Damage by a Lesion Nematode, *Pratylenchus vulnus,* to *Prunus* Rootstocks

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ABSTRACT

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The effects of *Pratylenchus vulnus* on growth and nutrition of seven *Prunus* rootstocks were evaluated in two field microplot experiments. Plant materials were experimental rootstocks in advanced stages of selection or new commercial introductions into the Spanish market. In a first trial, fresh shoot weights, shoot lengths, and root weights of D-3-5 almond (*Prunus dulcis*), Nemared peach (*P. persica*), and the peach-almond hybrid (*P. dulcis* × *P. persica*) G × N No. 9 were reduced in nematode-infested microplots compared with those in noninfested microplots at the end of the third growing season. Stem diameter was not affected by nematode infestation. In a second experiment lasting two growing seasons, most growth parameters were reduced in microplots infested with *P. vulnus* in Afgano (*P. dasycarpa*) and Myrobalan 29 C (*P. cerasifera*) plums, and in the peach-almond hybrid G × N No. 15. Damage was evident in the second year. Fresh top and root weights were reduced in Marianna GF 8-1 plum (*P. munsoniana* × *P. cerasifera*) at the end of the second growing season in nematode-infested microplots, whereas only shoot length was reduced in Nemared peach. No nutrient deficiencies were detected by foliar analysis in any of the rootstocks. Higher levels of phosphorus and zinc were found in *P. vulnus*–infested plants of G × N No. 15 than in control plants. All the tested rootstocks were good hosts for *P. vulnus*, which reached a high population density in the roots that fluctuated between 1,290 (G × N No. 15) and 5,311 (G × N No. 9) nematodes per g of root (fresh weight).

Additional keywords: pathogenicity, tolerance

The lesion nematode *Pratylenchus vulnus* Allen and Jensen has a wide geographic distribution (3,14) and is an important pest, attacking stone and pome fruit crops in warm Mediterranean environments (5,11,13,24). 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 extent of growth reduction caused by *P. vulnus* has been documented for Marianna 2624 (*Prunus cerasifera* Ehrh. × *P. munsoniana* W. Wight & Hedr.), Saint Julien 655-2, and PSM 101 plums (*P. insititia* L.) in Spain (23). Lesion nematodes also play an important role in the development of orchard replant problems (2,18). Lesion nematode resistance is unavailable in commercial *Prunus* rootstocks, although resistance has been detected in a few wild plums (*P. fremontii* Mer. and *P. tomentosa* Thunb.) and apricots (*P. armeniaca* L.). Tolerance has been identified in some rootstocks (4,12,15).

No information is available on the damage that *P. vulnus* causes to Nemared peach (*P. persica* (L.) Batsch.), Myrobalan 29 C (*P. cerasifera*), and Marianna GF 8-1 (*P. cerasifera* × *P. munsoniana*) plums, which have been recently introduced into Spain from the United States and France. These rootstocks are becoming widely used in replant situations in Spain. Myrobalan 29 C and Marianna GF 8-1, together with two new peach-almond hybrid (*P. dulcis* (Mill.) D. Webb × *P. persica*) genotypes (G × N selections) in advanced stages of selection, offer important agronomic advantages in that they adapt well to dry land, calcareous soils, and low fertility, conditions that are typical of Mediterranean environments. All of these rootstocks also have root-knot nematode resistance (6).

The purpose of this study was to determine the plant growth and nutritional responses to *P. vulnus,* and the pathogenicity of *P. vulnus,* in seven experimental and commercial *Prunus* rootstocks under field microplot conditions.

MATERIALS AND METHODS

Inoculum source. Five *P. vulnus* isolates from different geographic locations and hosts were obtained from several sources (Table 1). The isolates were cultured monoxenically on carrot (*Daucus carota* L.) disk cultures (16), and incubated at 21°C for three to six generations. Species identification was made by the Commonwealth Institute of Parasitology, St. Albans, U.K., and later confirmed by random amplified polymorphic DNA–polymerase chain reaction analysis (21). For soil infestation, nematodes were collected from flooded cultures with a pipette. The nematode suspension was stored at 20°C for 6 h before use.

