Evaluation of *Prunus* Rootstocks for Root-lesion Nematode Resistance

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Abstract. Fourteen *Prunus* rootstocks were evaluated against mixtures of several isolates of the root-lesion nematode *Pratylenchus vulnus* Allen and Jensen in three greenhouse experiments. Most of the tested rootstocks are new releases or materials in advanced stages of selection that also have incorporated root-knot nematode resistance. The plums Torinel (*Prunus domestica* L.) and Redglow (*P. salicina* Lindl. x *P. munsoniana* Wight and Hedrick cv. Jewel) showed a moderately resistant response; their final nematode population levels were lower or slightly higher than inoculation levels. Low nematode reproduction also was found in the peach–almond hybrid G x N No 22 [*P. persica* (L.) Batsch x *P. dulcis* (Mill.) D.A. Webb] and the plum Bruce (*P. salicina* x *P. angustifolia* Marsh.), and although these rootstocks did not perform as well as Torinel and Redglow, they also appear to be poor hosts for *P. vulnus*.

The root-lesion nematode *Pratylenchus vulnus* is an important pest attacking stone and pome fruit crops in the United States and southern Europe (Fernández et al., 1992; Lamberti, 1981; McElroy, 1972; Scotto la Massese, 1989). In Spain, *Pratylenchus vulnus* has been found in nurseries and commercial orchards and has been pathogenic on several *Prunus* rootstocks (Pinochet et al., 1991, 1993a). This migratory endoparasitic nematode causes the destruction of the root system, which results in loss of vigor and yield in young and mature trees. The economic importance of lesion nematodes in the development of orchard replant problems has been recognized for the last 40 years (Bertrand, 1989; Nyczepir and Halbrecht, 1993). Nematicides and tolerant rootstocks have been the main forms of nematode management (McKenry, 1988). In contrast, breeding for resistance has been one of the most neglected approaches toward achieving effective control of *P. vulnus* in stone fruit rootstocks adapted to warm Mediterranean environments.

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Most of the effort in incorporating host resistance to lesion nematodes in *Prunus* has been against *Pratylenchus penetrans* (Cobb) Filipjev and Schuurmans Stekhoven, a root-lesion nematode adapted to temperate environments. Resistance and tolerance for *P. penetrans* has been found in seedlings of the peach rootstock cultivars Rubira, Pisa, Rutgers Red Leaf, Tzim Pee Tao, and in hybrids of Rutgers Red Leaf x Tzim Pee Tao (Layne, 1987; Potter et al., 1984). In contrast, the search for resistance to *P. vulnus* has been less successful, even though potential sources have been detected in Bokhara and Shalil peach seedlings (Okie, 1987) and in a few wild plums, apricots, and interspecific hybrids (Ledbetter, 1994; Scotto La Massese, 1975).

A significant effort in incorporating host-plant resistance is being pursued against this pest in the United States (Ledbetter and Shonnard, 1991), France (Cossa-Raynaud and Audergon, 1987; Stalin et al., 1994), and in Spain (Marull and Pinochet, 1991; Pinochet et al., 1995). In general, resistance has been difficult to find (Ledbetter, 1994) and, even more, to transmit from wild *Prunus* into commercial rootstocks. Recent findings also indicate the existence of differences in pathogenicity among populations of *P. vulnus* (Pinochet, 1993b, 1994), a consideration that further complicates the evaluation of plant material in the search for resistance and tolerance.

The purpose of this study was to evaluate the host suitability of 14 *Prunus* rootstocks, some of which (peach and plums mainly) have been resistant (low reproduction) or suspected to be tolerant (nematode reproduces well but plant growth minimally affected) to this nema-

tode in the United States, France, Italy, and Spain, to mixtures of several isolates of *P. vulnus*. Nearly all the tested rootstocks have incorporated root-knot nematode resistance (Fernández et al., 1994); some are new cultivar releases or materials in advanced stages of selection.

Materials and Methods

Inoculum source and preparation. A total of seven *P. vulnus* isolates originating from diverse geographic locations and hosts were obtained from several sources (Table 1). The isolates were reared monoxenically on carrot (*Daucus carota* L.) disk cultures (Moody et al., 1973) and incubated at 21 °C for several generations. Species identification was made by the Commonwealth Institute of Parasitology, St. Albans, U. K., or confirmed by PCR-RAPD analysis (Pinochet et al., 1994). For inoculation procedures, nematodes from each isolate were recovered from cultures by adding water and collecting the nematode suspension with a pipette. In each experiment, inocula (mixtures of isolates) were adjusted to deliver 600 or 1100 individuals per plant through four holes located at 3 to 4 cm distance from the base of the plant. Most of the isolates used in this study have proven to be pathogenic on *Prunus*, *Malus*, *Pyrus*, and *Cydonia* rootstocks.

