Selection Against *Pratylenchus vulnus* Populations Attacking *Prunus* Rootstocks

Jorge Pinochet¹ and Carolina Fernández¹

Agromillora Catalana, S.A. El Rebato s/n, 08739 T.M. Subirats, Barcelona, Spain

Cinta Calvet² and Adriana Hernández-Dorrego³

Departamento de Protección Vegetal, Institut de Recerca i Tecnologia Agroalimentàries, Ctra. de Cabrils s/n 08348, Cabrils, Barcelona, Spain

Antonio Felipe⁴

Departamento de Fruticultura, Servicio de Investigación Agraria, Apartado 727, 50080 Zaragoza, Spain

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Abstract. Twenty-nine commercial and experimental *Prunus* rootstocks, most with incorporated root-knot nematode [*Meloidogyne javanica* (Traub.) Chitwood] resistance, were evaluated against mixtures comprising nine populations of the root-lesion nematode *Pratylenchus vulnus* Allen and Jensen. Nearly all tested materials were susceptible. Five cultivars with high resistant levels were further challenged with seven *P. vulnus* populations individually. ‘Redglow’ (*Prunus salicina* Lindl. x *P. munsoniana* Wight and Hedrick) was the only rootstock that showed broad resistance to all populations. The rootstocks ‘Torinel’ (*P. domestica* L.), AC-595 (*P. domestica* x *P. insititia* L.), ‘Marianna 4001’ (*P. cerasifera* Ehr. x *P. munsoniana*), and ‘Felinem’ [*P. dulcis* (Mill.) D. A. Webb x *P. persica* (L.) Batsch] showed resistance to one or a few *P. vulnus* populations. Several supposedly resistant sources proved to be susceptible. Tests of crosses made between parents of diverse genetic background with partial resistance to *P. vulnus* indicate that a descendant with potential *P. vulnus* resistance is difficult to obtain. Pathogenic diversity among *P. vulnus* populations appears to be high.

The root-lesion nematode *Pratylenchus vulnus* is regarded as economically important in stone fruit crops in warm Mediterranean environments. This species is widespread in Southern Europe (Lamberti, 1981; Scotto La Massese, 1989), California (McKenry, 1988), and the southeastern United States (Nyczepir and Becker, 1998) where it can play an important role in replant problems (Bertrand, 1989; García de Otazo, 1992; McElroy, 1972; Ricciardi et al., 1975). The extent of growth reduction caused by *P. vulnus* has been documented for almond [*P. dulcis* (Mill.) D. A. Webb], cherry (*P. avium* L.), peach [*P. persica* (L.) Batsch], and plum (*P. domestica* L.)

(Fliegel, 1969; McKenry, 1989; Pinochet et al., 1993a, 1996a). The nematode destroys the cortical parenchyma of the root, causing extensive lesions and a reduction of feeder roots, and predisposing tissue to secondary infections caused by fungi and bacteria (Marull and Pinochet, 1991). The symptoms are nonspecific and can be easily overlooked or mistaken for other soil-borne pathogens or abiotic stress factors (Nyczepir and Halbrendt, 1993). Damage can seriously affect early stages of plant development in the nursery or when rootstocks are transplanted into the field.

Most of the effort in incorporating host resistance to root-lesion nematodes in *Prunus* has been against *Pratylenchus penetrans* (Cobb) Filipjev and Schuurmans Stekhoven, a root-lesion nematode adapted to temperate environments. Resistance to and tolerance for *P. penetrans* have been found in seedlings of the peach rootstock cvs. ‘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; Ramm and Cociu, 1990). 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 plum and apricot species, and interspecific hybrids

(Alcañiz et al., 1996; Ledbetter, 1994; Scotto La Massese, 1975).

