Supplement to Journal of Nematology 25(4S):843–848. 1993.

Effects of Six *Pratylenchus vulnus* Isolates on the Growth of Peach–Almond Hybrid and Apple Rootstocks[1]

J. Pinochet,[2] C. Fernández,[2] D. Esmenjaud,[3] and M. Doucet[4]

Abstract: The effects of six geographic isolates of *Pratylenchus vulnus* on the growth of GF-677 peach–almond hybrid and M-26 apple rootstocks were determined under greenhouse conditions. Plantlets were obtained from micropropagated plant material, and nematode isolates were reared in monoxenic cultures. All isolates suppressed growth on GF-677 compared with the uninfected controls. Isolate PvRO-S from Spain affected top weight of GF-677 more adversely than PvAT-F from France. Final population densities (Pf) of all *P. vulnus* on GF-677 were greater than 14.7 times the initial densities (Pi). They increased 61.5-fold on plants infected by PvWA-U from the United States. PvWA-U-, PvAT-F, and geographic undetermined PvU-UK isolates did not affect the growth of M-26 apple rootstock compared with PvAP-S, PvRO-S (both from Spain), and PvWA-A from Argentina isolates, which severely suppressed shoot growth of this rootstock. On M-26, Pf of the more parasitically fit isolates PvWA-A, PvAP-S, and PvRO-S were greater than those of nondamaging PvWA-U, PvAT-F, and PvU-UK isolates (>41.4 vs. <14.7 times the Pi). PvWA-U and PvAT-F reproduced more on GF-677 than on M-26 (>28.6 vs. <6.5 times the Pi). Isolate PvRO-S reproduced well and was quite destructive on both rootstocks. Results confirm the existence of strains with different damage potentials among geographically separated populations of *P. vulnus*.

Key words: host response variability, *Malus silvestris* M-26, nematode reproduction, *Pratylenchus vulnus*, *Prunus persica* × *P. amygdalus* GF-677, root-lesion nematode.

The root-lesion nematode, *Pratylenchus vulnus*, is an important pathogen attacking fruit crops in the Mediterranean area (4,9, 12,22,25), California the southeastern United States (2,15–17,19), and Chile (1).

A host–parasite relationship study, conducted recently in Spain with a *P. vulnus* population isolated from rose (*Rosa multiflora*), showed that this isolate was highly destructive on apple (*Malus silvestris*) rootstock (6). In contrast, another *P. vulnus* population isolated from apple had no effect on growth of this host after 3 years of experimentation. This rose isolate does not reproduce on sour orange (*Citrus aurantium*), nor on six different citrus rootstocks (23), and differs in its host range from a *P. vulnus* population infecting citrus in Italy and the United States (8,11). Discrepancies in host preference among American and European populations on *Prunus* rootstocks have also been reported (13,22,25). This behavior suggests the possible existence within this species of different damaging strains and (or) physiological races, which complicate considerations in nematode management and in breeding programs against this pathogen. The existence of different host races or damaging strains in several species of migratory endoparasitic nematodes has been documented (20,21,24).

The purpose of this study was to compare the reproduction and effects on plant growth of six *P. vulnus* populations from different hosts and geographical origins (three continents) on a clonally propagated peach–almond hybrid and apple host.

Materials and Methods

Micropropagated peach–almond hybrid (*Prunus persica* × *P. amygdalus*) GF-677 and apple M-26 rootstock were obtained from Agromillora Catalana S.A., Sant Sadurní d' Anoia, Barcelona, Spain. In vitro plant-

Received for publication 22 July 1993.
[1] This research was supported by the Spanish Instituto Nacional de Investigaciones Agrarias, INIA, Grant No SC93-132.
[2] Research Nematologist and Graduate Assistant, Departamento de Patología Vegetal, Institut de Recerca i Tecnològia Agroalimentàries, IRTA, Crta. de Cabrils s/n, 08348 Cabrils, Barcelona, Spain.
[3] Research Nematologist, Laboratoire de Biologie des Invertébrés, Institut National de la Recherche Agronomique, INRA, B.P. 2078, 06606 Antibes Cedex, France.
[4] Research Nematologist, Centro de Zoología Aplicada, Universidad Nacional de Córdoba, CC 122, 5000 Córdoba, Argentina.
The authors are grateful to Dr. Saad Hafez for providing a nematode population and to the Dirección General de Investigación Científica y Técnica (DGICYT) of the Spanish Ministry of Education and Science for extending an invitation as Visiting Scientist to the fourth author.