Plant material. Seeds, herbaceous cuttings, and micropropagated material were provided by public research institutes and a private source (Table 2). Seeds of the peach rootstock Nemared and the autocom-

Table 1. Geographic and host origin of five isolates of *Pratylenchus vulnus* used in pathogenicity studies on *Prunus* rootstocks

Isolate	Geographic origin	Host	Source[z]
PvRO-S	Barcelona, Spain	Rose *(Rosa multiflora)*	IRTA
PvAP-S	Gerona, Spain	Apple *(Malus silvestris)*	IRTA
PvAT-F	Antibes, France	Apricot *(Prunus armeniaca)*	INRA
PvWA-A	Córdoba, Argentina	Walnut *(Juglans nigra)*	UNC
PvOL-I	Taranto, Italy	Olive *(Olea europea)*	INA

[z] IRTA = Institut de Recerca i Tecnologia Agroalimentàries; INRA = Institut National de la Recherches Agronomique; UNC = Universidad Nacional de Córdoba; INA = Instituto di Nematologia Agraria.

Table 2. Source of *Prunus* rootstocks and selections evaluated as hosts for *Pratylenchus vulnus*

Rootstock	Species/selection	Origin[y]
D-3-5	Almond *(Prunus dulcis)*	SIA, Spain
Nemared	Peach *(P. persica)*	USDA, USA
G × N No. 9	Peach-almond *(P. dulcis × P. persica)*	SIA, Spain
G × N No. 15	Peach-almond *(P. dulcis × P. persica)*	SIA, Spain
Afgano	Plum *(P. dasycarpa)*	SIA, Spain
Marianna GF 8-1	Plum *(P. munsoniana × P. cerasifera)*	INRA, France
Myrobalan 29 C	Plum *(P. cerasifera)*	USA[z]

[y] SIA = Servicio de Investigación Agraria de la Diputación General de Aragón; INRA = Institut National de la Recherche Agronomique; USDA = United States Department of Agriculture.
[z] Gregory Bros. Nursery, Brentwood, CA.

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patible almond D-3-5 were treated with a 5% solution of copper oxychloride for 24 h, rinsed with tap water, covered with moist paper towel, stratified in perlite trays, and kept in a storage room at 4°C for 45 days. Seeds were moved to an ambient temperature greenhouse (no heating or cooling) to induce germination. Herbaceous cuttings of the peach-almond hybrids (*P. dulcis* × *P. persica*) G × N No. 9, G × N No. 15, and Afgano plum (*P. dasycarpa* Ehrh.) were obtained from the Departamento de Fruticultura del Servicio de Investigación Agraria de la Diputación General de Aragón, Zaragoza, Spain. Herbaceous cuttings were treated for 6 to 10 s with a 50% ethanol solution that contained 2,000 ppm of indole butyric acid. Cuttings were planted in small pots (200 cm^3) containing a 3:1 (vol/vol) sand and peat mixture previously pasteurized with steam at 80°C. Micropropagated Marianna GF 8-1 and Myrobalan 29 C were received as plantlets from Agromillora Catalana S.A., Sant Sadurní d'Anoia, Barcelona, Spain. Plantlets were transferred from agar to 50-ml minipots with peat (Floratorf, Floraguard GmbH, Hamburg, Germany) and perlite (Iberperlita, Stavik S.A., Huesca, Spain) substrate, and placed in an 18°C, high humidity chamber (90 to 95% relative humidity) for 24 days. Plantlets with uniform growth arising from germinated seeds, rooted cuttings, and micropropagated material were transplanted to microplots.

Microplot experiments. Two microplot experiments were conducted. The first, lasting three growing seasons, was established in March 1991. Rooted material of the almond D-3-5, peach-almond G × N No. 9, and Nemared peach with 15 to 20 cm growth was transplanted individually into 40-cm-diameter-bucket microplots (1) containing a steam-pasteurized (75°C) sandy loam soil (74% sand, 20% silt, and 6% clay; pH 7.6; <1% organic matter; cation exchange capacity (CEC) < 10 meq per 100 g of soil). Microplots were established in a shaded area (54% shade) in the field and set with 1.2 m spacing between them. One month after transplanting, microplots with nematode treatments were infested with 500 juveniles and adult *P. vulnus* isolate Pv RO-S (equivalent to 21 per kg of soil) delivered through five to six holes located 4 to 5 cm from the base of the plant. Treatments (rootstocks and nematode infestations) were arranged in a completely randomized design with seven replications. Plants were irrigated as needed and fertilized with Osmocote Plus (15-10-12 + micronutrients, Sierra Grace España, S. A., Tarragona, Spain). In February 1993 (third year), the three rootstocks were chip grafted with the almond variety Moncayo.