A significant effort in incorporating host plant resistance against this pest is currently being pursued in the United States (Ledbetter and Shonnard, 1991), France (Crossa-Raynaud and Audergon, 1987; Stalin et al., 1998), and in Spain (Alcañiz et al., 1996; Marull and Pinochet, 1991; Pinochet, 1997). Resistance has been difficult to find (Ledbetter, 1994) and to transmit from wild *Prunus* into commercial rootstocks. Recent findings also indicate the existence of differences in pathogenicity among populations of *P. vulnus* (Pinochet et al., 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 susceptibility of 29 *Prunus* rootstocks (mainly peach and plum), some of which have been reported to be resistant (low reproduction) or suspected to be tolerant (nematode reproduces well but plant growth is minimally affected) to this nematode in the United States, France, Italy, and Spain to mixtures of several isolates of *P. vulnus*. A second objective was to determine the comparative reproduction of seven *P. vulnus* populations on five supposedly resistant *Prunus* rootstocks. Nearly all the tested rootstocks have incorporated root-knot nematode resistance (Esmenjaud et al., 1997; Fernández et al., 1994; Pinochet et al., 1999); many are new cultivar releases or materials in advanced stages of selection.

Materials and Methods

Plant material. Seeds, herbaceous and hardwood cuttings, and micropropagated material were supplied by public research institutes and private sources in Spain, France, and the United States (Table 1). Seeds of the peach rootstocks ‘Rutgers Red Leaf’, ‘Montclar’, and ‘Flordaguard’ were treated with a 5% solution of copper oxychloride for 24 h, rinsed with running water, covered with moist paper towel, stratified in perlite trays (Europerlite®; Dicalite Española, S.A., Barcelona, Spain), and kept in a cool chamber at 4 °C for 45 d. The seeds were then moved to an ambient temperature greenhouse (16 to 30 °C) to induce germination.

The plums ‘Bruce’ (*P. salicina* x *P. angustifolia* Marsh) and ‘Orotava’ (*P. salicina*) were propagated from herbaceous cuttings. ‘Bruce’, ‘Orotava’, and ‘Montizo’ (*P. insititia*) plum were obtained from the Departamento de Fruticultura del Servicio de Investigación Agraria de la Diputación General de Aragón, Zaragoza. ‘Adarcias’ (*P. dulcis* x *P. persica*) was supplied by the Estación Experimental Aula Dei, Consejo Superior de Investigaciones Científicas, Zaragoza. Hardwood cuttings of ‘Montizo’ and ‘Adarcias’ were treated for 10 s with a 50% alcohol solution that contained 2000 mg·L⁻¹ of indole butyric acid. Cuttings then were planted in small pots (200 cm³) containing a pasteurized 3 sand : 1 peat mixture (v:v). Peach ‘Cadaman’ *P. persica* x *P. davidiana* (Carr.) Franch., plum ‘Adara’ (*P. cerasifera*), ‘Torinel’, ‘Julior’ (*P. insititia* x *P.*

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¹Nematologist. E-mail address: agropinochet@ediho.es.

²Plant Pathologist.

³Graduate Assistant.

⁴Plant Breeder.

Table 1. *Prunus* rootstocks and selections evaluated against *Pratylenchus vulnus* in Spain.