lets were transferred from agar to 50-cm^3 minipots with peat substrate and acclimatized in a high humidity chamber for 24 days. Two-month-old GF-677 plantlets of 5–6 cm and M-26 of 4–5 cm height were then transferred to 3-L PVC pots that contained a pasteurized sandy loam soil (88% sand, 10% silt, 2% clay), pH 7.32, less than 2% organic matter, and a cation exchange capacity of less than 10 meq/100 g soil. Two greenhouse experiments were established in January and February 1993 for GF-677 and M-26 rootstocks, respectively.

Six populations of *P. vulnus* from different hosts and geographical origin were obtained from several sources, reared monoxenically on carrot (*Daucus carota*) disk cultures (18), and kept in an incubator at 23–24 C for several generations (Table 1). Species confirmation was made by the Commonwealth Institute of Parasitology, St. Albans, United Kingdom. In both experiments, seven treatments were established: 1) control (uninoculated), 2) PvRO-S (rose isolate of *P. vulnus*), 3) PvAP-S (apple isolate), 4) PvAT-F (apricot isolate), 5) PvU-UK (unknown host), 6) PvWA-A (walnut isolate), and 7) PvWA-U (walnut isolate). Nematodes were recovered from water added to stock cultures and collected with a pipette. For GF-677, inoculum (Pi) was 500 individuals of each isolate per plant (166 nematodes per kg soil) added through five holes located 4–5 cm from the base of the plant. For M-26, the Pi of each isolate was 100 nematodes per plant (33 nematodes per kg soil) due to the higher susceptibility of apple rootstock to *P. vulnus,* compared with *Prunus* sp. (6,22). Following inoculation, pots were placed in sand beds in a greenhouse to minimize temperature and humidity fluctuations. The ambient temperature range was 22 to 32 C during the growing season. Plants were watered daily or as needed and fertilized with Hoagland's (7) nutrient solution weekly.

At 135 days after inoculation, data on fresh shoot weights, fresh root weights, shoot length, and shoot diameter were taken. Shoot diameter measurements were made at 0.5 cm from the soil. Final nematode populations (Pf) and numbers of nematodes per gram of root were assessed at the end of each experiment. For nematode extraction, soil from each pot was separated from roots and placed in a large pan with water. Roots were washed in a second pan to remove soil particles, and the resulting suspension was added to the pan containing the soil. The soil solution was stirred thoroughly, and nematodes were extracted from a 250-cm^3 subsample of the slurry by differential sieving with 150, 74, and 38-μm-pore sieves (100, 200, and 400 mesh, respectively) and sugar flotation (10). For nematode extraction from roots, root systems were cut into small pieces (ca. 1 cm) and macerated with water in a commercial blender at 14,500 rpm for 30 seconds in three 10-second intervals. Nematodes were then collected with 150, 74, 32, and 25-μm-pore sieves (100, 200, 400, and 500 mesh, respectively). Root tissue and debris collected on the 150-μm-pore sieve were discarded. Nematodes retained on the remaining sieves were removed and counted.

TABLE 1. Geographic and host origin of six isolates of *Pratylenchus vulnus.*

Isolate	Geographic origin	Host (species)	Source†
PvRO-S	Barcelona, Spain	Rose (*Rosa multiflora*)	IRTA
PvAP-S	Gerona, Spain	Apple (*Malus silvestris*)	IRTA
PvAT-F	Antibes, France	Apricot (*Prunus armeniaca*)	INRA
PvU-UK	Unknown	Unknown	CABI
PvWA-A	Córdoba, Argentina	Walnut (*Juglans nigra*)	UNC
PvWA-U	Idaho, USA	Walnut (*Juglans regia*)	UI

† IRTA = Institut de Recerca i Tecnologia Agroalimentàries; INRA = Institut National de la Recherche Agronomique; CABI = Commonwealth Agricultural Bureaux International; UCN = Universidad Nacional de Còrdoba; UI = University of Idaho.