Plant material. Seeds, herbaceous and hardwood cuttings, and micropropagated material were supplied from sources in Spain, France, and the United States (Table 2). Seeds of the peach rootstock Rutgers Red Leaf were treated with a 5% solution of copperoxychloride for 24 h, rinsed with running water, covered with moist paper towels, stratified in perlite trays (Europerlite, Dicalite Española S.A., Barcelona, Spain), and kept in a chamber at 4 °C for 45 days. The seeds were then moved to an ambient-temperature greenhouse to induce germination. Buds of Bruce and Redglow were imported to Spain from the IR2/NRSP5 Fruit Tree Collection, Washington State Univ., Prosser. These were grafted on myrobalan plum for wood production (cuttings) the following year. This material, together with herbaceous and hardwood cuttings of the peach Cadaman, peach–almond hybrids G x N No 22 and GF-557, and the plums Montizo and Monpol, was obtained from the Departamento de Fruticultura del Servicio de Investigación Agraria de la Diputación General de Aragón, Zaragoza, Spain. Cuttings were treated for 10 s with a 50% alcohol solution that contained indolebutyric acid at 2000 mg·L⁻¹. Cuttings then were planted in small pots (200 cm³) containing a 3 sand : 1 peat (v/v) mixture previously pasteurized with steam at 80 °C. Micropropagated peach Barrier and plums AD-101, GF-31, Torinel, and Julior were received as plantlets from Agromillora Catalana S.A., Sant Sadurní d'Anoia, Barcelona, Spain. These were transferred from agar to 50-mL minipots with peat substrate and climatized in a high-humidity chamber for 24 days. The micropropagated plum P 2980 was supplied bare root from the Fruit Tree Crop Station of Institut

Table 1. Geographic and host origin of seven isolates of *Pratylenchus vulnus* used in three experiments.

Isolate	Geographic origin	Host	Source ^z	Expt. ^y
PvRO-S	Barcelona, Spain	Rose (<i>Rosa multiflora</i> Thunb.)	IRTA	1, 2, 3
PvAP-S	Gerona, Spain	Apple (<i>Pyrus malus</i> L.)	IRTA	1, 2
PvPL-S	Alicante, Spain	Plum (<i>Prunus domestica</i> L.)	IRTA	3
PvAT-F	Antibes, France	Apricot (<i>Prunus armeniaca</i> L.)	INRA	1, 2
PvWA-A	Córdoba, Argentina	Walnut (<i>Juglans nigra</i> L.)	UNC	1, 2, 3
PvAP-U	Idaho, U.S.A.	Apple (<i>Pyrus malus</i>)	UI	3
PvOL-I	Taranto, Italy	Olive (<i>Olea europea</i> L.)	INA	1, 2

^zIRTA = Institut de Recerca i Tecnologia Agroalimentàries; INRA = Institut National de la Recherche Agronomique; UNC = Universidad Nacional de Córdoba; UI = Univ. of Idaho; INA = Instituto di Nematologia Agraria.

^yUse of isolates in screening Expts. 1, 2, and 3.

Table 2. Source of *Prunus* rootstocks and selections evaluated against *Pratylenchus vulnus*.