Species	Rootstock/selection	Source ²
	<i>Plum</i>	
<i>P. salicina</i> × <i>P. angustifolia</i>	Bruce	Texas A&M
<i>P. besseyi</i> × <i>P. salicina</i>	Deep Purple	WSU
<i>P. salicina</i> × <i>P. munsoniana</i>	Redglow	Univ. of Minnesota
<i>P. salicina</i>	Orotava	Unknown, Canary Is., Spain
	Laroda F1OP	ISF Rome, Italy
<i>P. insititia</i>	Montizo	SIA-DGA, Spain
	AC-952	AC, Spain
<i>P. domestica</i>	Torinel	INRA, France
	Tetra	ISF Rome, Italy
	Penta	ISF Rome, Italy
<i>P. insititia</i> × <i>P. domestica</i>	Julior	INRA, France
	AC-959	AC, Spain
(<i>P. cerasifera</i> × <i>P. salicina</i>) × (<i>P. cerasifera</i> × <i>P. persica</i>)	Ishtara	INRA, France
<i>P. cerasifera</i> × <i>P. munsoniana</i>	Marianna 4001	Univ. of California
<i>P. cerasifera</i>	Myrocal	INRA, France
	Adara	CSIC, Spain
	<i>Peach-plum</i>	
<i>P. cerasifera</i> × (<i>P. dulcis</i> × <i>P. persica</i>)	AC-960	AC, Spain
	<i>Peach</i>	
<i>P. persica</i>	Montclar	INRA, France
	Rutgers Red Leaf	Rutgers Univ.
<i>P. persica</i> × <i>P. davidiana</i>	Cadaman	INRA, France
<i>P. persica</i>	Flordaguard	Univ. of Florida.
	<i>Peach-almond</i>	
<i>P. dulcis</i> × <i>P. persica</i>	Felinem	SIA-DGA, Spain
	AC-9000	AC, Spain
	AC-9501	AC, Spain
	AC-9502	AC, Spain
	Adarcias	CSIC, Spain
	Mayor	CIDA, Spain
<i>P. persica</i> × <i>P. dulcis</i>	Sirio	Univ. of Pisa, Italy
	<i>Cherry</i>	
<i>P. cerasus</i>	CAB-6P	Univ. of Bologna, Italy

²Texas A&M Univ., College Station; WSU = Washington State Univ., Prosser; Univ. of Minnesota, St. Paul; ISF = Istituto Sperimentale per la Fruticoltura di Roma; SIA-DGA = Servicio de Investigación Agraria de la Diputación General de Aragón, Zaragoza; AC = Agromillora Catalana, S.A., Barcelona; INRA = Institut National de la Recherche Agronomique, Bordeaux; Univ. of California, Davis; CSIC = Consejo Superior de Investigaciones Científicas, Zaragoza; Rutgers Univ., New Brunswick, N.J.; Univ. of Florida, Gainesville; CIDA = Centro de Investigación y Desarrollo Agrario, Murcia; Univ. of Pisa; Univ. of Bologna.

domestica), 'Ishtara' [(*P. cerasifera* × *P. salicina*) × (*P. cerasifera* × *P. persica*)], 'Deep Purple' (*P. besseyi* Bailey × *P. salicina*), 'Redglow', Laroda F1OP (*P. salicina*), AC-952 (*P. insititia*), AC-959 (*P. insititia* × *P. domestica*), AC-960 [*P. cerasifera* × (*P. dulcis* × *P. persica*)], 'Marianna 4001', and 'Myrocal' (*P. cerasifera*), the peach-almond hybrids (*P. dulcis* × *P. persica*) 'Felinem', AC-9000, AC-9501, AC-9502, and 'Mayor', and the cherry 'CAB 6P' (*P. cerasus* L.) were micropropagated at Agromillora Catalana S.A., Sant Sadurní d'Anoia, Barcelona, Spain. Plantlets were transferred from agar (Murashige and Skoog, 1962) to 50-mL minipot trays containing a moor peat and clay substrate (Stender Bassis Substrate 3, Blumenerdenwerke Stender, Schermbeck, Germany) and acclimatized in a high humidity chamber for 24–30 d. The rootstocks 'Tetra' (*P. domestica*), 'Penta' (*P. domestica*), and 'Sirio' (*P. persica* × *P. dulcis*), were received from Vivai Battistini, Diegario di Cesena, Italy.

Inoculum source and preparation. A total of 11 *P. vulnus* populations originating from different geographic locations and hosts were obtained from several sources (Table 2). The

populations 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) and total protein analysis by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Jaumot et al., 1997). For inoculation procedures, nematodes from each population were recovered from carrot cultures by adding water and collecting the nematode suspension with a pipette. In each experiment, inocula (mixtures of populations) were adjusted to deliver 900 individuals per plant through five holes located 3–4 cm from the base of the plant. Most of the populations used in this study have proven to be pathogenic on *Prunus*, *Malus*, *Pyrus*, and *Cydonia* rootstocks.

