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Reaction of *Prunus* Rootstocks to *Meloidogyne incognita* and *M. arenaria* in Spain[1]

J. MARULL, J. PINOCHET, S. VERDEJO, AND A. SOLER[2]

Abstract: *Prunus* rootstocks were evaluated for their reaction to *Meloidogyne incognita* and *M. arenaria.* Most rootstocks were peach–almond hybrids of Spanish origin. In one experiment three selections of Garfi × Nemared (G × N) and Hansen-5 were highly resistant to *M. incognita,* but four other rootstocks were susceptible showing high galling indices and population increases. In two experiments with *M. arenaria,* the hybrid selections G × N nos. 1 and 9 were immune, GF-305 and Hansen-5 were resistant, but nine other rootstocks expressed various degrees of susceptibility. All Spanish rootstocks were susceptible to both *Meloidogyne* species except for the three G × N selections. The root-knot nematode resistant peach Nemared used as a male parent with Garfi was found to transmit a high degree of resistance to *M. incognita* and immunity to *M. arenaria.* Progenies of *P. davidiana* (Ga × D no. 3), a known source of resistance to root-knot nematodes, were susceptible.
Key words: *Meloidogyne arenaria, M. incognita,* nematode, *Prunus,* resistance, root-knot nematode, rootstock.

In Spain, peach, *Prunus persica* Stock., is cultivated in ca. 60,000 ha in the Ebro Valley, Levante, and Andalucia. Almond (*Prunus amygdalus* Batsch) production is concentrated in the Mediterranean region and Baleares Islands with a cultivated area of 600,000 ha (7). Seedlings have been used to propagate both fruit tree species in the country, although in the last decade, the use of peach–almond hybrids has increased rapidly.

The presence of *Meloidogyne* spp. in orchards appears to be common in Spain (1,6,10). Losses caused by root-knot nematodes may be similar to those occurring in other Mediterranean areas (16). While there is ample information on the relative degree of resistance in *Prunus* rootstocks to *Meloidogyne* spp. in the United States (3,13,22,23), France (21), Israel (14,17), and Italy (5,24,25,27), there is only limited information on the reaction of peach–almond hybrids to root-knot nematodes in Spain.

Root-knot nematode resistant rootstocks, such as Nemaguard, Nemared, Hansen-2, and Hansen-5, are available in Spain. There is a growing interest in their use, although commercial distribution is still limited because these materials do not adapt well to growing conditions found in many areas of Spain. Some of these rootstocks suffer badly from iron chlorosis (8) and are more or less susceptible to root rots caused by *Armillaria* sp., *Phythophthora* spp., or *Agrobacterium tumefaciens.* Recent studies (9,18) suggest that some new Spanish rootstocks with desirable agronomic features are quite susceptible to *Meloidogyne* spp., which could generate serious production problems in some replant situations. The purpose of this study was to evaluate the reaction of *Prunus* rootstocks to *Meloidogyne incognita* (Kofoid & White) Chitwood and to *M. arenaria* (Neal) Chitwood, under greenhouse conditions.

MATERIALS AND METHODS

Plant materials evaluated included 11 peach–almond hybrids, two almonds, one peach seedling, and one wild *Prunus* species, *P. webbii* (Table 1). Plant material was supplied by the Programa de Fruticultura of the Servicio de Investigación Agraria of the Diputación General de Aragón in Zaragoza and by the Departamento de Arboricultura Mediterránea of the Institut de Recerca i Tecnologia Agroalimentàries in Mas Bové, Tarragona. The majority of the rootstocks are of Spanish origin, several

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[2] Graduate Assistant, Research Nematologists, and Assistant, Departamento de Patología Vegetal, Institut de Recerca i Tecnologia Agroalimentàries, IRTA, Crta. de Cabrils s/n 08348, Cabrils, Barcelona, Spain.

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TABLE 1. Origin and features of 15 *Prunus* rootstocks evaluated for reaction to *M. incognita* and *M. arenaria* in Spain.

