach orchards and was reported to be associated with reduced tree vigor (Fliegel, 1969). Our results demonstrate that this nematode reproduces on, and is a pathogen of, peach rootstocks used in Georgia, including Guardian. Guardian also has been

reported to be susceptible to *P. penetrans* in greenhouse tests in Canada (McFadden-Smith et al., 1998), although *P. penetrans* reproduction on Guardian was not as vigorous as on some of the other rootstocks tested.

*Pratylenchus vulnus* should not be ignored as a pest on peach in the Southeast. Proper management practices must be implemented when establishing an orchard on a site known to be infested with this nematode. Incidence of *Pratylenchus* spp. (i.e., *P. vulnus* and other species known to cause damage on *Prunus* worldwide) in the major peach-growing regions of the southeastern United States needs to be investigated further.

#### LITERATURE CITED

- Alcaniz, E., J. Pinochet, C. Fernandez, D. Esmenjaud, and A. Felipe. 1996. Evaluation of *Prunus* rootstocks for root-lesion nematode resistance. *HortScience* 31:1013–1016.
- Barker, K. R. 1985. Design of greenhouse and microplot experiments for evaluation of plant resistance to nematodes. Pp. 107–113 in B. M. Zuckerman, W. F. Mai, and M. B. Harrison, eds. *Plant nematology laboratory manual*. Amherst, MA: University of Massachusetts Agricultural Experiment Station.
- Byrd, D. W., Jr., K. R. Barker, H. Ferris, C. J. Nusbaum, W. E. Griffin, R. H. Small, and C. A. Stone. 1976. Two semi-automatic elutriators for extracting nematodes and certain fungi from soil. *Journal of Nematology* 8:206–212.
- Clean Air Act. 1990. Title VI. Stratospheric Ozone Protection Pub L. 101-549, Section 6001. U.S. Congress, Washington, DC.
- Crossa-Raynaud, P., and J. M. Audergon. 1987. Apricot rootstocks. Pp. 295–320 in R. C. Rom and R. F. Carson, eds. *Rootstocks for fruit crops*. New York, NY: John Wiley & Sons.
- Culver, D. J., D. W. Ramming, and M. V. McKenry. 1989. Procedures for field and greenhouse screening of *Prunus* genotypes for resistance and tolerance to root-lesion nematode. *Journal of the American Society for Horticultural Science* 114:30–35.
- Fliegel, P. 1969. Population dynamics and pathogenicity of three species of *Pratylenchus* on peach. *Phytopathology* 59:120–124.
- Hopper, D. J. 1970. Extraction of nematodes from plant material. Pp. 34–38 in J. F. Southey, ed. *Laboratory methods for work with plant and soil nematodes*. Technical Bulletin 2. London: Her Majesty's Stationery Office.
- Jenkins, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. *Plant Disease Reporter* 48:692.
- Ledbetter, C. L. 1994. Techniques for screening *Prunus* rootstocks for resistance to *Pratylenchus vulnus*. Pp. 34–36 in *Proceedings of the Sixth Stone Fruit Decline Workshop*, 26–28 October 1992, Fort Val-

ley, GA ARS-122. Springfield, VA: U.S. Department of Agriculture, Agricultural Research Service, National Technical Information Service.

Marull, J., and J. Pinochet. 1991. Host suitability of *Prunus* rootstocks to four *Meloidogyne* species and *Pratylenchus vulnus* in Spain. *Nematropica* 21:185–195.

McFadden-Smith, W., N. W. Miles, and J. W. Potter. 1998. Greenhouse evaluation of *Prunus* rootstocks for resistance or tolerance to the root-lesion nematode (*Pratylenchus penetrans*). *Acta Horticulturae* 465:723–729.

McKenry, M. V. 1989. Nematodes. Pp. 139–147 in J. H. LaRue and R. S. Johnson, eds. *Peaches, plums, and nectarines. Growing and handling for fresh market.* Publ. 3331. Oakland, CA: University of California, Division of Agriculture and Natural Resources.

Moody, E. H., B. F. Lownsbery, and J. M. Ahmed. 1973. Culture of the root-lesion nematode *Pratylenchus vulnus* on carrot disks. *Journal of Nematology* 5:225–226.

Nyczepir, A. P. 1991. Nematode management strategies in stone fruits in the United States. *Journal of Nematology* 23:334–341.

Nyczepir, A. P., and J. Ole Becker. 1998. Fruit and citrus trees. Pp. 637–684 in K. R. Barker, G. A. Pederson, and G. L. Windham, eds. *Plant nematode interactions.* Agronomy monograph no. 36. Madison, WI: American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America.

Nyczepir, A. P., and T. G. Beckman. 2000. Host status of Guardian peach rootstock to *Meloidogyne* sp. and *M. javanica*. *HortScience* 35:772.

Nyczepir, A. P., T. G. Beckman, and G. L. Reighard. 1999. Reproduction and development of *Meloidogyne incognita* and *M. javanica* on Guardian peach rootstock. *Journal of Nematology* 31:334–340.

Nyczepir, A. P., P. F. Bertrand, R. W. Miller, and R. E. Motsinger. 1985. Incidence of *Criconebella* spp. and peach orchard histories in short-life and non-short-life sites in Georgia and South Carolina. *Plant Disease* 69:874–877.

Nyczepir, A. P., C. C. Reilly, and W. R. Okie. 1987. Effect of initial

population density of *Criconebella xenoplax* on reducing sugars, free amino acids, and survival of peach seedlings over time. *Journal of Nematology* 19:296–303.

Nyczepir, A. P., D. Ritchie, and R. W. Miller. 2000. Nematode control on peaches. Pp. 27 in D. Horton, C. Gorsuch, and D. Ritchie, eds. 2000 Southern peach, nectarine, and plum pest management and culture guide. Bulletin 1171, University of Georgia Cooperative Extension Service, Athens.

Okie, W. R., G. L. Reighard, T. G. Beckman, A. P. Nyczepir, C. C. Reilly, E. I. Zehr, W. C. Newall, Jr., and D. W. Cain. 1994. Field screening *Prunus* for longevity in the southeastern United States. *HortScience* 29:673–677.

Pinochet, J., L. Cenis, C. Fernandez, M. Douchet, and J. Marull. 1994. Reproductive fitness and random amplified polymorphic DNA variation among isolates of *Pratylenchus vulnus*. *Journal of Nematology* 26:271–277.

Pinochet, J., C. Fernandez, C. Calvet, A. Hernandez-Dorrego, and A. Felipe. 2000. Selection against *Pratylenchus vulnus* populations attacking *Prunus* rootstocks. *HortScience* 35:1333–1337.

Pinochet, J., C. Fernandez, D. Esmenjaud, and M. Douchet. 1993. Effects of six *Pratylenchus vulnus* isolates on the growth of peach-almond hybrid and apple rootstocks. Supplement to the *Journal of Nematology* 25:843–848.

Pinochet, J., S. Verdejo, A. Soler, and J. Canals. 1992. Host range of a population of *Pratylenchus vulnus* in commercial fruit, nut, citrus, and grape rootstocks in Spain. Supplement to the *Journal of Nematology* 24:693–698.

Reighard, G. L., W. C. Newall, Jr., E. I. Zehr, T. G. Beckman, W. R. Okie, and A. P. Nyczepir. 1997. Field performance of *Prunus* rootstock cultivars and selections on replant soils in South Carolina. *Acta Horticulturae* 451:243–249.

Viglierchio, D. R., and R. V. Schmitt. 1983. On the methodology of nematode extraction from field samples: Baermann funnel modifications. *Journal of Nematology* 15:438–444.