Host susceptibility experiments. Three trials lasting 120 d were conducted in three successive years (1996 to 1998). In all the experiments, plants were transplanted to 4.5-L pots that contained a pasteurized sandy loam soil (78% sand, 15% silt, and 7% clay), pH 7.4,

<3% organic matter content and a cation exchange capacity (CEC) of <120 meq·kg⁻¹.

Plants with uniform growth of ≈20 cm height (10–15 leaves) were inoculated with a suspension of 900 nematodes per plant at 6 weeks after transplanting. In order to assure a desired pathogenic diversity for testing plant material (Pinochet et al., 1993b), inoculum consisted of a mixture of equal proportions of nine *P. vulnus* populations. The populations used in the first (1996) and second (1997) trial were: Pv RO-S, Pv PL-S, Pv WA-A, Pv OL-I, Pv PL-F, Pv WA-US, Pv AP-US, Pv AP-S, and Pv ST-J (Table 2). In the third trial (1998), nine *P. vulnus* isolates were also used, but two isolates used in the previous trials were not available and were replaced by two new *P. vulnus* isolates resulting in the following inoculum mixture: Pv RO-S, Pv PL-S, Pv WA-A, Pv OL-I, Pv PL-F, Pv AT-F, Pv PE-US, Pv AP-US, and Pv ST-J (Table 2).

Inoculated plants were placed in a sand bed to avoid fluctuations in temperature and humidity. Each rootstock was replicated seven or eight times in a completely randomized design. Plants were watered as needed and fertilized weekly with half-strength Hoagland's (Hoagland and Arnon, 1950) nutrient solution. The rootstocks 'Ishtara' or 'Cadaman' were used as susceptible reference rootstocks, whereas 'Torinel' was included as the resistant reference rootstock in each trial. Ambient temperature in the greenhouse fluctuated between 12 and 35 °C.

Experiment number four (1999) was established to confirm results of rootstocks that had shown a resistant response or a low host susceptibility (nonhost or poor host) in the three previous host susceptibility trials. These rootstocks were 'Bruce', 'Deep Purple', 'Torinel', and 'Redglow' plums, and the peach-almond hybrid 'Felinem'. In this experiment, seven *P. vulnus* populations were used, but in contrast with previous trials, each rootstock was challenged with individual populations. These populations were Pv RO-S, Pv PL-S, Pv WA-A, Pv OL-I, Pv AT-F, Pv PE-US, and Pv ST-J. Rootstocks were inoculated with a suspension of 400 nematodes per plant 7 weeks after transplanting. Nematode reproduction was assessed 150 d after inoculation on a per gram root basis, regarded as the most reliable criteria for measuring host suitability or susceptibility (Culver et al., 1989). Greenhouse and experimental conditions were the same as those described for the host susceptibility trials.

Final nematode populations in roots and numbers of nematodes per gram of root were assessed at the end of each experiment. Nematodes were extracted by cutting the whole root system into 1-cm-long pieces and macerating them with water in a commercial blender at 14,500 g_n for a total of 30 s given as 10-s blendings. The nematode suspension was then concentrated through 150-, 74-, and 20-μm-pore sieves (100, 200, and 600 mesh, respectively). Root tissue and debris collected on the 150-μm-pore sieve were discarded. Nematodes were extracted from the remaining sample by sugar flotation (Jenkins, 1964).

