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Prunus Rootstock Evaluation to Root-knot and Lesion Nematodes in Spain[1]

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Abstract: Two screening and one resistance verification trial involving 20 *Prunus* rootstocks were conducted under greenhouse conditions against *Meloidogyne* spp. and *Pratylenchus vulnus*. Most of the rootstocks were experimental genotypes or new commercial peach and plums of Spanish and French origin. Nearly all are interspecific hybrid rootstocks. In the first trial, the rootstocks Bruce, Cadaman, Mirac, G × N No. 15, Cachirulo × (G × N No. 9), and *P. myra* × peach were immune or resistant to a mixture of seven isolates of *M. incognita*. In the second screening trial, the hybrid plum P 2588 was a poor host to a mixture of four isolates of *P. vulnus*. The remaining seven rootstocks were good hosts to the root-lesion nematode. In the resistance verification trial GF-31, G × N No. 15, Torinel, AD-101, Monpol, Nemaguard, and Cadaman maintained a high level of resistance when tested against a mixture of 17 isolates comprising *M. incognita, M. javanica, M. arenaria, M. hapla,* and *M. hispanica*. Barrier peach suffered a partial loss of resistance not detected in previous tests.
Key words: lesion nematode, *Meloidogyne* spp., *Pratylenchus vulnus, Prunus,* resistance, root-knot nematode, rootstock.

Several species of root-knot nematodes (*Meloidogyne* spp.) and the lesion nematode *Pratylenchus vulnus* can become the limiting factor in stone fruit production in warm environments (18,19,21,22). Root-knot nematodes are more common than *P. vulnus* in the Mediterranean region, but the root-lesion nematode appears to be damaging when present, especially in replant situations (10,27). In the last decade a significant effort has been made in Spain and France to incorporate root-knot nematode resistance into new rootstocks, mainly peach-almond hybrids, plums, and interspecific *Prunus* rootstocks (1,6,9,17), replacing widely used seedling stocks, which often lack homogeneity, tend to suffer badly from root asphixia and calcareous soils, and are susceptible to root-knot and lesion nematodes (7).

Sources of resistance against root-knot nematodes are available in wild and commercial *Prunus*. The nematode-resistant trait is easy to transmit by hybridization and apparently is determined by one or a few dominant genes (3,15). Screening and resistance verification procedures in rootstock selection involve repeated testing of plant material with single nematode populations (5,16) and mixtures of populations involving several species of *Meloidogyne* to assure broad resistance (4,15,28). In contrast, resistance against *P. vulnus* has been difficult to find (14) and transmit the trait from wild *Prunus* into commercial rootstocks. Recent findings also indicate the existence of differences in pathogenicity among *P. vulnus* populations (23,24). This complicates the evaluation of plant material in the search for resistance and tolerance in *Prunus* rootstocks against this pathogen.

The purpose of this study was to evaluate *Prunus* genotypes in their late stages of selection from Spanish and French breeding programs, as well as some new commercial introductions into southern Europe against both root-knot and lesion nematodes. Secondly, root-knot nematode resistance was tested using mixtures of isolates from several *Meloidogyne* spp. (large pathogenic diversity) in rootstocks that have shown different levels of resistance with single species (or isolate) inoculations in previous testing in Spain.