Data on plant growth (fresh top weight, shoot length, stem diameter, and root weight) and nematode reproduction were assessed at the end of the third growing season (October 1993). Trunk diameter measurements were made 3 cm from the soil line. Final nematode population density in roots and soil and numbers of nematodes per gram of root fresh weight 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-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 (9). Nematodes in roots were extracted from whole root systems, cut into 1-cm-long pieces, and macerated in water in a commercial blender at 14,500 rpm (Waring blender, Waring Products Corp., New York) for 30 s (bursts of 10-s intervals). The nematode suspension was concentrated with 150-, 74-, and 25-µm-pore sieves (100, 200, and 500 mesh, respectively). Root tissue and debris collected on the 150-µm-pore sieve were discarded. Nematodes were recovered in the remaining sample by sugar flotation (9).

The second experiment was established in February 1993 and terminated in October 1994. Rooted plant material of Afgano, Nemared, G × N No. 15, Marianna GF 8-1, and Myrobalan 29C was transplanted individually into 36-cm-diameter containers containing the same soil mixture as described for the first experiment. Containers were buried in a field and were spaced 1 m apart, in a bucket microplot setup (1). The *P. vulnus* inoculum was adjusted to deliver 1,000 juvenile and adult nematodes per plant (equivalent to 46 per kg of soil). Inoculum was prepared from equal proportions of five *P. vulnus* isolates (Pv RO-S, Pv AT-F, Pv OL-I, Pv WA-A, and Pv AP-S). Each treatment was performed eight times in a completely randomized design. Plants were irrigated and fertilized as described for the first microplot experiment. Plant growth was measured at the end of the first and second growing season. Nematode reproduction was assessed at harvest as described for the first experiment. Daily ambient temperature readings in both microplot experiments fluctuated between 1 and 4°C in winter (coldest) and 23 to 35°C in summer (warmest).

Plant nutrition study. The concentrations in leaves of N, P, K, Fe, and Zn were determined in the second microplot experiment during the second growing season according to procedures established for the different *Prunus* species (10,19). Analyses for all elements except N were made with a F586-587 Varian Liberty 220 inductively coupled plasma (ICP) emission spectrometer (7,17). Nitrogen content was determined according to the Kjeldahl procedure (26).

Data on plant growth and element content in leaves were subjected to analysis of variance. Where F values were significant ($P \leq 0.05$), differences between means were compared with Fisher's least significant difference test.

RESULTS AND DISCUSSION

In the first microplot experiment, D-3-5, Nemared, and G × N No. 9 rootstocks grown in *P. vulnus*–infested soil had lower fresh shoot weights, shoot lengths, and fresh root weights than the same cultivars grown in noninfested soil at the end of the third growing season, but stem diameter was unaffected by nematode infestation (Table 3). D-3-5, Nemared, and G × N No. 9 were good hosts for the nematode, although G × N No. 9 supported a higher final nematode population density than D-3-5 (Table 4).

In the second microplot experiment, the peach-almond hybrid G × N No. 15 was

Table 3. Plant growth and nematode reproduction in three *Prunus* rootstocks, 3 years after inoculation with 500 *Pratylenchus vulnus,* per plant in field microplots near Cabrils, Barcelona

Rootstock	Treatment	Fresh shoot weight (g)	Stem diameter (cm)	Shoot length (mm)	Fresh root weight (g)
D-3-5	Control	291 a[z]	12.7 a	462 a	163 a
	P. vulnus	125 b	11.3 a	312 b	83 b
Nemared	Control	439 a	15.5 a	882 a	238 a
	P. vulnus	325 b	14.8 a	608 b	170 b
G × N No. 9	Control	574 a	19.6 a	1,136 a	288 a
	P. vulnus	333 b	16.9 a	688 b	177 b

[z] Data are means of seven replications. Paired means in the same columns followed by the same letter do not differ according to Fisher's least significant difference test ($P \leq 0.05$).

Table 4. Reproduction of *Pratylenchus vulnus* on three *Prunus* rootstocks, 3 years after inoculation with 500 nematodes, per plant in field microplots near Cabrils, Barcelona

Rootstock	Final population per microplot (soil and roots)	Nematodes per g of root(fresh weight)
D-3-5	369,520 a[z]	3,630 a
Nemared	796,400 ab	3,930 a
G × N No. 9	1,092,290 b	5,310 a

[z] Data are means of seven replications. Means in the same columns followed by the same letter do not differ according to Fisher's least significant difference test ($P \leq 0.05$).