Data on nematode reproduction were analyzed by a one-way analysis of variance. Data were $\log_{10}$ transformed ($x + 1$) for analyses. When F values were significant, differences between means were compared using Fisher's LSD test ($P \leq 0.05$). The reproduction factor: Rf = final population density (Pf) of all the life stages divided by the initial population density (Pi) was calculated as a measure of host susceptibility among the different treatments. Host rating used in relation to Rf values were the following: Rf = 0 (nonhost); Rf = 0.01–1.99 (poor host); Rf = 2.0+ (suitable host)

Results

Host susceptibility experiments. In the first trial (1996), 'Felinem' showed the lowest final nematode population, followed by 'Torinel', 'Bruce', and 'Deep Purple', although 'Deep Purple' did not differ from 'Julior'. The remaining rootstocks, 'Orotava', 'Myrocal', 'Montclar', 'Montizo', and 'Ishtara' presented high levels of population buildup in the roots (Table 3). 'Felinem', and 'Torinel' showed a significantly lower number of nematodes per gram of roots, 128 and 225, respectively. 'Bruce' did not differ from 'Torinel' or 'Deep Purple'. Significantly higher levels of high nematode parasitism were found among the remaining rootstocks. Nematode reproduction factor was low for 'Felinem' (Rf value of 1.47) but highest in 'Ishtara' (Rf value of 57.11).

In the second trial (1997), 'Torinel' showed the lowest final nematode population, followed by 'Penta', 'Tetra', 'CAB-6P', and 'Laroda F1OP', although these last three rootstocks did not differ from the rest of the tested materials with the exception of 'Mayor', which had the highest nematode reproduction. 'Torinel' also had the lowest numbers of nematodes per gram of root, followed by 'Tetra', 'Adara', and 'Cadaman'. 'Penta', 'CAB-6P', 'Laroda F1OP', 'Sirio', and 'Rutgers Red Leaf' showed a significantly higher level of parasitism but did not differ from 'Cadaman'. The peach-almond hybrid 'Mayor' had the highest nematode density of 10,160 nematodes per gram of roots. Reproduction factor was low for 'Torinel' (Rf = 1.42) and highest in 'Mayor' (Rf = 33.85).

In the third trial (1998), the final nematode population in roots was lowest in 'Redglow' and 'Torinel', although the latter did not differ from AC-959, followed by 'Marianna 4001' and AC-9000. 'Bruce' was similar to AC-9000 but differed from the remaining rootstocks, which ranged from 11,270 nematodes in 'Adarcias' to 93,260 in AC-952. 'Redglow', 'Torinel', and AC-959 had the lowest numbers of nematodes per g root, followed by 'Marianna 4001', although 'Marianna 4001' did not differ from AC-959 or 'Bruce'. The remaining rootstocks showed varying levels of high nematode densities in the roots with 'Ishtara' (4960) and AC-952 (6230) having the highest. Reproduction factors were low in 'Redglow' (0.23), 'Torinel' (0.85), AC-959 (1.71) and 'Marianna 4001' (2.68). In contrast, AC-952 had the highest Rf (103.62).

Table 2. Origin of eleven populations of *Pratylenchus vulnus* used in this study.

Populations	Geographic origin	Host	Source ²
PvRO-S	Barcelona, Spain	Rose (<i>Rosa multiflora</i> Thunb.)	IRTA
PvPL-S	Alicante, Spain	Plum (<i>Prunus domestica</i>)	IRTA
PvAP-S	Girona, Spain	Apple (<i>Malus × domestica</i>)	IRTA
PvAT-F	Antibes, France	Apricot (<i>Prunus armeniaca</i> L.)	INRA
PvPL-F	Dorogne, France	Plum (<i>Prunus domestica</i>)	INRA
PvWA-A	Córdoba, Argentina	Walnut (<i>Juglans regia</i> L.)	UNC
PvOL-I	Taranto, Italy	Olive (<i>Olea europea</i> L.)	INA
PvPE-U	Georgia, USA	Peach (<i>Prunus persica</i>)	USDA
PvWA-US	California, USA	Walnut (<i>Juglans regia</i>)	UCR
PvAP-US	Idaho, USA	Apple (<i>Malus × domestica</i>)	UI
PvST-J	Kyushu, Japan	Strawberry (<i>Fragaria</i> sp.)	UCR

¹IRTA = Institut de Recerca i Tecnologia Agroalimentàries, Barcelona, Spain; INRA = Institut National de la Recherche Agronomique, Antibes, France; INA = Instituto di Nematologia Agraria, Bari, Italy; UNC = Universidad Nacional de Córdoba, Córdoba, Argentina; U.S. Department of Agriculture, Byron, Ga.; UCR = Univ. of California, Riverside; UI = Univ. of Idaho, Parma.