Rootstock	Species or selection	Origin†	Main agronomic features
Adafuel	Natural peach–almond hybrid	C.S.I.C., Zaragoza, Spain	Good vigor, resistant to iron chlorosis, adapted to poor soils
Bergasa	Natural peach–almond hybrid	C.S.I.C., Zaragoza, Spain	Good vigor
Fermoselle	Natural peach–almond hybrid	C.S.I.C., Zaragoza, Spain	Good vigor
G × N no. 1	*P. amygdalus* × Nemared peach	S.I.A., Zaragoza, Spain	Good vigor, resistant to *M. incognita* and *M. arenaria*
G × N no. 3	*P. amygdalus* × Nemared peach	S.I.A., Zaragoza, Spain	Good vigor, resistant to *M. incognita*
G × N no. 9	*P. amygdalus* × Nemared peach	S.I.A., Zaragoza, Spain	Good vigor, resistant to *M. incognita* and *M. arenaria*
Ga × D no. 3	*P. amygdalus* × *P. davidiana*	S.I.A., Zaragoza, Spain	Good vigor, adapted to poor soil conditions
MB 1-35	Peach–almond hybrid	I.R.T.A., Tarragona, Spain	Good vigor, easy propagation
MB 2-2	Peach–almond hybrid	I.R.T.A., Tarragona, Spain	Good vigor, easy propagation
GF-677	Natural peach–almond hybrid	I.N.R.A., France	Good vigor, high compatibility, resistant to iron chlorosis
Hansen-5	Peach–almond hybrid	U. of California, Davis, U.S.A.	Good vigor, early production, resistant to *Meloidogyne* spp.
GF-305	*P. persica*	I.N.R.A., France	Homogeneous rootstock, resistant to some *Meloidogyne* spp.
Garrigues	*P. amygdalus*	Unknown, originally from Murcia, Spain	Good vigor, adapted to poor soil conditions, high productivity
Moncayo	*P. amygdalus*	S.I.A., Zaragoza, Spain	Good vigor, self compatible, late variety
P. webbii	*P. webbii*	Wild *Prunus* of Mediterranean origin	Adapted to dryland conditions, source of autocompatibility in almond

† C.S.I.C. = Consejo Superior de Investigaciones Cientificas; I.R.T.A. = Institut de Recerca i Tecnologia Agroalimentàries; S.I.A. = Servicio de Investigación Agraria; I.N.R.A. = Institut National de la Recherche Agronomique.

being new releases or materials in the process of selection.

Peach–almond hybrids were propagated from wood cuttings, whereas *P. webbii*, the peach GF-305, and the almond selections Garrigues and Moncayo originated from seed. Wood cuttings were treated for 6–10 seconds with a 50% alcohol and water solution containing 2,000 ppm of indole butyric acid. Almond, peach, and *P. webbii* seeds were kept in water for 3 days, stratified in perlite, covered with moist paper, and maintained in the dark at 5 C for 45 days until radicle emergence. Seeds of the peach rootstock GF-305 were maintained at 5 C for 120 days. Both germinated seeds and wood cuttings were planted in 200-cm^3 pots containing a 1:1 (v:v) sand and peat mixture previously pasteurized at 80 C. Rooted plant material was transplanted to 2-liter pots containing a pasteurized sandy loam (73% sand, 21% silt, 6% clay).

The isolate of *M. incognita* race 1 was collected from kiwi (*Actinidia deliciosa* (A. Chev.) Liang & Ferguson), in Tordera, Barcelona, and the isolate of *M. arenaria* came from tomato (*Lycopersicon esculentum* Mill.), in Canet, Barcelona. Both isolates were increased from single egg mass cultures on the tomato cultivar Roma. Both root-knot isolates were quite aggressive in previous *Prunus* screening tests (26). In three separate greenhouse experiments, plants with uniform growth were inoculated with a suspension of 5,000 eggs of *M. incognita* or *M. arenaria* per plant 60–80 days after transplanting. Nematode inoculum was prepared by macerating infected

TABLE 2. Gall indices and final population densities of *Meloidogyne incognita* on eight *Prunus* rootstocks at 4 months after inoculation with 5,000 nematodes/plant.

