Resistance among New *Prunus* Rootstocks and Selections to Root-knot Nematodes in Spain and France

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Abstract. New *Prunus* rootstocks and selections were evaluated for their reaction to *Meloidogyne arenaria* (Neal) Chitwood, *M. incognita* (Kofoid & White Chitwood), or *M. javanica* (Treub) Chitwood. Most of the clones were peach-almond hybrids (P-AHs) [*P. persica* (L.) Batsch x *P. dulcis* (Mill.) D.A. Webb] or plums of Spanish and French origin. In a first experiment, the P-AH Hansen 2-168 and plums GF-31 (*P. cerasifera* Ehr.) and GF 8-1 (*P. cerasifera* x *P. munsoniana* Weighth et Hedr.) were highly resistant to the mixture of five isolates of *M. javanica*. The P-AHs Barrier and Titan x Nemared were resistant and moderately resistant, respectively; GF-677, MB 3-13, MB 2-2, and MB 2-6 were susceptible. In the second and third experiment, the plums P 1079, P 2175, the hybrids Afgano (*P. dasycarpa* Ehrh.), G x N No 22, and G x N No 15, both P-AHs, and Nemared peach were highly resistant to mixtures of five isolates of *M. incognita* or *M. arenaria*. The plums P 2980 (*P. cerasifera*) and GF 8-1 tested against either root-knot species were also highly resistant. Cachirulo x (G x N No 9), a P-AH, showed less resistance to *M. arenaria* than to *M. incognita*. Montclar (*P. persica*) and the P-AHs Torrents AC and GF-677 were susceptible to both species.

The *Meloidogyne* spp. are common in nurseries and *Prunus* orchards in Spain and in southern France (De Guiran, 1993; García de Otazo, 1992; Pinochet et al., 1992; Scotto La Massese, 1989). Losses caused by root-knot nematodes in peach, plum (*Prunus* spp.), and almond [*P. dulcis* (Mill.) Webb] production are unknown, although they are likely to be similar to those that occur in other production areas that share similar environmental and agronomic conditions (Lamberti, 1981; McKenry, 1989; Nyczepir, 1991). Root-knot-resistant rootstocks that are available commercially, such as Nemaguard, Hansen 2-168, Titan x Nemaguard, Nemared, and Saint Julien

655-2, do not always adapt well to Mediterranean growing conditions, suffering badly from iron chlorosis, root asphyxia, and root diseases (Felipe, 1989; Socías, 1990).

In the last decade, a significant effort has been made in Spain and France to incorporate resistance against root-knot nematodes into new *Prunus* rootstocks, mainly peach-almond hybrids (P-AHs) and plums (Bernhard et al., 1985; Felipe et al., 1990). These are replacing widely used seedling stocks, which, in general, lack homogeneity and are susceptible to root-knot nematodes. Several interesting genotypes in their late stages of selection from breeding programs of Spanish and French research centers have shown a high degree of resistance with single isolate inoculations (Esmenjaud et al., 1994; Marull et al., 1991; Pinochet et al., 1989). Nevertheless, the responses of new advanced breeding lines, as well as new commercial introductions to this pest, are not known. The high intraspecific variability in *Meloidogyne* spp. clearly indicates the need for testing plant material to several populations of the same species to assure broad resistance (Esmenjaud et al., 1994). To achieve this goal, inoculations with

mixed populations of the same root-knot species were performed. This investigation was conducted under greenhouse conditions to determine the comparative reaction of 19 experimental and commercial *Prunus* rootstocks to the three most common root-knot nematode species in the Mediterranean region, *Meloidogyne arenaria*, *M. incognita*, and *M. javanica*.

Materials and Methods

Seeds, herbaceous and hardwood cuttings, and micropropagated material were supplied by public research institutes and private sources in Spain and France. The main horticultural features of each rootstock are given in Table 1. Seeds of the peach rootstocks Nemared, Montclar, and P-AH Titan x Nemared were treated with a 5% solution of copper oxychloride for 24 h, rinsed with running water, covered with a moist paper towel, and stratified in perlite on trays that were kept in a storage room at 4°C for 45 days. Then, seed material was moved to ambient temperature in a greenhouse to induce germination. Herbaceous and hardwood cuttings of G x N No 15, G x N No 22, MB 2-2, MB 2-6, MB 3-13, Cachirulo x (G x N No 9), and Afgano were treated for 6 to 10 sec with a 50% ethanol solution that contained 2000 ppm of indole butyric acid (Hansen and Hartman, 1967). Cuttings then were planted in small pots (200 cm³) containing a 3 sand : 1 peat mixture (v/v) previously pasteurized at 80°C. Micropropagated P-AHs GF-677, Barrier, Torrents AC, Hansen 2-168, and plums GF-31 and GF 8-1 were received as plantlets from Agromillora Catalana S.A., Sant Sadurní d'Anoia, Barcelona, Spain; transferred from agar to 50-cm³ minipots with peat substrate; and climatized in a high-humidity chamber for 24 days. The micropropagated plums P 1079, P 2175, and P 2980 were supplied bare root from the Fruit Breeding Stations of the Institut National de la Recherche Agronomique in Bordeaux and Avignon, France.