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Materials and Methods

Rootstock material: Seeds, herbaceous and hardwood cuttings, and micropropagated material were supplied by Spanish and French public research institutes, and private sources (Table 1). Seeds of the peach rootstocks Rutgers Red Leaf and Nemaguard were treated with a 5% solution of copper oxychloride for 24 hours, rinsed with running water, covered with a moist paper towel, and stratified in perlite on trays kept in a storage room at 4°C for 60 days. Seeds then were moved to ambient temperature in a greenhouse to induce germination. Herbaceous and hardwood cuttings of the rootstocks Balones, G × N No. 15, Cachirulo × (G × N No. 9), Mirac, Montizo, Monpol, Bruce, and *P. myra* × peach were treated for 6–10 seconds with a 50% alcohol solution that contained 1,000 ppm of indole butyric acid (11). Cuttings then were planted in 6-cm-diam. pots containing 200 cm^3 of a pasteurized (80°C) sand:peat mixture (2:1/v:v). Both seed and cuttings were provided by the Departamento de Fruticultura of the Servicio de Investigación Agraria in Zaragoza, Spain. Micropropagated GF-677, Barrier, GF-31, Cadaman, Torinel, Julior, AD-101, and Ma × Ma were received as plantlets in 8-cm-diam. pots containing 300 cm^3 from Agromillora Catalana S. A., Saint Sadurní d'Anoia, Barcelona, Spain, in a sand:peat substrate (1:1/v:v). Bare-root micropropagated plum genotypes P 2481 and P 2588 were supplied by the Fruit Breeding Station of the Institut National de la Recherche Argonomique (INRA) in Bordeaux, France.

Plantlets with uniform growth from germinated seeds, rooted cuttings, and micropropagated material were transplanted into 3 liters of soil in 18-cm-diam. pots filled with a pasteurized sandy loam textured soil (85% sand, 12% silt, 3% clay; pH 7.3; <2% organic matter; and a cation-exchange-capacity <10 meq/100 g soil). Plants were kept in the greenhouse for 4 weeks to allow rooting before inoculation.

Inoculum source and preparation: Twenty nematode isolates comprising five *Meloi-*

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

Rootstock	Species-selection	Source[a]
Barrier	Peach (*P. persica* × *P. davidiana*)	CNR, Italy
Cachirulo × (G × N No 9)	Peach-almond (*P. persica* × *P. dulcis* × *P. persica*)	SIA, Spain
Cadaman	Peach (*P. persica* × *P. davidiana*)	INRA, France
Nemaguard	Peach (*P. persica* × *P. davidiana*)	USDA, USA
GF-677	Peach-almond (*P. persica* × *P. dulcis*)	INRA, France
Rutgers Red Leaf	Peach (*P. persica*)	Rutgers U., USA
G × N No 15	Peach-almond (*P. dulcis* × *P. persica*)	SIA, Spain
AD-101	Plum (*P. insititia*)	CSIC, Spain
Balones	Peach-almond (*P. persica* × *P. dulcis*)	CIDA, Spain
Bruce	Plum (*P. salicina* × *P. angustifolia*)	Unknown, USA
GF-31	Plum (*P. cerasifera* × *P. salicina*)	INRA, France
Ma × Ma	Cherry (*P. avium* × *P. mahaleb*)	Unknown, USA
Montizo	Plum (*P. insititia*)	SIA, Spain
Monpol	Plum (*P. insititia*)	SIA, Spain
P. myra × peach	Plum (*P. myra* × *P. persica*)	INRA, France
Mirac	Plum-peach (*P. cerasifera* × *P. persica*)	SIA, Spain
P 2481	Plum (*P. besseyi* × *P. salicina*)	INRA, France
P 2588	Plum (*P. besseyi* × *P. maritima*)	INRA, France
Torinel	Plum (*P. domestica*)	INRA, France
Julior	Plum (*P. insititia* × *P. domestica*)	INRA, France

[a] SIA = Servicio de Investigación Agraria; CNR = Consejo Nationale de Recerca; INRA = Institut National de la Recherche Agronomique; USDA = United States Department of Agriculture; CSIC = Consejo Superior de Investigaciones Científicas; CIDA = Centro de Investigación y Desarrollo Agrario.

dogyne spp. were used in two separate experiments. Nematodes were originally collected from different hosts (mainly *Prunus*) and geographical localities (Table 2). Isolates were increased on tomato (*Lycopersicon esculentum* Mill. cv. Rutgers Marglobe) from single-egg-mass cultures. Identification of isolates was made by perineal patterns (20 females per population) and confirmed by Random Amplified Polymorphic DNA technique (2). In cases of discrepancy in species identification between methods, the molecular technique was considered the reliable criterion. Inoculum of each isolate was prepared by macerating infested tomato roots in a blender for 10-second in a 0.12–0.15% solution of NaOCl. Eggs were concentrated on a 25 µm-pore sieve (500 mesh) and rinsed with tap water before inoculation (12).