Table 3. Reproduction of *Pratylenchus vulnus* on 29 *Prunus* rootstocks 120 d after inoculation with 900 nematodes per plant in three greenhouse trials.

Rootstock	Final nematode population in roots ²	Nematodes per g of root	Rf ³
<i>Trial 1 (1996)</i>			
Felinem	1,330 a ^x	128 a	1.47
Torinel TM	3,360 b	225 ab	3.73
Bruce	3,600 b	810 bc	4.00
Deep Purple	3,900 bc	1,100 c	4.33
Julior	7,590 cd	1,400 d	8.43
Orotava	12,570 de	2,010 de	13.96
Myrocal	26,860 ef	2,680 e	29.94
Montclar	29,700 f	2,940 e	33.00
Montizo	38,850 f	2,340 e	43.17
Ishtara	51,400 f	4,290 e	57.11
<i>Trial 2 (1997)</i>			
Torinel	1,280 a ^x	105 a	1.42
Penta	6,210 b	1,330 c	6.90
Tetra	10,740 bc	500 b	11.93
CAB-6P	11,780 bc	2,990 c	13.08
Laroda F1OP	12,030 bc	1,420 c	13.36
Sirio	16,250 c	1,060 c	18.05
Adara	17,240 c	650 b	19.15
Cadaman	19,510 c	940 bc	21.67
Rutgers Red Leaf	19,630 c	1,770 c	21.80
Mayor	30,470 d	10,160 d	33.85
<i>Trial 3 (1998)</i>			
Redglow	211 a ^x	32 a	0.23
Torinel	770 ab	140 a	0.85
AC-959	1,540 bc	190 ab	1.71
Marianna 4001	2,420 cd	360 bc	2.68
AC-9000	4,350 de	2,070 d	4.83
Bruce	5,050 ef	1,120 cd	5.61
Adarcias	11,270 ghi	1,900 d	12.52
Deep Purple	18,180 ijk	3,710 def	20.20
AC-9501	23,310 jkl	2,960 def	25.90
AC-9502	31,620 jkl	2,714 def	35.13
Flordaguard	38,300 lm	2,080 de	42.55
Felinem	47,200 lm	2,210 de	52.44
Ishtara	53,490 lm	4,960 ef	59.43
AC-960	54,170 lm	2,820 de	60.18
AC-952	93,260 m	6,230 f	103.62

²*Pratylenchus vulnus* populations used in trial 1 (1996) and 2 (1997): Pv RO-S, Pv PL-S, Pv WA-A, Pv OL-I, Pv PL-F, Pv WA-US, Pv AP-US, Pv ST-J, and Pv AP-S. *P. vulnus* populations used in trial 3 (1998): Pv RO-S, Pv PL-S, Pv WA-A, Pv OL-I, Pv PL-FR, Pv AT-FR, Pv PE-US, Pv AP-US, and Pv ST-J.

³Rf: Reproductive factor (Pf/Pi) where Pi = initial population density was equivalent to 20 nematodes per 100 cm³ of soil.

⁴Data are means of seven or eight replications. Actual data are presented, but data were transformed to $\log_{10}$ ($x + 1$) for analysis. Mean separation within columns and trials by Fisher's LSD test ($P \leq 0.05$).

Table 4. Reproduction of seven populations of *Pratylenchus vulnus* on five *Prunus* rootstocks 150 d after inoculation with 400 nematodes per plant.