Rootstock	Species/selection	Origin ^z	Expt. ^y
Barrier	Peach [<i>P. persica</i> (L.) Batsch x <i>P. davidiana</i> (Carr.) Franch.]	CNR, Italy	1, 3
Cadaman	Peach (<i>P. persica</i> x <i>P. davidiana</i>)	INRA, France	2
GF-557	Peach [<i>P. persica</i> x <i>P. dulcis</i> (Mill.) D.A. Webb]	INRA, France	2
Rutgers Red Leaf	Peach (<i>P. persica</i>)	Rutgers Univ., U.S.A.	2, 3
G x N No 22	Peach-almond (<i>P. dulcis</i> x <i>P. persica</i>)	SIA, Spain	2, 3
AD-101	Plum (<i>P. insititia</i> L.)	CSIC, Spain	1
Bruce	Plum (<i>P. salicina</i> Lindl. x <i>P. angustifolia</i> Marsh)	Texas A&M Univ., U.S.A. ^z	2, 3
GF-31	Plum (<i>P. cerasifera</i> Ehrh. x <i>P. salicina</i>)	INRA, France	1
Montizo	Plum (<i>P. insititia</i>)	SIA, Spain	2
Monpol	Plum (<i>P. insititia</i>)	SIA, Spain	1, 3
P 2980	Plum (<i>P. cerasifera</i>)	INRA, France	2
Torinel	Plum (<i>P. domestica</i>)	INRA, France	1, 3
Redglow	Plum (<i>P. salicina</i> x <i>P. munsoniana</i> Wight and Hedrick)	Univ. of Minnesota, U.S.A. ^z	3
Julior	Plum (<i>P. insititia</i> x <i>P. domestica</i>)	INRA, France	3

^zSIA = Servicio de Investigación Agraria; CNR = Consejo Nacional de Recerca; INRA = Institut National de la Recherche Agronomique; CSIC = Consejo Superior de Investigaciones Científicas.

^yEvaluation of plant material in screening Expts. 1, 2, and 3.

^zPlant material was obtained from IR-2, Prosser, Wash.

National de la Recherche Agronomique, Bordeaux, France.

Greenhouse experiments. Two screening experiments lasting 120 days and one screening experiment lasting 110 days were established; the first two were performed in 1994 and the third in 1995. In all the experiments, plant material arising from germinated seeds, rooted cuttings, and micropropagated rootstocks were transplanted to 3-L PVC pots that contained a pasteurized sandy loam soil (78% sand, 15% silt, and 7% clay), pH 7.4, <3% organic matter content with a cation exchange capacity (CEC) <12 meq/100 g of soil.

Plants with uniform growth, ≈20 cm tall (10 to 15 leaves), were inoculated with a suspension of 1100 nematodes per plant at 6 weeks after transplanting. To assure a desired pathogenic diversity for testing plant material in both greenhouse screening experiments (Pinochet et al., 1993b), inoculum consisted of a mixture of five *P. vulnus* isolates that included 220 nematodes from each isolate. The *P. vulnus* isolates used in both screening trials were Pv RO-S, Pv AP-S, Pv AT-F, Pv OL-I, and Pv WA-A (Table 1). Inoculated plants were placed in a sand bed to avoid temperature and humidity fluctuations. Each rootstock was replicated eight and 10 times in completely randomized designs in the first and second screening experiment, respectively. Plants were watered as needed and fertilized weekly with

half-strength Hoagland's (Hoagland and Arnon, 1950) nutrient solution. The rootstocks AD-101 and Montizo were used as susceptible control rootstocks in the first and second experiments, respectively. Ambient air in the greenhouse fluctuated between 14 and 35 °C.

Final nematode population in roots and soil and numbers of nematodes per gram of root were assessed at the end of each experiment. 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 and stirred thoroughly. Nematodes in soil were extracted from a 250-cm³ subsample of the slurry by differential sieving using 150-, 74-, and 38-μm pore sieves (100, 200, and 400 mesh, respectively) and sugar flotation (Jenkins, 1964). Nematodes in roots were extracted by cutting the whole root system into 1-cm-long pieces and macerating it with water in a commercial blender at 14,500 g_n for 30 s at 10-s intervals. The nematode suspension was then concentrated using 150-, 25-, and 20-μm sieves (100, 500, and 600 mesh, respectively). Root tissue and debris collected on the 150-μm sieve were discarded. Nematodes recovered were then counted under a light microscope.

The third experiment was established to confirm results with rootstocks that had shown a resistant response (low nematode reproduc-

tion) in the first and second experiments, as well as with plant material found to be resistant to *P. vulnus* in the United States and Europe. These evaluated rootstocks were Bruce, Julior, Redglow, and Torinel plums; Rutgers Red Leaf and Barrier peaches; and the peach-almond hybrid G x N No 22. Monpol plum was used as a susceptible control rootstock. Inoculum consisted of a mixture of four *P. vulnus* isolates (Pv RO-S, Pv PL-S, Pv WA-A, and Pv AP-U) that included 150 nematodes from each isolate (600 nematodes per plant). Each rootstock was replicated six times in a completely randomized design.