In the experiments with peach–almond hybrid and apple, each treatment (isolates and uninoculated control) was represented by seven and eight replications, respectively. Experiments were arranged in a completely randomized design. Data on plant growth and nematode reproduction were analyzed by a one-way ANOVA. Data were $\log_{10}$ transformed $(x + 1)$ for analysis. When F values were significant, means were compared with Fisher's LSD ($P \leqslant 0.05$).

Results and Discussion

One or more growth parameters of GF-677 rootstock were adversely affected by *P. vulnus* isolates (Table 2). Plants inoculated with PvRO-S isolate showed significantly lower fresh shoot weights in comparison to those infected by PvAT-F. No differences were detected among the remaining inoculated plants for this growth parameter. Also, no differences were noted among inoculated treatments for shoot length, stem diameter, or fresh root weights.

On GF-677, isolate PvWA-U showed the highest population increase, which was greater than that of the PvWA-A and PvU-UK isolates (61.5 vs. 14.3 and 16.5 times the Pi, respectively) (Fig. 1). On this same host, PvWA-U also showed higher numbers of nematodes per gram root than PvWA-A and PvU-UK ($P \leqslant 0.05$).

There were no differences in fresh shoot weights between the controls and plants inoculated with PvWA-U, PvAT-F, or PvU-UK in M-26 apple rootstock. However, shoot growth of M-26 was suppressed by PvAP-S, PvRO-S, and PvWA-A. The South American strain PvWA-A and PvRO-S caused the greatest growth restriction of M-26. A similar situation was observed for shoot length of M-26. Only nematode strain PvWA-A had a significant impact on stem diameter and root growth.

On M-26, Pf of PvRO-S, PvAP-S, and PvWA-A were greater than those of PvWA-U, PvAT-F, and PvU-UK (Fig. 2). The reproduction factor (Pf/Pi) for PvRO-S was 234.5. The Pf of PvWA-U differed from the Pf of PvU-UK, but not PvAT-F.

The present findings support the existence of strains of *P. vulnus* with different parasitic fitness. These differences are likely to be broader than those detected

TABLE 2. Effects of six isolates of *Pratylenchus vulnus* on the growth of GF-677 peach–almond hybrid and M-26 apple rootstock 135 days after inoculation with 500 or 100 nematodes per plant for GF-677 and M-26, respectively.

Treatment (Isolate)	Fresh shoot weights (g)	Shoot length (cm)	Stem diameter (mm)	Fresh root weights (g)
		GF-677 Peach–almond hybrid:		
Control	6.85 a	14.9 a	3.84 a	12.50 a
PvAT-F	4.62 ab	11.2 ab	3.48 ab	7.53 b
PvU-UK	4.25 bc	10.4 ab	3.14 b	5.86 b
PvWA-A	3.73 bc	9.9 ab	3.14 b	5.23 b
PvAP-S	3.66 bc	9.1 ab	3.31 ab	6.19 b
PvWA-U	3.64 bc	8.0 b	3.16 b	5.23 b
PvRO-S	3.14 c	8.1 b	3.14 b	5.46 b
		M-26 apple:		
Control	4.52 a	17.9 a	4.18 a	3.67 ab
PvWA-U	4.32 a	17.0 a	4.24 a	4.60 a
PvAT-F	4.16 a	15.6 ab	4.11 a	3.68 ab
PvU-UK	3.78 ab	13.2 ab	4.06 ab	3.87 ab
PvAP-S	2.97 bc	11.6 bc	3.74 ab	3.28 abc
PvRO-S	2.36 cd	7.7 cd	4.11 a	2.93 bc
PvWA-A	1.85 d	5.0 d	3.47 b	2.37 c

Data are means of seven (GF-677 and eight replications (M-26). Actual data are presented for peach–almond hybrid and M-26 apple, respectively, but data were transformed to $\log_{10}$ $(x + 1)$ for analysis. Means in column for each isolate followed by the same letter do not differ according to Fisher's LSD test ($P \leqslant 0.05$).

