Resistance of Peach and Plum Rootstocks from Spain, France, and Italy to Root-knot Nematode *Meloidogyne javanica*

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Abstract. Two trials involving 20 *Prunus* rootstocks were conducted under greenhouse conditions to screen for resistance to root-knot nematode [*Meloidogyne javanica* (Treub.) Chitwood]. Many of the tested materials are interspecific hybrid rootstocks and represent new commercial peach (*P. persica* Batsch) and plum (*Prunus* sp.) releases or experimental genotypes of Spanish, French, and Italian origin. In the first trial, the rootstocks Adesoto 101 (*P. insititia* L.), Bruce (*P. salicina* Lindl. x *P. angustifolia* Marsh.), Ishtara, AC-952 (*P. insititia*), Garnem [*P. dulcis* (Mill.) D.A. Webb x *P. persica*], Cadaman [*P. persica* x *P. davidiana* (Carr.) Franch], and Orotava (*P. salicina*) were immune or resistant to a mixture of 10 isolates of *M. javanica*. The remaining rootstocks, Myrocal (*P. cerasifera* Ehr.), Montclar (*P. persica*), and Adafuel (*P. dulcis* x *P. persica*), were susceptible. In the second screening trial, the plum rootstocks Adesoto 101, Adara (*P. cerasifera*), Myro-10 (*P. cerasifera*), Constantí (*P. domestica* L.), and AD 105 (*P. insititia*) were immune to the root-knot nematode. Cadaman, G x N No. 17 (*P. dulcis* x *P. persica*), and Tetra (*P. domestica*) were resistant. Laroda F1OP (*P. salicina*), Myro-almond (*P. cerasifera* x *P. dulcis*), and the peach–almond hybrids Mayor, Adafuel, and Sirio were susceptible.

Root-knot nematodes (*Meloidogyne* sp.) can become a limiting factor in peach and plum production in Mediterranean environments (Lamberti, 1981; McKenry, 1989; Nyczepir and Halbrendt, 1993; Pinochet, 1997), especially in replant sites that have a history of nematode infestation (García de Otaño, 1992; Scotto La Massese, 1989). Several species of root-knot nematodes are present in Spain, but *M. javanica* is predominant in the warmer areas of the Levante Region (Valencia, Alicante and Murcia) and Andalucía, where most of the peaches, nectarines, and plums for export are produced. The area of production, especially for peach, has steadily increased

since the late 1980s and seems to have stabilized in the mid-1990s (Badenes et al., 1998). In general, root-knot nematodes are common and tend to be a serious problem in warm, well-drained, sandy soils. High susceptibility of peach and plum seedling rootstocks, and the widespread use of the susceptible peach–almond hybrid GF-677 as a rootstock have significantly contributed to an increase in the incidence of replant problems caused by root-knot nematodes in Spain, thus seriously affecting the competitiveness of the fresh peach and plum industry.

A significant effort has been made in Spain to incorporate resistance to root-knot nematodes into new peach, peach–almond hybrid, plum, and interspecific *Prunus* rootstocks (Esmenjaud et al., 1993, 1994; Fernández et al., 1994; Marull et al., 1991), with the intention of replacing the widely used seedling rootstocks. Seedling rootstocks often lack homogeneity, tend to suffer badly from root asphyxia and iron chlorosis, and are susceptible to root-knot infestation (Felipe, 1989; Socías, 1990). This breeding effort has been constant in the last decade resulting in the introduction of new material that has multiple

resistance and/or tolerance to several biotic and abiotic stresses (Felipe et al., 1990).

The root-knot nematode resistance trait is easy to transmit by hybridization. It is apparently controlled by one major gene or a few dominant genes (Cook and Evans, 1987; Esmenjaud et al., 1997). Screening procedures in rootstock selection involve repeated testing of plant material with mixtures of populations involving one or several species of *Meloidogyne* to ensure broad resistance (De Guiran, 1993; Marull et al., 1994; Scotto La Massese et al., 1984). Some of the rootstocks tested in this study have been bred specifically for root-knot nematode resistance and have been evaluated in the past against *M. incognita* Kofoid and White (Pinochet et al., 1996) but not against *M. javanica*. This last species attacks rootstocks that are supposedly resistant to root-knot nematode, especially peach and peach–almond hybrids, indicating differences in virulence between *Meloidogyne* species (Esmenjaud et al., 1994).

The purpose of our study was to evaluate new commercial releases and experimental *Prunus* genotypes in their late stages of selection from Spanish, French, and Italian breeding programs to determine their response to *M. javanica*, using mixtures of many isolates of the nematode (large pathogenic diversity).