Four populations of *P. vulnus* were obtained from different sources (Table 2). Isolates were established from 5 to 10 female specimens reared on carrot (*Daucus carota* L.) disk cultures (20) and incubated at 21 °C for several generations. Species identification was made by the Commonwealth Institute of Parasitology, St. Albans, United Kingdom, or confirmed by PCR-RAPDs analysis (23). All *P. vulnus* isolates used in this study have proven to be pathogenic on *Prunus, Malus, Pyrus,* and *Cydonia* rootstocks.

Greenhouse experiments: Two screening trials, one with *M. incognita* and another with *P. vulnus,* and a resistance verification experiment for testing resistant rootstock material to several *Meloidogyne* species were conducted under greenhouse conditions. The responses of the *Prunus* rootstocks Balones, Bruce, Cachirulo × (G × N No. 9), Cadaman, G × N No. 15, GF-677, Mirac, *P. myra* × peach, and Rutgers Red Leaf to a mixture of seven isolates of *M. incognita* were evaluated in the first experiment. The peach-almond hybrids G × N No. 15 and GF-677 were used as resistant and susceptible reference rootstocks, respectively. Plants of uniform growth (20–25 cm height; 10 to 20 leaves) were inocu-

TABLE 2. Isolates of five *Meloidogyne* species and *Pratylenchus vulnus* used in evaluating *Prunus* rootstocks in Spain.

Species	Isolate	Host	Origin	Test[a]
Meloidogyne incognita	Mi VR-ES	Peach-almond	Sevilla, Spain	Mi ST & RVT
	Mi AN-ES	Plum	Jaén, Spain	Mi ST & RVT
	Mi CP-ES	Peach	Zaragoza, Spain	Mi ST & RVT
	Mi AD-ES	Peach	Zaragoza, Spain	Mi ST & RVT
	Mi PR-FR	Peach	Provence, France	Mi ST & RVT
	Mi CA-GU	Tomato	Cayene, F. Guayana	Mi ST
	Mi NC-EU	Tomato	North Carolina, USA	Mi ST
M. javanica	Mj CA-ES	Fig	Barcelona, Spain	RVT
	Mj MB-ES	Almond	Tarragona, Spain	RVT
	Mj CN-EU	Tomato	North Carolina, USA	RVT
	Mj DE-TU	Peach	Anatolia, Turkey	RVT
	Mj AL-PO	Almond	El Algarve, Portugal	RVT
M. arenaria	Ma CN-ES	Tomato	Barcelona, Spain	RVT
	Ma CB-ES	Tomato	Barcelona, Spain	RVT
	Ma LP-ES	Carnation	Sevilla, Spain	RVT
	Ma AL-ES	Peach	Murcia, Spain	RVT
	Ma SA-ES	Peach	Tarragona, Spain	RVT
M. hapla	Mh CA-ES	Kiwi	Barcelona, Spain	RVT
M. hispanica	Mhi BR-ES	Peach	Sevilla, Spain	RVT
Pratylenchus vulnus	Pv RO-S	Rose	Barcelona, Spain	Pv ST
	Pv PL-S	Plum	Alicante, Spain	Pv ST
	Pv WA-A	Walnut	Córdoda, Argentina	Pv ST
	Pv AP-U	Apple	Idaho, USA	Pv ST

[a] Mi ST = *Meloidogyne incognita* screening test (seven isolates); RVT = Resistance verification test (17 *Meloidogyne* isolates); Pv ST = *Pratylenchus vulnus* screening test (four isolates).