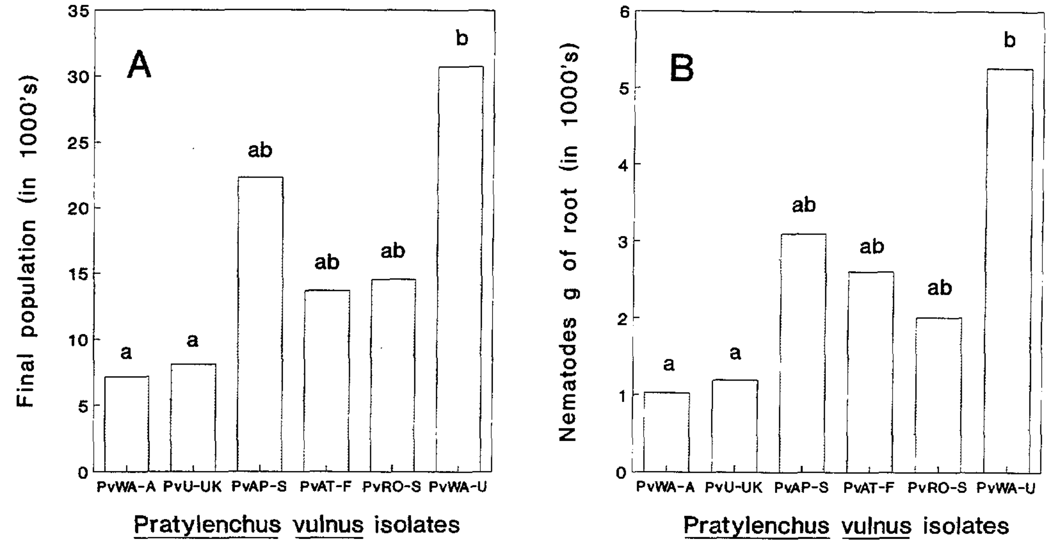

FIG. 1. Increase of six isolates of *Pratylenchus vulnus* on GF-677 peach–almond hybrid rootstock 135 days after inoculation with 500 nematodes (Pi) per plant. A) Combined final nematode population densities (Pf) in roots and soil. B) Nematodes per gram root. Data are means of seven replications. Actual data are presented, but data were transformed to $\log_{10}(x + 1)$ for analysis. Means represented by bars for each isolate with different letters above them are different according to Fisher's LSD test ($P \leq 0.05$).

with the six isolates tested this study. However, the results herein do not allow separation of these isolates because nematode races are mainly based on plant host ranges (27).

All isolates damaged GF-677. Except for PvRO-S, differences in parasitic fitness among *P. vulnus* isolates on GF-677 were not evident (PvRO-S caused higher shoot growth suppression than PvAT-F). Per-