Materials and Methods

Rootstock material. Seeds, hardwood cuttings, and micropropagated material were supplied by Spanish and French public research institutes and private sources (Table 1). Seeds of the peach rootstock Montclar were treated with a 5% solution of copper oxychloride for 36 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 60 d. Seeds were then moved to ambient temperature in a greenhouse to induce germination. Hardwood cuttings of the rootstocks G x N No. 17, AD 105, Constantí, Bruce, Orotava, Myro-almond, Myro-10, and Adara were treated for 6–10 s with a 50% alcohol solution that contained 1000 mg·L⁻¹ of indole butyric acid (Hansen and Hartman, 1967). Cuttings then were planted in 200-mL minipots containing a pasteurized (80 °C) 2 sand : 1 peat (v/v) mixture. Montclar seeds were provided by Viveros Orero, Villaverde del Río, Sevilla. Hardwood cuttings were provided by the Dept. de Fruticultura of the Servicio de Investigación Agraria in Zaragoza, Spain, and by the Dpto. de Fruticultura, Estación Experimental Aula Dei, Zaragoza, Spain. Micropropagated Adafuel, Cadaman, Garnem, Adesoto 101, Mayor, Myrocal, Ishtara, Laroda F1OP, and AC-952 were provided as plantlets in 300-mL minipots by Agromillora Catalana S.A., Sant Sadurní d'Anoia, Barcelona, Spain, in a Moor peat and clay substrate (Stender Bassis Substrate 3, Blumenerdenwerke Stender® GmbH, Schermbeck, Germany). Micropropagated Tetra and Sirio rootstocks were supplied by Vivai Battistini, Diegaro di Cesena, Italy.

Plantlets with uniform growth arising from germinated seeds, rooted cuttings or micro-

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Table 1. Peach and plum rootstocks and selections evaluated against *Meloidogyne javanica* in Spain.

Rootstock	Species/ selection	Source ^a
Montclar	Peach (<i>P. persica</i>)	INRA, France
Cadaman	Peach (<i>P. persica</i> × <i>P. davidiana</i>)	INRA, France
Adafuel	Peach-almond (<i>P. dulcis</i> × <i>P. persica</i>)	CSIC, Spain
Sirio	Peach-almond (<i>P. persica</i> × <i>P. dulcis</i>)	U. Pisa, Italy
G × N No. 17	Peach-almond (<i>P. dulcis</i> × <i>P. persica</i>)	SIA-DGA, Spain
Garnem	Peach-almond (<i>P. dulcis</i> × <i>P. persica</i>)	SIA-DGA, Spain
Mayor	Peach-almond (<i>P. persica</i> × <i>P. dulcis</i>)	CIDA, Spain
Adesoto 101	Plum (<i>P. insititia</i>)	CSIC, Spain
AD-105	Plum (<i>P. insititia</i>)	CSIC, Spain
AC-952	Plum (<i>P. insititia</i>)	AC, Spain
Tetra	Plum (<i>P. domestica</i>)	ISF, Italy
Constantí	Plum (<i>P. domestica</i>)	CSIC, Spain
Orotava	Plum (<i>P. salicina</i>)	Unknown, Spain
Laroda F1OP	Plum (<i>P. salicina</i>)	IFS, Italy
Myrocal	Plum (<i>P. cerasifera</i>)	INRA, France
Ishtara	Plum [<i>P. belsiana</i> × (<i>P. cerasifera</i> × <i>P. persica</i>)]	INRA, France
Bruce	Plum (<i>P. salicina</i> × <i>P. angustifolia</i>)	Texas A&M, USA
Myro-almond	Plum (<i>P. cerasifera</i> × <i>P. dulcis</i>)	CSIC, Spain
Myro-10	Plum (<i>P. cerasifera</i>)	SIA-DGA, Spain
Adara	Plum (<i>P. cerasifera</i>)	CSIC, Spain

^aINRA = Institut National de la Recherche Agronomique, Bordeaux, France; CSIC = Consejo Superior de Investigaciones Científicas, Zaragoza, Spain; U. Pisa = Univ. of Pisa, Pisa, Italy; SIA-DGA = Servicio de Investigación Agraria de la Diputación General de Aragón, Zaragoza, Spain; CIDA = Centro de Investigación y Desarrollo Agrario, Murcia, Spain; AC = Agromillora Catalana, S.A., Barcelona, Spain; ISF = Istituto Sperimentale per la Fruticoltura di Roma, Italy; Texas A&M Univ., College Station, Texas, USA.

propagated material were transplanted to 4.5-L containers filled with a pasteurized sandy loam textured soil (85% sand, 12% silt, 3% 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 4 weeks to allow root growth before inoculation.