Plantlets with uniform growth arising from germinated seeds, rooted cuttings, and micropropagated material were transplanted to 3-liter containers filled with a pasteurized sandy loam-textured soil (84% sand, 14% silt, 2% clay) with pH 7.3, <2% organic matter, and a cation-exchange capacity <10 meq/100 g soil. Plants were kept in the greenhouse for 6 weeks before inoculation.

Three root-knot nematode species (five isolates per species) were originally collected from various hosts (mainly *Prunus*) and geographical localities (Table 2). Isolates were increased on tomato (*Lycopersicon esculentum* Mill. cv. 'Redondo Liso') 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 (Cenis, 1993). In cases of discrepancy in species identification between methods, the molecular technique was considered as a more reliable identification criterion. To assure a desired pathogenic diversity for testing plant material (Marull et al., 1994; Scotto La Massese et al., 1984),

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Table 1. Information of 19 *Prunus* rootstocks and selections evaluated against *Meloidogyne arenaria*, *M. incognita*, and *M. javanica*.

Rootstock	Species/selection	Origin ²	Main horticultural features
GF-677	Natural peach–almond hybrid	INRA, France	Good vigor, high compatibility, and resistant to iron chlorosis
G x N No 15	Peach–almond hybrid	SIA, Spain	Good vigor, easy propagation, and resistant to <i>M. arenaria</i> , <i>M. incognita</i> , and <i>M. javanica</i>
G x N No 22	Peach–almond hybrid	SIA, Spain	Good vigor, resistant to iron chlorosis and <i>M. javanica</i>
Hansen 2-168	Peach–almond hybrid	Univ. of Calif., USA	Good vigor, easy propagation, and resistant to <i>M. arenaria</i> , <i>M. incognita</i> , and <i>M. javanica</i>
Barrier	<i>P. persica</i> x <i>P. davidiana</i>	Univ. of Florence, Italy	Good vigor, early production, resistant to <i>Meloidogyne</i> spp., and cold sensitive
Torrents AC	Natural peach–almond hybrid	AC, Spain	Medium vigor and increased fruit size
Cachirulo x (G x N No 9)	Peach–almond hybrid	SIA, Spain	Medium vigor, red leaf, and easy propagation
MB 2-2	Peach–almond hybrid	IRTA, Spain	Good vigor and easy propagation
MB 2-6	Peach–almond hybrid	IRTA, Spain	Good vigor and easy propagation
MB 3-13	Peach–almond hybrid	IRTA, Spain	Good vigor and easy propagation
Titan x Nemared	Peach–almond hybrid	USDA, Calif., USA	Medium vigor, irregular in development, and resistant to <i>Meloidogyne</i> spp.
Nemared	<i>P. persica</i>	USDA, USA	Red leaf, resistant to <i>M. arenaria</i> , <i>M. hapla</i> , <i>M. incognita</i> , and <i>M. javanica</i>
Montclar	<i>P. persica</i>	INRA, France	Good vigor and good compatibility
GF 8-1	<i>P. marrianna</i>	INRA, France	Resistant to root asphixia
P 1079	<i>P. cerasifera</i>	INRA, France	Good vigor, resistant to <i>M. arenaria</i> , <i>M. hapla</i> , <i>M. hispanica</i> , <i>M. incognita</i> , and <i>M. javanica</i> . Low compatibility with peach varieties
P 2175	<i>P. cerasifera</i>	INRA, France	Good vigor, resistant to <i>M. arenaria</i> , <i>M. hapla</i> , <i>M. hispanica</i> , <i>M. incognita</i> , and <i>M. javanica</i> . Good compatibility with most peach varieties
P 2980	<i>P. cerasifera</i>	INRA, France	Good vigor, resistant to <i>M. arenaria</i> , <i>M. hispanica</i> , <i>M. incognita</i> , and <i>M. javanica</i> . Good compatibility with most peach varieties
GF-31	<i>P. cerasifera</i> x <i>P. salicina</i>	INRA, France	Good compatibility with apricot varieties
Afgano	<i>P. dasycarpa</i>	SIA, Spain	Easy propagation, resistant to iron chlorosis and drought

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inoculum for each nematode species consisted of a mixture of five isolates that included 1000 nematodes from each isolate (5000 nematodes per plant). Simultaneously to the *Prunus* inoculations, 1000 nematodes from each isolate were inoculated separately onto four 'Roma' tomato plants to confirm the infectivity of each isolate at inoculation time.