Population	Final nematode population (No. per g of root)				
	Torinel	Deep Purple	Bruce	Felinem	Redglow
Pv ST-J	30 a A ^z	110 a B	5 a A	180 ab B	25 a A
Pv PE-U	195 b A	2480 c C	890 c B	4700 c C	26 a A
Pv WA-A	370 b B	3440 d C	1180 c C	260 ab B	18 a A
Pv RO-S	590 b B	1610 c B	1100 c B	100 a A	9 a A
Pv PL-S	400 b B	940 bc BC	1400 c C	460 b B	22 a A
Pv AT-F	650 b B	2510 c C	120 b A	30 a A	---
Pv OL-I	390 b B	580 b B	0 a A	700 b B	17 a AB

^zData are means for eight replications. Actual data are presented but data were transformed to $\log_{10}(x + 1)$ for analysis. Mean separation within rows (upper case letters) and columns (lower case letters) by Fisher's LSD test ($P \leq 0.05$).

When individual isolates were inoculated on the five rootstocks that had exhibited a low host suitability or resistance in previous trials ('Torinel', 'Deep Purple', 'Bruce', 'Felinem', and 'Redglow'), the Japanese population (Pv ST-J) reproduced significantly less than did other populations in 'Torinel' (Table 4). 'Deep Purple' showed different levels of parasitism ranging from a low of 110 nematodes per g with Pv ST-J, to a very high level of 3440 nematodes per g root when inoculated with the Pv WA-A from Argentina. 'Bruce' was a poor and nonhost to populations Pv ST-J and Pv OL-I, respectively. The French isolate Pv AT-F had lower reproduction on 'Bruce' than did the other nematode isolates. On 'Felinem', Pv AT-F and Pv RO-S reproduced less than did Pv PL-S, Pv OL-I, and Pv PE-U, but did not differ from Pv ST-J or Pv WA-A. The U.S. population Pv PE-U exhibited the highest number of nematodes per g root (4700). Parasitism in 'Redglow' was very low for all *P. vulnus* populations. No differences among populations were detected. Although significant differences in reproduction on the various genotypes were evidenced for each *P. vulnus* population, no relationship was found for a given population in its capacity to multiply evenly on the five rootstocks.

Discussion

Nearly all the evaluated rootstocks were suitable hosts for *P. vulnus* as indicated by high population densities in the roots. However, a few cultivars proved to be poor hosts with final population levels lower or slightly higher than the initial inoculation levels. 'Torinel', AC-959, 'Marianna 4001', and 'Redglow' appear to be the most interesting materials tested so far, although AC-959 and 'Marianna 4001' will require repeated evaluation to verify the results obtained under greenhouse conditions. As difficult as it is to accurately determine plant host resistance to root-lesion nematode, the number of nematodes per gram of root continues to be regarded as the most reliable parameter in measuring host suitability, and probable susceptibility.

'Redglow' (*P. salicina* 'Burbank' × *P. munsoniana* 'Jewell') is an American plum selected in 1919 at the Univ. of Minnesota (American Society for Horticultural Science, 1997). This cultivar is dwarf, hardy, and

productive, but of little or no commercial value. However, it was the most promising rootstock from the point of view of nematode resistance, and perhaps the best source of broad resistance to *P. vulnus* so far detected in a commercial *Prunus* rootstock. 'Redglow' supported the lowest numbers of nematodes in the roots and was resistant to all *P. vulnus* populations tested. The few specimens extracted from the roots were always adult forms (no larvae or eggs were detected), suggesting that they may have been remnants of the original inoculum that penetrated the roots but were incapable of multiplying in the root tissue. 'Redglow' is resistant to this nematode in the United States (Ledbetter, pers. comm.).

The agronomic potential of 'Redglow' as a plum or peach rootstock appears to be limited. Little is known about its response to other soil-borne pathogens and abiotic stress factors. The main problem with 'Redglow' is that it is difficult to propagate by hardwood or herbaceous cuttings; with some success, around 25% to 30% survival (unpublished) has been observed with in vitro techniques.

