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Growth Response of Peach and Plum Rootstocks Infected with *Pratylenchus vulnus* in Microplots

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Abstract: The effects of *Pratylenchus vulnus* on growth and nutrition of Cadaman peach and Ishtara and Julior plum rootstocks were evaluated in a microplot experiment lasting two growing seasons. Cadaman peach was the only rootstock that showed suppressed growth for all growth parameters at the end of the first year. At the end of the second growing season, dry and fresh shoot weights as well as shoot length and root weights of Cadaman peach were reduced in nematode-inoculated microplots in comparison to uninoculated treatments. Stem diameter was not affected. Dry and fresh shoot weights were the only growth parameters affected by the nematode in Ishtara plum at the end of the second growing season, whereas Julior was not affected by *P. vulnus* infection. No nutrient deficiencies were detected by foliar analysis in any of the rootstocks and treatments. All the tested rootstocks were good hosts for *P. vulnus*, whose mean root population ranged from 1,670 (Cadaman) to 2,895 (Julior) nematodes/g of root.
Key words: nematode, nutrients, pathogenicity, peach, plum, *Pratylenchus vulnus, Prunus,* rootstocks, root-lesion nematode, tolerance.

The root-lesion nematode *Pratylenchus vulnus* is an important pathogen attacking stone and pome fruit crops in warm Mediterranean environments (Marull and Pinochet, 1991; Fernández et al., 1992; Lamberti, 1981; Nyczepir and Becker, 1998; Scotto La Massèse, 1989). This migratory endoparasitic nematode destroys the cortical parenchyma of the root causing cavities and lesions, and predisposes the parasitized tissue to secondary infections by fungi and bacteria. These symptoms are non-specific and can be easily overlooked or mistaken for damage caused by other soil pathogens. Reduction in the amount of feeder roots usually results in loss of vigor and yield in young and mature trees (McKenry, 1988; Nyczepir and Halbrendt, 1993). The extent of damage has been documented for several peach, plum, and cherry rootstocks in Spain (Pinochet et al., 1993, 1995, 1996). Root-lesion nematodes also play an important role in the development of orchard replant problems (Bertrand, 1989; García de Otazo, 1992).

Cadaman (*Prunus persica* × *P. davidiana*), Julior (*P. insititia* × *P. domestica*), and Ishtara (*P. belsiana* × *P. cerasifera*) are three rootstocks for peach and plum released by INRA (Institut National de la Recherche Agronomique) in France in the last decade. These rootstocks offer important agronomic advantages—adapting well to dry land, calcareous soils, and low fertility (conditions that are typical of Mediterranean environments). The rootstocks also have incorporated root-knot nematode resistance. The three rootstocks have been found to be good hosts for *P. vulnus* (Alcañiz et al., 1996) in screening tests, but information on the damage that this nematode species causes to Cadaman, Julior, and Ishtara is unknown. Julior and Ishtara have been recently introduced from France into Spain. Cadaman has been available for many years and is becoming widely used in replant situations where root-knot nematodes are a limitation in peach, nectarine, and plum production. In many cases, mixed populations of root-knot and root-lesion nematodes cohabit in peach and plum production areas in the Levante region and in southern Spain.

The purpose of this study was to determine the growth and nutritional response of Cadaman, Julior, and Ishtara *Prunus* root-

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stocks to *P. vulnus* under microplot field conditions during the first 2 years of establishment.

Materials and Methods

Inoculum source: A *P. vulnus* population was originally isolated from a plum orchard in Alicante, Spain. The population was reared monoxenically on carrot (*Daucus carota* L.) disk cultures (Moody et al., 1973) and incubated at 22 °C for several generations. Species identification was made by the Commonwealth Institute of Parasitology, St. Albans, United Kingdom, and later confirmed by SDS-PAGE analysis (Jaumot et al., 1997). For inoculation procedures, nematodes were recovered from cultures by adding water and collecting the nematode suspension with a pipet. Inoculum was adjusted to deliver 3,000 individuals/plant into 7 or 8 holes 4 to 5 cm distant from the rootstock.