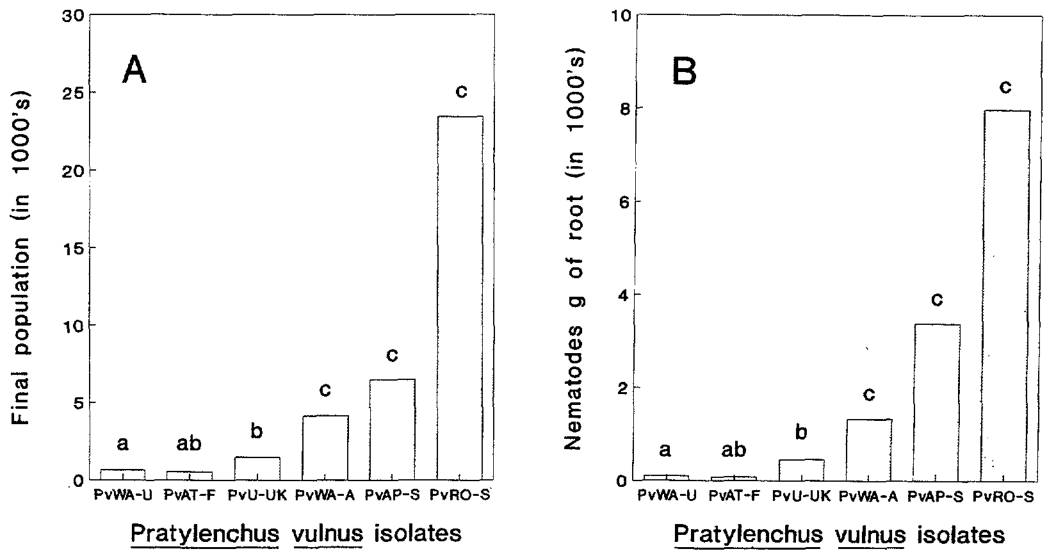

FIG. 2. Increase of six isolates of *Pratylenchus vulnus* on M-26 apple rootstock 135 days after inoculation with 100 nematodes (Pi) per plant. A) Final nematode population (roots and soil). B) Nematodes per gram root. Data are means of eight replications. Actual data are presented, but data were transformed to $\log_{10}(x + 1)$ for analysis. Means represented by bars for each isolate with different letters above them are different according to Fisher's LSD test ($P \leq 0.05$).

haps a longer period of exposure of the nematode to the rootstock would have allowed determination of differences in damage levels among isolates. In contrast, the host response induced by the six *P. vulnus* isolates on M-26 apple rootstock was more variable. The North American (PvWA-U), French (PvAT-F), and geographically undefined (PvU-UK) isolates did not have any adverse effect on M-26, whereas the two Spanish (PvAP-S, PvRO-S) and Argentine (PvWA-A) isolates were injurious. PvWA-A was highly destructive on M-26 apple.

All isolates increased well on GF-677 hybrid with reproduction factors ranging from 14.3 (PvWA-A) to 61.5 (PvWA-U) (data for this parameter not given). On apple, the parasitically fit forms (PvAP-S, PvRO-S, and PvWA-A) reproduced considerably more than the nondamaging isolates (PvWA-U, PvAT-F, and PvU-UK).

Of the three potentially damaging strains, plants with the Argentine isolate (PvWA-A) had lower numbers of nematodes per gram root than plants with PvRO-S. However, the apparently higher reproductive fitness of PvRO-S should be taken with reserve because lower root weights and poorer plant development in plants inoculated with PvWA-A suggest premature destruction of the root system caused by this isolate. Therefore, a clear correlation between the reproduction of PvWA-A and damage was not established.

It is noteworthy that the North American isolate (PvWA-U) reached the highest final nematode population (32,500) in GF-677. However, this same isolate attained the lowest Pf on apple (660). The French isolate (PvAT-F) showed a similar pattern. In contrast, the Spanish isolate, originally isolated from rose (PvRO-S), reproduced fairly well and was quite destructive on both rootstocks.

Resistance to *P. vulnus* is available in a few wild *Prunus* (25). Unfortunately, these wild species are difficult to cross or graft with commercial material. The search for new sources of resistance and its incorporation into commercial rootstocks is currently being vigorously pursued in California (5; Ledbetter, USDA, Fresno, CA, pers. comm.), France (26), and Spain (14,23). The practical implications of this study are important in that it underscores the necessity of knowing as much as possible about the existing diversity and potential variability of the nematode pathogen against which one is trying to breed (3). Thus, plant-breeding material must be tested with several isolates or mixtures of *P. vulnus* isolates to assure broad resistance.

Literature Cited

1. Allen, M. W., E. M. Noffsinger, and A. Valenzuela. 1971. Nematodos en huertos y viñedos de Chile. Agricultura Técnica 31:115–119.

2. Bertrand, P. F. 1989. Peach nematode management in the Southeastern United States. Pp. 751–757 *in* N. F. Childers and W. B. Sherman, eds. The peach. Gainesville, FL: Horticultural Publications.

3. Buddenhaggen, I. 1987. Disease susceptibility and genetics in relation to breeding bananas and plantains. Pp. 95–109 *in* G. J. Persley, and E. A. De Langhe, eds. Banana and plantain breeding strategies. ACIAR Proceedings No 21, Canberra, Australia.