lated with a suspension of 2,000 eggs per plant through four holes in the soil 3 cm from the base of the plant. Inoculations contained an equal proportion of seven isolates of *M. incognita.*

In the second screening trial, the rootstocks Cahirulo × (G × N No. 9), Cadaman, Mirac, *P. myra* × peach, Ma × Ma, Nemaguard, P 2481, and P 2588 were evaluated against a mixture of four *P. vulnus* isolates. For inoculation procedures, nematodes from each isolate were recovered from carrot cultures by adding water and collecting the nematode suspension with a pipette. Inoculum (mixtures of four isolates in equal proportion) was adjusted to deliver 1,000 nematodes per plant. In this trial, Nemaguard peach and the Ma × Ma cherry were used as low and high susceptible reference rootstocks, respectively.

In the resistance verification experiment, rootstocks G × N No. 15, GF-31, Torinel, AD-101, Monpol, Nemaguard, Cadaman, Barrier, and GF-677 were evaluated against a mixture of 17 isolates in equal proportion comprising *M. incognita, M. javanica, M. arenaria, M. hapla,* and *M. hispanica.* Inoculum preparation and inoculation procedures were conducted as previously described herein, but the inoculation level was adjusted to deliver 5,000 eggs per plant. The peach-almond hybrids G × N No. 15 and GF-677 were used as resistant and susceptible reference rootstocks, respectively.

Plants were watered daily or as needed, and fertilized twice with Osmocote Plus (15-10-12; N:P:K + micros, Sierra Grace España, S. A.). Inoculated pots were maintained in sand beds in a greenhouse to minimize temperature and humidity fluctuations. Ambient temperatures in the greenhouse fluctuated between 16 and 37 °C. In the *M. incognita* experiment, each material was replicated eight times; in the *P. vulnus,* six times; and in the resistance verification trial, eight times. The three experiments were arranged in a completely randomized design.

Plants were harvested 120 days after inoculation. In the root-knot nematode trials, the total number of galls, final nematode population per plant (soil and roots), and the numbers of nematodes per gram of root were determined. Soil nematodes were extracted from a 250-cm^3 sub-sample by centrifugal flotation (13). Nematode eggs in roots were extracted as previously described (12). However, the entire root system was weighed, cut into 1-cm pieces, and macerated in a blender at 14,500 rpm in a stronger solution of NaOCl (0.25–0.30%) for three periods of 15-second, separated by two 5-second, intervals. Nematodes were concentrated using 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. Resistance rating for root-knot nematodes for each rootstock was estimated based on reproduction and root galling: I = immune (no nematodes in roots and an absence of root galls); HR = highly resistant (nematode may or may not invade root with little or no reproduction; very low galling); R = resistant (limited reproduction with final nematode population lower than initial; low galling); MR = moderately resistant (final population lower or similar than initial; galling scarce, although noticeable); and S = susceptible (nematode densities greatly increased and abundant galling). *P. vulnus* reproduction was assessed at harvest as described for *Meloidogyne,* but without NaOCl. A host rating system was established based on initial population/final population ratios (Pf/Pi): NH = non host (nematode not found in the roots); PH = poor host (Pf/Pi <3); H = host (Pf/Pi >3).

Data were subjected to a one-way analysis of variance. Number of galls per plant, final nematode population, and nematodes per gram of root data were transformed by $\log_{10}(x + 1)$ before analysis. When F values were significant, means were compared by Fisher's LSD test ($P \leq 0.05$).

Results

Greenhouse—M. incognita: Bruce, Cadaman, Mirac, G × N No. 15, Cachirulo × (G

× N No. 9), and *P. myra* × peach rootstocks exhibited lower ($P \leq 0.05$) galling and numbers of nematodes per gram of root than susceptible Rutgers Red Leaf, Balones, and GF-677 rootstocks (Table 3). Galling and nematodes were highest in GF-677. Bruce showed a lower final nematode population than Cachirulo × (G × N No. 9), *P. myra* × peach, and the three susceptible rootstocks. Cadaman also differed from *P. myra* × peach and the three susceptible rootstocks.