Microplot experiment: Micropropagated Cadaman peach, Julior, and Ishtara plums were received as 30-cm-tall rootstocks from Agromillora Catalana S.A. (Sant Sadurní d'Anoia, Barcelona, Spain). These plants had been previously propagated in vitro and transferred from agar 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 days. Plantlets were then transferred to 300-ml pots, grown for 90 days in a greenhouse, and micro-grafted with the peach cultivar Andross.

A microplot experiment lasting two growing seasons was established in March 1997. Rooted plants were transplanted individually into 35-cm-diam. and 55-cm-deep bucket microplots (Barker, 1985) containing a steam-pasteurized (70 °C for 4 hours) sandy loam soil (76% sand, 14% silt, 1% clay, pH 7.5, <1% organic matter, C.E.C. < 10 meq/100 g of soil). Microplots were established in a shaded area (54% shade) in the field and were set at 1.2-m spacing. Forty-five days after transplant, microplots were infested with 3,000 nematodes (equivalent to 80–90 nematodes/kg of soil). Each treatment (infested and uninfested) was represented by 8 microplots for each rootstock arranged in a completely randomized design. Plants were irrigated as needed and fertilized with Osmocote Plus [15-10-12 (N-P-K) + micronutrients] (Sierra Grace, Valencia, Spain).

Data on plant growth (fresh and dry top weights, shoot length, and stem diameter) were gathered at the end of the first (November 1997) and second (October 1998) growing seasons. Fresh root weight was determined at the end of the experiment following destructive harvest. Trunk-diameter measurements were made 3 cm above the soil line. Final nematode populations in roots and soil, and numbers of nematodes per gram of root also were assessed at the end of the experiment. Soil from each pot

Table 1. Fresh and dry shoot weights, shoot length, stem diameter, and root weights of Cadaman, Ishtara, and Julior rootstocks inoculated with 3,000 *Pratylenchus vulnus*/plant at 7 (first season) and 19 months (second season) after nematode inoculation.

		First season (1997)			Second season (1998)				
Rootstock[a]	Treatment	Fresh shoot weight (g)	Dry shoot weight (g)	Shoot length (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	Stem diameter (mm)	Shoot length (cm)	Fresh root weight (g)
Cadaman	Control	125.6 a	50.8 a	470.8 a	422.3 a	184.7 a	14.9 a	1,374.6 a	245.4 a
	P. vulnus	93.8 b	38.9 b	338.8 b	375.4 b	166.1 a	14.3 a	1,193.5 b	208.0 b
Ishtara	Control	58.2 a	22.3 a	123.6 a	340.0 a	141.6 a	14.2 a	719.0 a	271.1 a
	P. vulnus	58.3 a	23.1 a	148.5 a	291.3 b	121.1 b	14.3 a	656.5 a	219.7 a
Julior	Control	71.3 a	27.1 a	193.5 a	347.8 a	140.8 a	13.5 a	908.4 a	236.8 a
	P. vulnus	73.5 a	27.8 a	197.2 a	334.4 a	135.6 a	13.7 a	842.9 a	221.5 a

[a] Data are means of 8 replications. Paired means in the same column followed by the same letter do not differ according to Student's *t*-test ($P \leq 0.05$).

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, 38, and 25-µm-pore sieves (100, 200, 400, and 500 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 them with water in a commercial blender at 14,500 g for 30 seconds given at 10-second intervals. The nematode suspension was then concentrated through 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 extracted from the remaining sample with sugar flotation.

Plant nutrition study: Macro-(N, P, K) and micro-elements (Ca, Mg, Fe, and Zn) were determined during the second growing season. Leaf samples from 30 to 35 mid-shoot leaves were taken from each tree in mid-August, avoiding apical shoots and senescent or necrotic tissue. Leaf samples of the same treatment were composited from two trees, resulting in 4 replications to obtain a minimum of 4 to 5 g of dry matter as required for element analysis. Leaves were thoroughly washed in mild detergent and rinsed twice in distilled water. Samples were then dehydrated in a temperature-controlled, fan-ventilated oven at 70 °C ± 1 °C for 48 hours, ground in a ball mill, and digested in wet acid (Jones et al., 1991) with nitric and perchloric acids. Analysis for all elements except nitrogen was made with an F586-587 Varian Liberty 220 inductively coupled plasma (ICP) emission spectrometer (Munter and Grande, 1981). Nitrogen content was determined according to the Kjeldahl procedure (Rund, 1984). Two readings were taken per sample.