Three experiments were conducted, one in 1992 and two in 1993. The reaction of the *Prunus* rootstocks Barrier, Titan x Nemared, Hansen 2-168, MB 2-2, MB 2-6, MB-3-13, GF-677, GF-31, and GF 8-1 against a mixture of five isolates of *M. javanica* were evaluated

in the first experiment (1992). The P-AHs Hansen 2-168 and GF-677 were used as resistant and susceptible reference rootstocks, respectively. Plants with uniform growth of ≈ 30 cm in height (25 to 30 leaves) were inoculated with a suspension of 5000 eggs per plant through four holes in the soil, 3 cm from the base of the plant. Inoculum of each isolate was prepared by macerating infested tomato roots in a blender for 10 sec in a 0.12% to 0.15% solution of NaOCl to disperse egg masses. Eggs were concentrated in a 25 μ m-pore sieve (500 mesh) and rinsed with tap water before inoculation (Hussey and Barker, 1973).

In the second experiment, rootstocks G x N No 15, G x N No 22, Cachirulo x (G x N No 9), Torrents AC, GF-677, Afgano, Nemared, Montclar, P 1079, P 2175, and P 2980 were evaluated against a mixture of five isolates of *M. incognita*. In the third experiment, the rootstocks G x N No 15, G x N No 22, Cachirulo x (G x N No 9), Torrents AC, Titan x Nemared, Afgano, Nemared, Montclar, P 1079, P 2175, and GF 8-1 were evaluated against a mixture of five isolates of *M. arenaria*. In both experiments conducted in 1993, Nemared and Montclar peach were used as resistant and susceptible reference rootstocks, respectively. Inocula and inoculation were prepared as described for the first experiment. Most of the rootstocks tested against *M. javanica* were not included in evaluations to *M. arenaria* or *M. incognita*, since they had already been tested (Marull et al., 1991) or were not worth testing due to high susceptibility to *M. javanica*.

Plants were harvested 120 days after inoculation. Total number of galls, final nematode population per plant (soil and roots), and the numbers of nematodes per gram of root were determined. Nematodes in soil were obtained by placing the soil from each container in a large pan. Roots were washed free of soil particles in a second pan with a known volume of water. Contents of both pans were mixed and stirred for 1 min, and 250 cm³ of the resulting slurry was used as a sample (Marull and Pinochet, 1991). Nematodes then were extracted by differential sieving and sugar flotation (Jenkins, 1964). Nematode densities in the soil were calculated based on 250-cm³ aliquots. Nematodes in roots were extracted in the same manner as was the inoculum, but the entire root system was weighed, cut into pieces with shears, and macerated in a blender at 14,500 rpm in a stronger solution of NaOCl (0.25% to 0.30%) for three periods of 15 sec each, separated by two 5-sec intervals. This extra blending was needed to free eggs and second-stage juveniles embedded in the lignified root tissue. Nematodes then were concentrated using 150-, 74-, and 25- μ m pore sieves (100, 200, and 500 mesh, respectively). Root tissue and debris collected on the 100-mesh sieve were discarded. The resistance rating of each rootstock was estimated according to nematode reproduction and root galling: HR = highly resistant (nematode may or may not invade root, but there was little or no reproduction); R = resistant (limited reproduction with final nematode population lower than initial; incipient galling); MR = moderately resistant (final population lower or slightly higher than initial; galling scarce, although noticeable); and S = susceptible (nematode densities increased rapidly, causing abundant galling).

Plants were watered daily or as needed and fertilized with a full-strength Hoagland's (Hoagland and Arnon, 1950) nutrient solution once a week. Experiments were conducted in a greenhouse with controlled temperature range (23 to 32C). Inoculated containers were placed in a sand bed to avoid temperature and humidity fluctuations. In all experiments, each material was replicated eight times in a completely randomized design. Data were analyzed by a

Table 2. Information on host and geographic origin of 15 *Meloidogyne* isolates used for testing *Prunus* rootstocks.