'Felinem', previously known as G × N No. 22, is a red-leaved peach-almond hybrid selected recently in Spain. It was resistant to *P. vulnus* in previous evaluations (Alcañiz et al., 1996) and in our 1996 trial. However, results obtained in 1998 were disappointing, with 2210 nematodes per g root, a density typical of a highly susceptible host. Individual population inoculations revealed that population Pv PE-U readily multiplied on 'Felinem' (Table 4), whereas most of the *P. vulnus* populations were unsuitable or poor hosts for the nematode. Population Pv PE-U, isolated from peach in Georgia, had not been used in previous trials, which would explain the discrepant results between trials. This case also indicates the pathogenic diversity of this species on a given rootstock. In addition, little is known as to how the resistance trait of *P. vulnus* is transmitted.

Our results suggest that pooling genes from several sources with partial resistance to one or several *P. vulnus* populations, such as those found in 'Bruce', 'Deep Purple', 'Torinel' and 'Felinem', is a sound approach for obtaining a broader spectrum of resistance. However, this task has proved to be difficult because of the lack of genetic compatibility of the *Prunus* species involved (Okie

and Weinberger, 1996). In Spring 1999, crosses between several of these parents that have partial resistance to several populations were carried out. A total of 1220 pollinations of 'Deep Purple' by 'Felinem' produced three hybrid seedlings (*P. salicina* × *P. besseyi*) × (*P. dulcis* × *P. persica*). Several hundred additional pollinations made between 'Redglow' × 'Felinem', 'Deep Purple' × 'Redglow', and 'Bruce' × 'Felinem', produced two more seeds, but the seedlings died following germination, underscoring the difficulty in achieving a descendant with these potential sources of resistance.

From the agronomic standpoint, the most interesting rootstock tested was 'Torinel'. It is a commercial European plum (*P. domestica*), released in France in 1989 by INRA-CTIFL as a plum rootstock for apricot cultivars. 'Torinel' consistently proved to be a very poor host in mixtures and when challenged individually to most populations of *P. vulnus*. Testing carried out by Alcañiz et al. (1996) confirmed its low host suitability. The second most interesting rootstock was the experimental hybrid plum selection AC-959 (*P. domestica* × *P. insititia*) which proved to be a poor host for *P. vulnus*, although it was evaluated in only one experiment (Table 3). Both 'Torinel' and AC-959 adapt well to heavy and calcareous soils, and are also immune to root-knot nematodes (Pinochet et al., 1996b, and unpublished).

Breeding for resistance against *P. vulnus* has been a difficult task, and perhaps one of the most neglected areas of research in rootstock pathology. In general, little progress has been made in identifying sources of resistance in both wild and commercial *Prunus* and then, incorporating these sources into new rootstock material. Results have been limited, in relation to the efforts undertaken in the last 40 years, when compared, for example, with the success achieved with developing root-knot nematode resistance in peach and plum. Nevertheless, in spite of the few sources detected, our findings are encouraging, some in commercial plums, because we have a better understanding of the breeding strategies to be adopted in the future to allow for the pathogenic diversity of this pest.

The prohibition of methyl bromide fumigant in the year 2005 (Hansen, 1999) because of environmental concerns should accelerate research into developing rootstocks resistant to *P. vulnus*. Methyl bromide is commonly used in nursery operations worldwide and in many areas for orchard establishment, such as the Central Valley of California, where *P. vulnus* is widespread (McKenry, 1988). The banning of methyl bromide will have less effect on the control of root-knot nematodes, which can be effectively controlled by commercially available rootstocks (Nyczepir and Becker, 1998; Pinochet et al., 1999). In contrast, root-lesion nematode control will have to rely on less effective measures, such as fallow and the use of nonvolatile nematicides in granular formulations. At present, the use of tolerant rootstocks would seem to be the

best management alternative, although it may be insufficient in the future, as replant activity takes place with less effective fumigants.

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