Greenhouse—P. vulnus: Comparative reproduction of *P. vulnus* was gradual among rootstocks. The plum hybrid P 2588 showed a significantly lower final nematode population than all except Mirac and P 2481 (Table 4). Cadaman and Ma × Ma achieved the highest values in nematode reproduction and differed from P 2588 and Mirac. No differences were found between most of the tested rootstocks. P 2588 and Mirac also supported fewer numbers of nematodes per gram of root but did not differ from P 2481. Parasitism was also lower in P 2481 when compared to Cachirulo × (G × N No. 9), Cadaman, and Ma × Ma. The hybrid plum P 2588 had the lowest Pf/Pi ratio (3.0) and Ma × Ma the highest (13.3).

Resistance verification: All plant materials tested, with exception of GF-677, exhibited different levels of resistance ranging from immunity (GF-31) to moderate resistance (Barrier) (Table 5). GF-31, G × N No. 15, Torinel, AD-101, Monpol, and Cadaman showed little or no galling ($P \leq 0.05$) as compared to Nemaguard, Barrier, and GF-677. The peach-almond hybrid GF-677 was heavily galled. Significant differences were found in the final nematode populations among resistant rootstocks. These ranged from 0 in GF-31 to 1,080 per g of root in Barrier (Pf/Pi < 1). Only GF-677 showed a high nematode reproduction. Nematodes per gram of root presented the same general pattern of significance as root galling.

Discussion

The plum hybrids Bruce and Mirac, and Cadaman peach were the most interesting rootstocks tested in the first screening trial (Table 3). These materials showed no galling and an absence of nematodes in the roots, indicating that *M. incognita* was incapable of reproducing on these materials (immunity). Furthermore, Bruce was a poor host to *P. vulnus* in two previous trials conducted in Spain using mixtures of several *P. vulnus* isolates (unpubl.). Bruce also has been found to be resistant to *P. vulnus* in the United States (Ledbetter, pers.

TABLE 3. Galling and reproduction of a mixture of seven isolates of *Meloidogyne incognita* on interspecific *Prunus* rootstocks at 120 days after inoculation with 2,000 nematodes per plant in 3 liters of soil.

Rootstock[a]	Number of galls per plant	Final nematode population (roots and soil)	Nematodes per gram of root	Resistance rating[b]
Bruce	0 a	0 a	0 a	I
Cadaman	0 a	5 ab	0 a	I
Mirac	0 a	19 abc	0 a	I
G × N No 15	3 a	20 abc	9 a	HR
Cachirulo × (G × N No 9)	6 a	84 bc	10 a	HR
P. myra × peach	11 a	124 c	11 a	HR
Rutgers Red Leaf	65 b	2,550 d	65 b	S
Balones	93 c	5,410 d	93 b	S
GF-677	170 d	9,790 d	170 c	S

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

[b]I = immune (no nematodes or galls); HR = highly resistant (nematode may invade root, little or no reproduction, very low galling); S = susceptible (nematode reproduces well with abundant galling).

TABLE 4. Reproduction of *Pratylenchus vulnus* on interspecific *Prunus* rootstocks 120 days after inoculation with 600 nematodes per plant in 3 liters of soil.

Rootstock[a]	Final nematode population (roots and soil)	Nematodes per gram of root	Pf/Pi	Host rating[b]
P 2588	1,800 a	150 a	3.0	PH
Mirac	3,540 ab	150 a	5.9	H
P 2481	3,880 abc	320 ab	6.5	H
P. myra × peach	4,330 bc	630 bc	7.2	H
Nemaguard	5,140 bc	690 bc	8.5	H
Cahirulo × (G × N No 9)	6,690 bc	980 cd	11.2	H
Cadaman	7,650 c	1,220 de	12.7	H
Ma × Ma	9,773 c	2,810 e	13.3	H