4. Corbett, D. C. M. 1974. *Pratylenchus vulnus*. C. I. H. Descriptions of plant-parasitic nematodes set 3, No. 37. St. Albans, UK: Commonwealth Institute of Helminthology.

5. 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.

6. Fernández, C., J. Pinochet, and R. Dolcet. 1992. Host parasite relationship of *Pratylenchus vulnus* on apple and pear rootstocks. Nematropica 22:227–236.

7. Hoagland, D., and D. I. Arnon. 1950. The water culture method for growing plants without soil. California Agricultural Experimental Station Circular No 347. Berkeley: University of California.

8. Inserra, R. N., and N. Vovlas. 1974. Danni da *Pratylenchus vulnus* su arancio amaro in Puglia. Nematologia Mediterranea 2:183–185.

9. Inserra, R. N., A. Zepp, and N. Vovlas. 1979. I *Pratylenchus* dell'Italia meridionale. Nematologia Mediterranea 7:137–162.

10. Jenkins, W. R. 1964. A rapid centrifugal flotation technique for separating nematodes from soil. Plant Disease Reporter 48:692.

11. Jensen, H. 1953. Experimental greenhouse host range studies of two root-lesion nematodes, *Pratylenchus vulnus* and *Pratylenchus penetrans*. Plant Disease Reporter 37:384–387.

12. Lamberti, F. 1981. Plant nematode problems in the Mediterranean region. Helminthological Abstracts 50B:145–166.

13. Lownsbery, B. F., and E. F. Serr. 1963. Fruit and nut tree rootstocks as hosts for a root-lesion nematode, *Pratylenchus vulnus.* Proceedings of the American Society for Horticultural Science 82:250–254.

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

15. McElroy, F. D. 1972. Nematodes of tree fruits and small fruits. Pp. 335–376 *in* J. M. Webster, ed. Economic nematology. London: Academic Press.

16. McKenry, M. V. 1987. Survey of nematodes associated with almond production in California. Plant Disease 71:71–73.

17. McKenry, J. V. 1988. Nematodes. Pp. 139–147 *in* J. H. La Rue and R. S. Johnson, eds. Peaches, plums and nectarines. Growing and handling for fresh market. Cooperative Extension, University of California. Division of Agriculture and Natural Resources. Publication 3331.

18. 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 19:125–134.

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

20. Olthof, T. H. A. 1968. Races of *Pratylenchus penetrans* and their effect on black root rot resistance of tobacco. Nematologica 14:482–488.

21. Pinochet, J. 1979. Comparison of four isolates of *Radopholus similis* from Central America on Valery bananas. Nematropica 9:40–43.

22. Pinochet, J., S. Verdejo, and J. Marull, J. 1991. Host suitability of eight *Prunus* spp. and one *Pyrus communis* rootstocks to *Pratylenchus vulnus, P. neglectus* and *P. thornei.* Supplement to the Journal of Nematology 23:570–575.

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

24. Sarah, J. L., C. Sabatini, and M. Boisseau. 1993. Differences in pathogenicity to banana (*Musa* sp., cv. Poyo) among isolates of *Radopholus similis* from different production areas of the world. Nematropica 23:75–79.

25. Scotto La Massese, C. 1975. Tests d'hôtes de quelques porte-greffe et variétés fruitières à l'égard de *Pratylenchus vulnus* Allen et Jensen. Comptes Rendus del'Academie d'Agriculture de France 61:1088–1095.

26. Scotto La Massese, C. 1989. Les problèmes posés par les nématodes phytophages à l'amandier. Pp. 33–38 *in* A. J. Felipe and R. Socías, eds. Options méditerranéennes. Séminaire du GREMPA sur les porte-greffe de l'amandier, CIHEAM, Zaragoza, España.

27. Triantaphyllou, A. C. 1987. Genetics of nematode parasitism on plants. Pp. 354–363 *in* J. A. Veech and D. W. Dickson, eds. Vistas in nematology. Society of Nematologists.