Data on plant growth and element content in leaves were analyzed with one-way ANOVA. Paired means were compared with Student's *t*-test ($P \leq 0.05$). Data on nematode reproduction were $\log_{10}$-transformed (x + 1) for analysis. When *F* values were significant, means were compared with Fisher's LSD test ($P \leq 0.05$).

Results and Discussion

At the end of the first growing season (October 1997), Cadaman showed significant (*P*

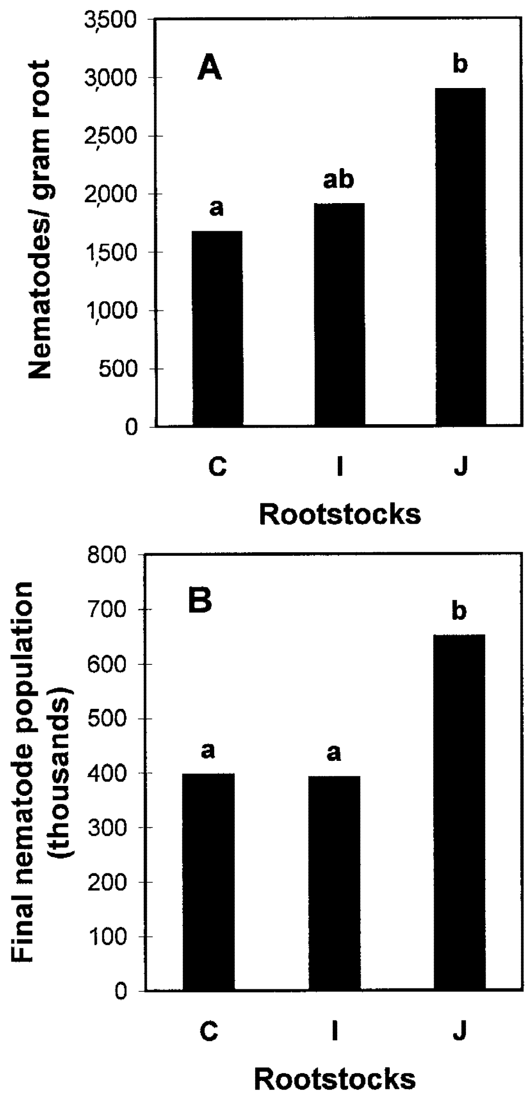

Fig. 1. Reproduction of *Pratylenchus vulnus* in Cadaman (C), Ishtara (I), and Julior (J) *Prunus* rootstocks at 19 months after inoculation with 3,000 nematodes in a microplot experiment. A) Nematodes per gram of root. B) Final nematode population (roots and soil). Data are means of 8 replications. Actual data are presented, but data were transformed with $\log_{10}$ (x + 1) for analysis. Within a graph, means represented by bars for each rootstock with the same letter do not differ according to Fisher's LSD test ($P \leq 0.05$).

≤ 0.05) reduction for fresh and dry top weights and for shoot length in *P. vulnus*-infested microplots compared to controls. No differences were recorded for growth parameters in Ishtara and Julior for the same period (Table 1). At the end of the second growing season, *P. vulnus*-inoculated Cadaman showed lower fresh shoot weight, shoot length, and fresh root weight than uninoculated controls. Ishtara plum exhibited lower fresh and dry root weights in *P. vulnus* treatments, although no differences for plant height, stem diameter, and for fresh root weights between nematode-inoculated and uninoculated treatments were recorded. In contrast to Cadaman and Ishtara, Julior showed no growth reduction for any parameter after two growing seasons in nematode-infested microplots.

Ishtara showed no measurable damage in the first growing season, but growth suppression was evident at the end of the second season. This same pattern of damage has been observed with Garnem peach-almond hybrid (*Prunus dulcis* × *P. persica*), Myrobalan 29C, and Marianna GF-81 plums (*P. cerasifera*) in *P. vulnus*-infested soil in a similar microplot experiment (Pinochet et al., 1995).