Isolate/species ^z	Host	Geographic origin
<i>M. arenaria</i>	Carnation (<i>Dianthus caryophyllus</i> L.)	Seville, Spain
	Tomato (<i>Lycopersicon esculentum</i>)	Canet, Barcelona, Spain
	Peach (<i>Prunus persica</i>)	Sastre, Tarragona, Spain
	Peach	Abarán, Murcia, Spain
	Peach	Mendoza, Argentina
<i>M. incognita</i>	Peach	Caspe, Zaragoza, Spain
	Peach-almond hybrid (<i>P. persica</i> x <i>P. dulcis</i>)	Villaverde del Río, Seville, Spain
	Plum (<i>Prunus cerasifera</i>)	Andújar, Jaen, Spain
	Tomato	Provence, France
	Tomato	Cayene, French Guayana
<i>M. javanica</i>	Fig (<i>Ficus carica</i> L.)	Cabrils, Barcelona, Spain
	Almond (<i>Prunus dulcis</i>)	Reus, Tarragona, Spain
	Peach-almond hybrid	San José de la Rinconada, Seville, Spain
	Unknown	California, USA
	Peach	Anatolia, Turkey

^zIsolates were identified by random amplified polymorphic DNA technique.

one-way analysis of variance. Data for galling, final nematode population, and nematodes per gram of root data were $\log_{10}(x + 1)$ transformed. Means were compared by Tukey's multiple range test ($P \leq 0.05$).

Results and Discussion

All tomato plants used for checking the viability of each isolate before mixing in aqueous suspension at inoculation time were heavily galled 60 days after inoculation, indicating that every isolate was highly infective.

In the first experiment, the P-AH Hansen 2-168 and plums GF-31 and GF 8-1 were free of galls, and the final population (soil and roots) at 4 months after inoculation indicated a high level of resistance to the mixture of five isolates of *M. javanica* (Table 3). These three rootstocks differed from the hybrids Titan x Nemared (P-AH) and Barrier [*P. persica* x *P. davidiana* (Carr.) Franch.], which showed low galling and reproduction (although lower than the Pi), indicating a lower level of resistance than Hansen 2-168, GF-31, and GF 8-1. However, the relative differences in resistance between Hansen 2-168, GF-31, GF 8-1 vs. Titan x Nemared and Barrier would appear to be minimal in terms of field performance. These three rootstocks (Hansen 2-168, GF-31, and GF 8-1) also differed from Titan x Nemared, but not from Barrier in the number of nematodes per gram of root. The susceptible control rootstock GF-677 and the experimental P-AHs MB 2-2, MB 2-6, and MB 3-13 showed a high level of susceptibility, with abundant galling and nematode reproduction. MB 2-6 reached the highest level of parasitism in this study (Table 3).

In the second experiment using inoculation with *M. incognita*, the *Prunus* rootstocks Afgano, Nemared, P 1079, P 2175, P 2980, G x N No 15, G x N No 22, and Cachirulo x (G x N No 9) were highly resistant to the nematode, whereas GF-677, Montclar, and Torrents AC showed different degrees of susceptibility (Table 4).

In the third experiment (inoculation with *M. arenaria*), the rootstocks Afgano, Nemared,

P 1079, P 2175, GF 8-1, G x N No 15, G x N No 22, and Titan x Nemared showed a highly resistant response (Table 5). The P-AH Cachirulo x (G x N No 9) was moderately resistant, while Montclar and Torrents AC were susceptible. Nemared and Cachirulo x (G x N No 9) presented some galling, although in the case of Nemared, nematode reproduction was suppressed, whereas in the P-AH Cachirulo x (G x N No 9) reproduction was low. In these two experiments, the nine rootstocks evaluated to *M. incognita* and *M. arenaria* mixtures (but not mixtures of both

species) showed a similar response to both species except that Cachirulo x (G x N No 9) was less resistant to the mixture of *M. arenaria* isolates. Perhaps one or several isolates in this mixture were capable of attacking this rootstock.

Occasionally, third- and fourth-stage juveniles (J3 and J4) of *M. arenaria* and *M. incognita* were detected in root tissues of G x N No 15, G x N No 22, P 1079, and GF 8-1 in the absence of galling, indicating that penetration and minor development took place. However, nematode development appeared to be suppressed, as with other *Prunus* rootstocks that are root-knot-nematode resistant (Malo, 1967; Marull et al., 1994).