Data on nematode reproduction were analyzed by a one-way analysis of variance. Data were log₁₀ transformed (x + 1) for analyses. When F values were significant, differences between means were evaluated using Fisher's LSD test ($P \leq 0.05$). Initial population : final population ratios (Pf : Pi) were calculated for each experiment (nematode reproduction rate).

Results

In the first greenhouse experiment, Barrier, Torinel, and GF-31 had similar final nematode populations (Table 3). The nematode count per gram of root in Barrier was significantly lower than that of the other four rootstocks (Table 3). *Pratylenchus vulnus* multiplication in Monpol was highest, reaching 10,215 nematodes per plant (roots and soil). Torinel, GF-31, and the susceptible AD-101 had similar nematode populations, whereas Monpol had the highest nematode density in the roots. Pf : Pi values for Barrier, Torinel, and GF-31 were low compared to values for AD-101 and Monpol.

In the second greenhouse experiment, the final nematode population (roots and soil) was significantly lower in Rutgers Red Leaf, G x N No 22, Bruce, and P 2980 than in Cadaman (Table 4). Rutgers Red Leaf and G x N No 22 differed from Cadaman and Montizo in having fewer nematodes per gram of roots.

The final nematode population for Torinel was significantly lower than for the other rootstocks in Expt. 3 (Table 5). Redglow, G x N No 22, and Bruce had significantly lower nematode populations than Rutgers Red Leaf, Monpol, Barrier, and Julior. Torinel had the lowest amount of nematodes per gram of root. There were no differences between Redglow, G x N No 22, and Bruce for this characteristic. Barrier differed from Rutgers Red Leaf and Julior but not from Monpol. Rutgers Red Leaf and Julior contained the highest numbers of nematodes per gram of root. Pf : Pi values were lowest for Torinel followed by Redglow, G x N No 22, and Bruce, fluctuating between 0.36 and 1.63. The remaining rootstocks achieved high Pf : Pi values (10.4 to 31.2).

Discussion

Our findings show that GF-557 is a suitable host and likely susceptible. Rutgers Red Leaf peach is susceptible to root-lesion nematode (*P. penetrans*) infection (Potter et al., 1984). Rutgers Red Leaf, together with Bar-

Table 3. Reproduction of *Pratylenchus vulnus* on five *Prunus* rootstocks 120 days after inoculation with 1100 nematodes per plant in the first greenhouse experiment.

Rootstock	Final nematode population ² (roots and soil)	Nematodes/ g of root	Pf : Pi ³
Barrier	2,166 a ⁴	37 a	1.4
Torinel	2,285 ab	144 b	2.1
GF-31	2,492 ab	102 b	2.3
AD-101	4,441 b	382 b	4.0
Monpol	10,215 c	1,975 c	9.3

²*Pratylenchus vulnus* isolates used in this experiment: Pv RO-S, Pv AP-S, Pv AT-F, Pv WA-A, and Pv OL-I.

³Pf : Pi: final *P. vulnus* population : initial *P. vulnus* population (reproduction rate).

⁴Data are means of 10 replications. Actual data are presented, but data were transformed to $\log_{10}(x + 1)$ for analysis. Means in the same columns followed by the same letter do not differ significantly according to Fisher's LSD test ($P \leq 0.05$).

Table 4. Reproduction of *Pratylenchus vulnus* on seven *Prunus* rootstocks 120 days after inoculation with 1100 nematodes per plant in the second greenhouse experiment.

Rootstock	Final nematode population ² (roots and soil)	Nematodes/ g of root	Pf : Pi ³
Rutgers Red Leaf	1,707 a ⁴	176 a	1.6
G x N No 22	3,411 a	326 a	3.1
Bruce	3,530 a	521 ab	3.2
P-2980	3,915 a	746 ab	3.6
GF-557	6,130 ab	881 ab	5.6
Montizo	10,484 ab	3,158 b	9.5
Cadaman	18,105 b	3,349 b	16.5

²*Pratylenchus vulnus* isolates used in this experiment: Pv RO-S, Pv AP-S, Pv AT-F, Pv WA-A, and Pv OL-I.

³Pf : Pi: final *P. vulnus* population : initial *P. vulnus* population (reproduction rate).

⁴Data are means of eight replications. Actual data are presented, but data were transformed to $\log_{10}(x + 1)$ for analysis. Means in the same columns followed by the same letter do not differ significantly according to Fisher's LSD test ($P \leq 0.05$).