[a] Data are means of six replications. Actual data are presented, but data were transformed by $\log_{10}(x + 1)$ before analysis. Means in the same columns followed by the same letter do not differ significantly according to Fisher's LSD test ($P \leqslant 0.05$).
[b] PH = poor host (Pf/Pi lower than 3); H = host (Pf/Pi higher than 3.1).

comm.). This plant genotype is one of the few rootstock materials that exhibit different levels of resistance to both *M. incognita* and *P. vulnus* (multiple resistance). Bruce propagates easily, although not much is known about its agronomic adaptation to Mediterranean environments. Its future use in plant breeding is of high interest.

Detecting resistance and tolerance in both wild and commercial *Prunus* spp. against *P. vulnus* has had limited success in Spain (16,24–26). The plum hybrid P 2588 was a poor host for *P. vulnus* and is considered the most promising rootstock evaluated in this trial (Table 4). This experimental genotype requires further testing to both single and multiple *P. vulnus* isolate inoculations that might allow the detection of partial sources of resistance and confirm the poor host response observed in these studies. The rest of the tested rootstocks were suitable hosts, and likely susceptible.

Using multiple *Meloidogyne* spp. isolate inoculation within a rootstock selection scheme is considered rigorous and reliable. The majority of the rootstocks evaluated in the resistance verification test maintained their high level of resistance observed in previous screening tests with single-species or isolate inoculations (9). However, Barrier peach was inconsistent

TABLE 5. Galling and reproduction of *Meloidogyne* on *Prunus* rootstocks at 120 days after inoculation with 5,000 nematodes per plant in 3 liters of soil. The inoculant included a mixture of 17 isolates of *M. incognita* (5), *M. javanica* (5), *M. arenaria* (5), *M. hapla* (1), and *M. hispanica* (1).

Rootstock[a]	Number of galls per plant	Final nematode population (roots and soil)	Nematodes per gram of root	Resistance rating[b]
GF-31	0 a	0 a	0 a	I
G × N No 15	0 a	50 ab	5 a	HR
Torinel	0 a	170 abc	0 a	HR-I
AD-101	0 a	190 bcd	17 a	HR
Monpol	2 a	350 cd	17 a	HR
Cadaman	0 a	470 de	0 a	HR-I
Nemaguard	13 b	370 de	7 a	HR
Barrier	24 b	1,080 e	195 b	MR
GF-677	104 c	18,540 f	2,010 c	S

[a] Data are means of eight replications. Actual data are presented, but data were transformed by $\log_{10}(x + 1)$ before analysis. Means in the same columns followed by the same letter do not differ significantly according to Fisher's LSD test ($P \leqslant 0.05$).
[b] I = immune (no nematodes or galls); HR = highly resistant (nematode may invade root, little or no reproduction, very low galling); MR = moderately resistant (population lower or similar than initial; galling scarce, although noticeable); S = susceptible (nematode reproduces well with abundant galling).

and disappointing (moderately resistant) in relation to results obtained previously. After subjecting it to a larger pathogenic diversity of root-knot nematodes as in this experiment, its poor behavior suggests that one or several isolates were capable of reproducing on Barrier peach. This underscores the importance of using as many isolates as possible to ensure that the high level of resistance observed in screening trials will be maintained and prevail in warm Mediterranean environments.

Many commercial and experimental *Prunus* genotypes (almond, peach, peach-almond hybrids, plums, and interspecific hybrids) have been evaluated and studied against root-knot nematodes in recent years (9,17). Some of these genotypes have been bred for specific agronomic traits such as resistance to chlorosis, dryland conditions, vigor, ease of propagation, and root-knot nematode resistance (8). Most of the materials tested in this study incorporate new sources of resistance in interspecific *Prunus* and plums against the most common root-knot nematode species of the Mediterranean region. Such sources of nematode resistance will allow a higher diversification in stone fruit production zones, taking into account a better adaptation of plant material to dryland and calcareous soil conditions.

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