Table 5. Fresh shoot weight, shoot length, stem diameter, and root weight of five *Prunus* rootstocks inoculated with 1,000 *Pratylenchus vulnus* per plant at 7 (1993) and 19 months (1994) after nematode inoculation in field microplots near Cabrils, Barcelona

		1993			1994			
Rootstock	Treatment	Fresh shoot weight (g)	Stem diameter (mm)	Shoot length (cm)	Fresh shoot weight (g)	Stem diameter (mm)	Shoot length (cm)	Fresh root weight (g)
Afgano	Control	10.2 a[z]	5.6 a	55.4 a	35.6 a	6.6 a	217.7 a	20.2 a
	P. vulnus	7.2 a	5.2 a	45.6 a	23.9 b	5.6 b	132.2 b	13.1 b
Nemared	Control	20.1 a	5.8 a	101.8 a	107.4 a	8.0 a	676.0 a	34.5 a
	P. vulnus	20.0 a	5.9 a	99.0 a	89.8 a	7.5 a	529.0 b	29.9 a
G × N No. 15	Control	30.1 a	12.2 a	80.6 a	172.1 a	14.8 a	263.8 a	84.5 a
	P. vulnus	18.2 b	10.4 b	50.3 b	115.5 b	12.7 b	209.1 b	45.2 b
Myrobalan 29 C	Control	10.7 a	5.8 a	54.6 a	82.2 a	9.0 a	475.1 a	54.2 a
	P. vulnus	13.0 a	6.0 a	72.1 a	43.1 b	7.3 b	293.9 b	31.3 b
Marianna GF 8-1	Control	6.8 a	5.1 a	39.5 a	49.9 a	7.4 a	302.1 a	37.7 a
	P. vulnus	3.3 a	4.5 a	25.1 a	39.3 b	6.4 a	278.6 a	20.6 b

[z] Data are means of eight replications. Paired means in the same columns followed by the same letter do not differ according to Fisher's least significant difference test ($P \leq 0.05$).

Table 6. Reproduction of *Pratylenchus vulnus* on five *Prunus* rootstocks, 19 months after inoculation with 1,000 nematodes, per plant in a microplot experiment

Rootstock	Final population per microplot (soil and roots)	Nematodes per g of root
Marianna GF 8-1	93,830 a[z]	2,940 b
Nemared	127,350 ab	2,900 b
Myrobalan 29 C	153,960 b	2,200 b
G × N No. 15	165,780 b	1,290 a
Afgano	187,250 b	4,430 c

[z] Data are means of eight replications. Means in the same columns followed by the same letter do not differ according to Fisher's least significant differnce test ($P \leq 0.05$).

the only rootstock with suppressed growth for all measured parameters in the presence of *P. vulnus* at the end of the first and second growing seasons (Table 5). Growth in the remaining rootstocks was unaffected in the presence of *P. vulnus* at the end of the first year. At the end of the second growing season (October 1994), all growth parameters were lower for Myrobalan 29 C and Afgano plums grown in *P. vulnus*–infested microplots. Marianna GF 8-1 and Nemared were less affected by *P. vulnus* than were the other plant cultivars. The nematode reproduced well on all five rootstocks, although the final nematode population density was lower on Marianna GF 8-1 than on Myrobalan 29 C, Afgano, or G × N No. 15. In contrast, G × N No. 15 supported the lowest, and Afgano the highest, numbers of nematodes per g of root (Table 6). Visual examination of nematode-infected rootstocks revealed darker root systems and a lack of feeder roots in general.

Our results indicate that testing for one growing season might be insufficient to determine the susceptibility of *Prunus* rootstocks to *P. vulnus* as previously reported with Marianna 2624 and PSM 101 (23), and with Afgano, Marianna GF-81, and Nemared in the present study, in which damage became evident in the second year. Furthermore, whereas Nemared peach was apparently less affected by nematode parasitism in the 2-year microplot experiment (only shoot length was reduced), it showed a clear susceptible response in the 3-year experiment; thus, a longer period of exposure to the nematode was needed for damage to be measurable.

The use of a mixture of five *P. vulnus* isolates in the second microplot experiment was based on recent findings of differences in pathogenicity among populations of *P. vulnus* (21,22) to ensure pathogenic diversity for plant damage evaluation. This information was not available when the first microplot experiment was established; however, the *P. vulnus* rose isolate (Pv RO-S) used in the first microplot experiment was damaging to all three rootstock cultivars tested.