Rootstock	Gall index†	Final nematode population (soil and root)‡	Nematodes/g root	Resistance rating§
G × N no. 1	1.0 a	34 a	0 a	HR
G × N no. 3	1.0 a	74 a	10 a	HR
G × N no. 9	1.0 a	83 a	24 a	HR
Hansen-5	2.2 a	140 a	23 a	R
Bergasa	6.0 c	5,700 ab	790 ab	S
Prunus webbii	5.1 b	21,370 c	1,600 b	S
Adafuel	6.0 c	14,090 bc	1,940 b	S
Ga × D no. 3	6.0 c	55,560 d	8,050 c	S

Data are means of seven replications. Values in a column followed by the same letter do not differ significantly according to Duncan's multiple-range test ($P = 0.05$).
† 1 = no galls, 2 = 1–10 galls, 3 = 11–30, 4 = 31–70, 5 = 71–90, 6 = 91–100 galls per plant.
‡ Total numbers of nematodes per plant.
‡ HR = highly resistant; R = resistant; S = susceptible.

tomato roots in a blender for 10 seconds in a 0.12–0.15% NaOCl solution (11). Eggs were concentrated in a 25-μm-pore sieve (500 mesh) and rinsed with tap water before inoculation. Each experiment was set up in a completely randomized design with seven replications per treatment.

Gall index, final nematode population per plant (soil and roots), and numbers of nematodes per gram of root were determined 120 days after inoculation. Gall indices were established using a 1–6 scale (2): 1 = 0 galls; 2 = 1–10 galls; 3 = 11–30 galls; 4 = 31–70 galls; 5 = 71–90 galls; 6 = 91–100 galls per plant. Nematodes in soil were obtained by removing soil from pots and placing it in a large pan. Roots were washed free of soil particles in a second pan. Contents of both pans were mixed and stirred for 1 minute. A 250-cm^3 subsample of the slurry was removed and extracted by differential sieving and sugar flotation (12).

Nematode extraction from the roots was similar to that described for inoculum preparation, except that the entire root system was cut into pieces and macerated in a blender for three periods of 15 seconds separated by two intervals of 10 seconds in a 0.25–0.30% NaOCl solution. Nematodes were then concentrated using 150 μm-pore, 75 μm-pore, and 25 μm-pore sieves (100, 200, and 500 mesh, respectively). Root tissue and debris collected on the 0.150-mm-pore sieve were discarded. The resistance rating of each rootstock was estimated according to the scale of Taylor and Sasser (28), based on nematode reproduction and root galling. In this scale, I = immune (plant does not allow penetration of the nematode); HR = highly resistant (nematode invades root but there is little or no reproduction); R = resistant (limited reproduction with final nematode populations lower than initial, incipient galling); MR = moderately resistant (final populations equal or slightly higher than the initial, galling scarce although noticeable); S = susceptible (nematode densities increase rapidly, causing abundant galling).

Plants were watered daily or as needed and fertilized with full-strength Hoagland's solution once a week. Data on gall index, total nematode population, and nematodes per gram of root were log transformed ($x + 1$) and analyzed by one-way analysis of variance. Means were compared by Duncan's multiple-range test ($P = 0.05$).

RESULTS

In the first experiment, the three G × N selections (nos. 1, 3, and 9) were highly resistant to *M. incognita* (Table 2). Galls were not observed on these rootstocks at the end of the experiment. Galling indices on the three G × N selections and Hansen-5 differed ($P = 0.05$) from the four remaining susceptible rootstocks. The G × N selections and Hansen-5 had significantly

TABLE 3. Gall indices and final population densities of *Meloidogyne arenaria* on eight *Prunus* rootstocks at 4 months after inoculation with 5,000 nematodes/plant.

Rootstock	Gall index†	Final nematode population (soil and root)‡	Nematodes/g root	Resistance rating§
G × N no. 1	1.0 a	0 a	0 a	I
GF-305	2.0 b	430 a	7 b	R
Fermoselle	3.6 c	21,240 b	2,637 cd	S
Bergasa	5.9 d	25,000 b	1,613 c	S
GF-677	6.0 d	23,260 b	1,771 c	S
Garrigues	5.0 d	53,920 c	2,849 cd	S
Moncayo	6.0 d	60,490 c	3,023 cd	S
Adafuel	5.3 d	56,450 c	4,985 d	S

Data are means of seven replications. Values in a column followed by the same letter do not differ significantly according to Duncan's multiple-range test ($P = 0.05$).
† Index given in Table 2.
‡ Total numbers of nematodes per plant.
§ I = immune; R = resistant; S = susceptible.

lower final populations than Bergasa, Adafuel, *P. webbii,* and Ga × D no. 3. The hybrid selection Ga × D no. 3, a cross between the almond Garrigues and *Prunus davidiana* Franch., proved to be more susceptible ($P = 0.05$) than the rest of the tested materials.