The plums P 1079 and P 2175 had been previously tested in France against single-isolate inoculations to a wide range of populations belonging to *M. arenaria*, *M. incognita*, *M. hapla* Chitwood, *M. hispanica* Hirschmann, and *M. javanica* (Esmenjaud et al., 1993; Esmenjaud et al., 1994). The expression of resistance in *Prunus* rootstocks infected with *Meloidogyne* spp. can be affected by high soil temperatures (Canals et al., 1992; Fernández et al., 1993; Wehunt, 1972). In this study, both plum rootstocks were evaluated under higher temperature regimes than those in France, but they maintained their high level of resistance and broad resistance to a mixture of root-knot nematodes. This study also confirms the broad resistance of G x N No 15 (Marull et al., 1994) and provides new information on the high level of resistance of G x N No 22, a selection

Table 3. Galling and reproduction of *Meloidogyne javanica* on nine *Prunus* rootstocks or selections 120 days after inoculation with 5000 nematodes per plant.

Rootstock or selection ^z	No. galls/plant	Final nematode population (soil and roots)	Nematodes/g root	Resistance rating ^y
Hansen 2-168	0 a	0 a	0 a	HR
GF-31	0 a	0 a	0 a	HR
GF 8-1	0 a	0 a	0 a	HR
Barrier	13 b	440 b	20 ab	R
Titan x Nemared	8 b	2,820 b	350 b	MR
GF-677	116 c	16,680 c	1,220 c	S
MB 3-13	129 c	23,630 c	5,440 cd	S
MB 2-2	76 c	33,970 c	8,250 d	S
MB 2-6	80 c	77,330 c	23,630 d	S

^zData are means of eight replications. Actual data are presented, but data were transformed to $\log_{10}(x + 1)$ for analysis. Mean separation in columns by Tukey's multiple range test ($P \leq 0.05$).

^yHR = highly resistant; R = resistant; MR = moderately resistant; S = susceptible.

Table 4. Galling and reproduction of *Meloidogyne incognita* on 11 *Prunus* rootstocks or selections 120 days after inoculation with 5000 nematodes per plant.

Rootstock or selection ^z	No. galls/plant	Final nematode population (soil and roots)	Nematodes/g root	Resistance rating ^y
Afgano	0 a	0 a	1 a	HR
Nemared	0 a	0 a	0 a	HR
P 1079	0 a	0 a	0 a	HR
P 2175	0 a	0 a	0 a	HR
P 2980	0 a	0 a	0 a	HR
G x N No 15	0 a	20 a	3 a	HR
G x N No 22	0 a	10 a	2 a	HR
Cachirulo x (G x N No 9)	1 a	17 a	0 a	HR
GF-677	121 b	4,650 b	510 b	S
Montclar	186 c	14,300 b	3,660 bc	S
Torrents AC	185 c	58,780 b	5,490 c	S

^zData are means of eight replications. Actual data are presented, but data were transformed to $\log_{10}(x + 1)$ for analysis. Mean separation in columns by Tukey's multiple range test ($P \leq 0.05$).

^yHR = highly resistant; S = susceptible.

Table 5. Gallings and reproduction of *Meloidogyne arenaria* on 11 *Prunus* rootstocks or selections 120 days after inoculation with 5000 nematodes per plant.

Rootstock or selection ^a	No. galls/plant	Final nematode population (soil and roots)	Nematodes/g root	Resistance rating ^y
Afgano	0 a	0 a	1 a	HR
Nemared	6 ab	50 a	0 a	HR
P 1079	0 a	0 a	0 a	HR
P 2175	0 a	198 a	10 ab	HR
GF 8-1	0 a	32 a	3 ab	HR
G x N No 15	0 a	58 a	1 a	HR
G x N No 22	0 a	100 a	2 a	HR
Titan x Nemared	1 ab	50 a	4 ab	HR
Cachirulo x (G x N No 9)	5 b	1,210 a	220 b	MR
Montclar	138 c	11,420 b	690 c	S
Torrents AC	117 c	28,070 b	2,640 c	S

^aData are means of eight replications. Actual data are presented, but data were transformed to $\log_{10}(x + 1)$ for analysis. Mean separation in columns by Tukey's multiple range test ($P \leq 0.05$).

^yHR = highly resistant; MR = moderately resistant; S = susceptible.

that has also proved to adapt well to very calcareous soils. Both G x N selections derive from crosses between Nemared peach (Ramming and Tanner, 1983) and the Spanish almond Garfi (Felipe et al., 1990).

We conclude that several new *Prunus* rootstocks and selections, especially plums (Afgano, P 1079, and P 2175) and P-AHs (Titan x Nemared, G x N No 15, G x N No 22), have desirable resistance to at least two common species of *Meloidogyne*, and they offer an effective control alternative to this pest. Although Barrier and P 2980 were tested only against one root-knot species, they showed a good level of resistance. The majority of these materials also are well adapted agronomically to warm Mediterranean environments (Felipe et al., 1990). A few are available commercially, while others are in late stages of selection.

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