Both G × N selections were derived from crosses between the Spanish almond Garfí (female parent) and the Californian peach cultivar Nemared (male parent). These cultivars have red leaves, root-knot nematode resistance, and are vigorous and well adapted to dryland and calcareous soils (6). In the 3-year experiment, G × N No. 9 and Nemared showed comparable susceptible responses. In the 2-year experiment, however, growth of G × N No. 15 was suppressed by the nematode in the first and second growing season, indicating that this selection is more susceptible than its parent Nemared, which has higher tolerance to *P. vulnus*. Shoot length was the only growth parameter in Nemared affected by *P. vulnus* in this experiment.

In a study conducted in California to develop screening procedures for *Prunus* to lesion nematodes, Myrobalan 29 C plum was considered tolerant to *P. vulnus* (4). The number of nematodes per g of root reported in that study (4) was considerably lower than that observed in our study. Similarly, in a 7-year field study in California, McKenry (15) found only a 1.5% reduction in fruit weight between Myrobalan 29 C grown in *P. vulnus*–infested soil compared with nematode-free soil, indicating a high level of tolerance to the nematode. In our study, Myrobalan 29 C was susceptible and vegetative growth was suppressed, compared with noninoculated controls. These discrepancies point toward different experimental conditions and perhaps differences in pathogenicity among *P. vulnus* populations.

It is noteworthy that in the 2-year microplot experiment four plants died during the second year of evaluation. In the 3-year microplot experiment, two plants died in the second and third year, respectively. In all of these cases, the deaths were in *P. vulnus*–infested microplots. There were no deaths in uninfested plots.

Nearly all elemental contents in leaf samples for each rootstock with or without *P. vulnus* were sufficient for growth of almond, peach, and plum (10). Criteria for choosing Fe and Zn determinations were based on needs in local soils (calcareous) affecting fruit tree crops. Low levels of Fe (60 to 90 ppm) were found in all of the sampled rootstocks. No deficiency for any element was detected by foliar analysis. High levels of Zn were found in inoculated and noninoculated Afgano plum. Higher leaf contents of P and Zn were found in *P. vulnus*–inoculated G × N No. 15 hybrid rootstock (Table 7). These elements seem to be absorbed continuously and accumulate in plants with suppressed growth, thus increasing their concentration in leaf tissues. In contrast, the lower concentrations of these same elements in leaf tissues in treatments without the nematode are explained by a growth dilution effect (8). A similar pattern for these elements (increase in Zn, Mg, and N levels in *P. vulnus*–infected plants) has been described on rose (25) and apple (20) in plants stunted by *P. vulnus*. In a recent nematode-mycorrhizae interaction study with the arbuscular mycorrhizal fungus *Glomus mosseae* and *P. vulnus* on Nemared peach, the nematode-

Table 7. Mineral constituents of composite leaf samples from five *Prunus* rootstock 17 months after inoculation with 1,000 *Pratylenchus vulnus* per plant

Rootstock		Percentage of dry weight			ppm	
		N	K	P	Fe	Zn
Marianna GF 8-1	Control[z]	3.79 a	2.39 a	0.32 a	68 a	23 a
	P. vulnus	3.67 a	2.58 a	0.31 a	74 a	26 a
Myrobalan 29 C	Control	3.78 a	2.58 a	0.31 a	67 a	22 a
	P. vulnus	3.81 a	2.39 a	0.34 a	77 a	23 a
Afgano	Control	4.37 a	3.27 a	0.38 a	87 a	66 a
	P. vulnus	4.58 a	2.77 a	0.34 a	91 a	41 a
G × N No. 15	Control	...	2.33 a	0.17 a	59 a	14 a
	P. vulnus	...	2.61 a	0.27 b	60 a	17 b
Nemared	Control	3.93 a	2.80 a	0.27 a	75 a	22 a
	P. vulnus	3.86 a	2.80 a	0.30 a	74 a	21 a

[z] Data are means of eight replications. Paired means in the same columns followed by the same letter do not differ according to Fisher's least significant difference test ($P \leq 0.05$).

inoculated plants with or without the mycorrhizae showed an increase in leaf concentrations of Mg, Mn, Zn and Na, but a decrease in Fe and Cu (19). Under field and greenhouse conditions, Culver et al. (4) found that *P. vulnus*–infected Nemaguard peach Myrobalan 29 C plum had lower K content in leaves. Element content in tissues as criteria for evaluating host reaction to root-lesion nematodes is confusing and requires further research.

We found that all of the evaluated *Prunus* rootstocks are susceptible to *P. vulnus* and support high population densities in the roots. The majority of the tested rootstocks were damaged measurably after the first year. Estimated levels of tolerance for field conditions remain to be determined. The search for sources of resistance for com-mercial *Prunus* is a priority for rootstocks adapted to Mediterranean environments.

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