In the second experiment, G × N no. 1 was immune to *M. arenaria* (Table 3). Neither galls nor nematodes were detected in the soil or root tissues at the end of the evaluation. The peach rootstock, GF-305, had a lower ($P = 0.05$) gall index and final populations than all other rootstocks except G × N no. 1. The six susceptible rootstocks showed high levels of parasitism, ranging from 1,613 (Bergasa) to 4,955 (Adafuel) nematodes per gram of root.

In the third experiment, G × N no. 9 was immune and Hansen-5 resistant to *M. arenaria* (Table 4). Nematode numbers and gall indices on both peach–almond hybrids were less ($P = 0.05$) than on the susceptible rootstocks MB 1-35, GF-677, and MB 2-2.

DISCUSSION

Our results showed that there is resistance to *M. incognita* in the rootstocks G × N nos. 1, 3, and 9 and immunity to *M. arenaria* in G × N nos. 1 and 9. These are the only Spanish peach–almond hybrids selected for a high degree of resistance against root-knot nematodes, out of 25 entries evaluated in this and in previous studies (18,26; Marull, unpubl.). The G × N selections were derived from crosses be-

TABLE 4. Gall indices and final population densities of *Meloidogyne arenaria* on five *Prunus* rootstocks at 4 months after inoculation with 5,000 nematodes/plant.

Rootstock	Gall index†	Final nematode population (soil and root)‡	Nematodes/g root	Resistance rating§
G × N no. 9	1.0 a	0 a	0 a	I
Hansen-5	1.0 a	320 a	0 a	HR
MB 1-35	4.0 b	12,470 b	1,502 b	S
GF-677	4.9 c	12,110 b	2,380 b	S
MB 2-2	5.4 c	15,100 b	4,947c	S

Data are means of seven replications. Values in a column followed by the same letter do not differ significantly according to Duncan's multiple-range test ($P = 0.05$).
† Index given in Table 2.
‡ Total numbers of nematodes per plant.
§ I = immune; HR = highly resistant; S = susceptible.

tween the almond Garfi (female parent) and the root-knot nematode resistant peach Nemared (19). Garfi is a selection of Garrigues, a widely used almond rootstock which is vigorous and well adapted to dry and calcareous soils but highly susceptible to *M. incognita, M. arenaria, M. javanica,* and *M. hapla* (26). The resistance and immunity against two *Meloidogyne* species found in Nemared was transmitted to F1 progenies of G × N, suggesting that these traits are determined by one or a few dominant genes. This pattern of inheritance is similar to that found in other resistant peach and almond germplasm, like Nemaguard against *M. incognita* (4), Okinawa and Jusei against *M. incognita* and *M. javanica* (29), and bitter almond against *M. javanica* (15).

The peach–almond hybrid Ga × D no. 3 was the most susceptible to *M. incognita* (Table 2). It is derived from the susceptible almond Garrigues and the wild peach *P. davidiana,* which is resistant to several species of *Meloidogyne* (4,20,21).

In the Mediterranean region, the peach rootstock GF-305 has shown a variable response to several species of root-knot nematodes. In France and Spain, GF-305 was resistant to *M. hapla* but susceptible to *M. incognita* and *M. javanica* (20,17); it was also found to be susceptible to *M. arenaria* in France, although in our study it was found to be resistant to this nematode species. These differences may be due to an evaluation method in which a mixture of 17 isolates of *M. arenaria* from Europe and Africa was used in screening procedures (21). It is possible that differences in pathogenicity among isolates of *M. arenaria* accounted for this discrepancy. This procedure apparently simulates more closely the conditions found in field situations and may be a correct approach to rootstock selection against *Meloidogyne* spp.

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