Table 5. Reproduction of *Pratylenchus vulnus* on eight *Prunus* rootstocks 110 days after inoculation with 600 nematodes per plant in the third greenhouse experiment.

Rootstock	Final nematode population ² (roots and soil)	Nematodes/ g of root	Pf : Pi ³
Torinel	220 a ⁴	18 a	0.36
Red Glow	580 b	70 b	0.93
G x N No 22	750 b	85 bc	1.25
Bruce	980 b	110 bc	1.63
Rutgers Red Leaf	6,290 c	1,010 ef	10.48
Monpol	6,640 c	430 de	11.06
Barrier	7,110 c	480 d	11.85
Julior	18,750 c	1,630 f	31.25

²*Pratylenchus vulnus* isolates used in this experiment: Pv RO-S, Pv PL-S, Pv Wa-A, and Pv AP-U.

³Pf : Pi: final *P. vulnus* population : initial *P. vulnus* population (reproduction rate).

⁴Data are means of six replications. Actual data are presented, but data were transformed to $\log_{10}(x + 1)$ for analysis. Means in the same columns followed by the same letter do not differ significantly according to Fisher's LSD test ($P \leq 0.05$).

rier, presented an inconsistent response to *P. vulnus*. In the 1994 evaluation trials, both rootstocks achieved a low nematode infection that could be considered as a moderately resistant response. But when tested in the third experiment in 1995, they had a high nematode reproduction, similar to that obtained by the susceptible Monpol plum. However, Torinel, Bruce, and G x N No 22, which had a low nematode reproduction level for *P. vulnus* (Pf : Pi values of 2.1, 3.2, and 3.1, respectively) in the screening tests, performed better in the third experiment. Inconsistencies in the evaluation of the same plant material under similar experimental conditions points toward the presence of several nematode isolates in mixtures of the same pathogen. In the third experiment, we used only two of the isolates we had used

in the first two screening trials. Host and pathogenic differences among *P. vulnus* populations from different geographic regions have been described (Jensen, 1953; Pinochet et al., 1992, 1993b; Scotto la Massese, 1975) and would seem to complicate any effort in *Prunus* breeding and selection against this nematode. It is important to use several populations to assure broad resistance.

As difficult as it is to accurately measure plant host resistance against *P. vulnus*, Torinel and Redglow seem to be the only materials tested in this investigation that showed a moderately resistant response. These rootstocks are poor hosts to the nematode as final population levels were lower or slightly higher than inoculation levels. However, Redglow was not tested in the screening trials in 1994 be-

cause this rootstock was very difficult to propagate, and nearly all plant material died.

Although G x N No 22 and Bruce did not perform as well as Torinel and Redglow, they also appear to be poor hosts for *P. vulnus*. These four rootstocks represent the most promising plant materials tested so far in Spain where a total of 52 *Prunus* rootstocks have been evaluated unsuccessfully for resistance and tolerance to *P. vulnus* in the past 5 years (Marull et al., 1990; Marull and Pinochet, 1991; Pinochet et al., 1992, 1993a). GF-31, which performed well in one of the screening trials, was not tested in the third experiment. This rootstock, which is used for apricot cultivars, is tolerant to *P. vulnus* (Crossa-Raynaud and Audergon, 1987).

We conclude that all the evaluated rootstocks show varying degrees of host suitability to the root-lesion nematode. Of these, Torinel, Redglow, G x N No 22, and Bruce have low nematode reproduction. Immunity or a high level of resistance against *P. vulnus* has not been detected. Repeated evaluation is required to verify the results obtained under greenhouse conditions. Because of the good adaptation of Torinel and G x N No 22 to Mediterranean conditions and root-knot nematode resistance (*Meloidogyne* spp.) (Fernández et al., 1994), both rootstocks should be tested under field conditions in *P. vulnus*-infested soil to determine if the low nematode reproduction we observed is correlated with good vegetative growth and productivity traits in comparison to susceptible rootstocks. Bruce and Redglow would seem to be more valuable in plant breeding, especially in crossings with plum rootstocks that are highly susceptible to *P. vulnus*. The search for sources of resistance in commercial *Prunus*, or the detection of the trait in wild *Prunus* species for its incorporation into commercial genotypes, is a priority for rootstocks adapted to Mediterranean environments.

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