The nematode reproduced well on the three rootstocks. The final nematode population was significantly higher in Julior than in Cadaman and Ishtara (650,800 nematodes/plant in Julior vs. 397,100 in Cadaman and 392,000 in Ishtara) (Fig. 1B). Julior also had a significantly higher level of parasitism than Cadaman (2,895 nematodes/g of root vs. 1,675) but did not differ from Ishtara (1,905 nematodes/g of root) (Fig. 1A).

Most elements in leaf samples for each rootstock with or without *P. vulnus* were within sufficiency levels for peach (Jones et al., 1991) (Table 2). No deficiency was detected by foliar analysis. However, low levels were found for Ca and Fe on the peach cultivar Andross grafted on the three rootstocks except with non-inoculated Ishtara, which was within sufficiency level. McKenry (1989) reported reduced levels of Ca and Mg in petiole samples in Lovell (*P. persica*), Nemaguard, Myrobalan 29C, and Mariana 2624 (*P. cerasifera* × *P. munsoniana*) rootstocks in a *P. vulnus*-infested site. Other macroelements (N and P) were unaffected by the nematode.

Vegetative growth in Cadaman peach was affected more by the nematode than either plum, and Julior supported a high level of *P. vulnus* parasitism without affecting plant growth, nutrient uptake, or translocation of these elements during the early stages of plant development. Our findings indicate that Julior plum performed as a tolerant rootstock under these experimental conditions. Its true level of tolerance (and productivity) under broader field conditions remains to be determined. Tolerance should be considered by growers as a sound alternative (Nyczepir, 1991) in situations in which resistance is limited or unavailable, as

TABLE 2. Mineral constituents of composite leaf samples and Andross peach on Cadaman, Ishtara, and Julior rootstocks 17 months (second growing season) after inoculation with 3,000 *Pratylenchus vulnus*/plant.

		Percentage of dry weight					ppm	
Rootstock[a]	Treatment	N	P	K	Ca	Mg	Fe	Zn
Cadaman	Control[z]	4.17 a	0.20 a	2.63 a	1.14 a	0.45 a	83 a	32 a
	P. vulnus	4.19 a	0.21 a	2.65 a	1.11 a	0.42 a	90 a	36 a
Ishtara	Control	3.92 a	0.21 a	2.98 a	1.62 a	0.35 a	85 a	34 a
	P. vulnus	4.17 a	0.24 a	3.38 a	1.47 a	0.32 a	92 a	36 a
Julior	Control	3.76 a	0.17 a	3.31 a	1.35 a	0.31 a	89 a	36 a
	P. vulnus	3.61 a	0.18 a	3.27 a	1.40 a	0.32 a	90 a	39 a
Interpretative values	Low level	1.7–2.39	0.09–0.13	1.0–1.59	1.0–1.49	0.2–0.29	60–99	15–19
for foliar analysis	Suffic. level	2.4–3.0	0.14–0.25	1.6–3.0	1.5–3.0	0.3–0.8	100–250	20–50
in peach	High level	3.1–4.0	0.26–0.4	3.1–4.0	3.1–4.0	0.8–1.1	251–500	51–70

[a] Data are means of 4 replications. Paired means in the same columns followed by the same letter do not differ according to Fisher's LSD test $P \leq 0.05$).

is the case of root-lesion nematodes for stone fruit rootstocks. However, efforts to identify resistance (Alcañiz et al., 1996; Culver et al., 1989; Ledbetter, 1994; Pinochet, 1997) and incorporate the trait into commercial *Prunus* cultivars are ongoing. The forthcoming loss of methyl bromide, as well as other fumigants for orchard establishment, will probably increase efforts in the search for rootstocks tolerant and resistant to *P. vulnus*.

Pratylenchus vulnus is less widespread than root-knot nematodes in intensive peach-growing areas of Spain (Pinochet et al., 1998), although in Seville and Valencia both nematode pests can be found in mixed populations in peach and nectarine orchards. A similar situation occurs in the Central Valley of California (McKenry, 1988). From the practical standpoint, the high susceptibility of Cadaman to *P. vulnus* would suggest that this rootstock might not be the best choice for peach in areas infested with both root-knot and lesion nematodes, in spite of the high level of resistance exhibited to all root-knot nematode species.

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