Inoculum source and preparation. Twelve isolates of *M. javanica* were used in two experiments. Nematodes were originally collected from different hosts (mainly *Prunus*) and geographical localities (Table 2). Most of these isolates have proven to be highly pathogenic on *Prunus* in previous screening trials. 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 isozyme analysis (Esbenshade and Triantaphyllou, 1990). In cases of discrepancy in species identification between methods, the biochemical technique was considered the more reliable. Inoculum of each isolate was prepared by macerating infested tomato roots in a blender for 10 s in a 0.12% to 0.15% NaOCl solution. Nematode eggs and larvae (≈20:1) were concentrated in a 25-μm pore sieve (500-mesh) and rinsed with tap water before inoculation (Hussey and Barker, 1973). Inocula, consisting of mixtures of 10 isolates in equal proportion, were adjusted to deliver 1000 eggs and larvae per plant in each experiment.

Greenhouse experiments. Two screening trials, one conducted in 1997 and the other in 1998, were carried out under similar greenhouse conditions. The response of the *Prunus* rootstocks Bruce, Ishtara, AC-952, Adesoto 101, Myrocal, Garnem, Cadaman, Adafuel, Montclar, and Orotava to a mixture of 10 isolates of *M. javanica* were evaluated in the first trial. Cadaman and Adesoto 101 were used as resistant standards, and Montclar as a susceptible control. Plants with uniform height

of ≈15–20 cm (10 to 15 leaves) were inoculated through four holes in the soil 3 cm from the base of the plant with a suspension of 1000 nematodes per plant containing an equal proportion of 10 isolates of *M. javanica*.

In the second screening trial, the rootstocks Cadaman, Mayor, Sirio, G × N No. 17, Adafuel, Adara, Adesoto 101, Tetra, Myro-10, AD 105, Constantí, Laroda F1OP, and Myro-almond were also evaluated against a mixture of 10 isolates of *M. javanica*. The isolates Mj NC-US and Mj AN-TU used in the first trial were not available, and were replaced by isolates Mj MA-MX and Mj OU-M. As in the first trial, Cadaman and Adesoto 101 were used as resistant standards, while Adafuel was used as a susceptible control. Inoculation procedures were carried out as in the previous trial.

Plants were watered daily or as needed, and fertilized twice, at the beginning of the experiment and 3 months thereafter, with Osmocote® Plus (15N–10P–12K + micronutrients; Sierra Grace España, S.A.), a slow-release fertilizer. During the course of the study, inoculated pots were placed in sand beds in a greenhouse to minimize substantial fluctuations in temperature and humidity. Mean ambient temperature between day and night in the greenhouse fluctuated between 14 and 37 °C. In both experi-

ments, each rootstock was replicated seven times in a completely randomized design.

Plants were harvested 120 and 140 d after inoculation in the first and second trial, respectively. The percentage of galled root system (Barker, 1985), final nematode population in roots, and the numbers of nematodes per gram of fresh weight (FW) of roots were determined. Nematodes in roots were extracted in the same manner as described for the inoculum preparation, but the entire root system was weighed, cut into 1-cm pieces, and macerated in a blender at 14,500 rpm in a NaOCl solution (0.25% to 0.30%) for three periods of 15 s each, separated by two 5-s intervals. Nematodes then 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 (100 mesh) were discarded. Root-knot resistance ratings were 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 and incipient 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 increased rapidly causing abundant galling).

Data were analyzed by a one-way analysis of variance. Data for the final nematode population and the number of nematodes per gram of root data were log₁₀ (x+1) transformed for analysis. The percentage of galled root system were transformed to arcsin (√x/100) for analysis. When F values were significant, means were compared by Tukey's multiple range test (*P* ≤ 0.05).

Results and Discussion

Rootstock materials exhibited different levels of response ranging from immunity to susceptibility to *M. javanica*. In the first trial, the rootstocks Adesoto 101, Bruce, Ishtara, AC-952, Garnem, and Cadaman were free of galls and no nematode reproduction was recorded (Table 3). This group differed from Orotava plum, which showed minor galling and some reproduction, and which was rated as moderately resistant. The remaining rootstocks, Myrocal, Montclar, and Adafuel,

Table 2. Isolates of *Meloidogyne javanica* used for evaluating peach and plum rootstocks in Spain.

Isolate	Source host	Geographic origin
Mj CA-S	Fig (<i>Ficus carica</i> L.)	Cabrils, Barcelona, Spain
Mj MB-S	Almond	Mas Bové, Tarragona, Spain
Mj CN-US	Tomato (<i>Lycopersicon esculentum</i>)	Raleigh, N.C., USA
Mj AN-TU	Peach	Anatolia, Turkey
Mj AL-PO	Almond	El Algarve, Portugal
Mj SE-S	Peach	Camas, Seville, Spain
Mj LP-S	Peach-almond	Los Palacios, Seville, Spain
Mj TE-S	Banana (<i>Musa</i> AAA)	Tenerife, Canary Islands, Spain
Mj SJ-S	Peach-almond	S. José de la Rinconada, Seville, Spain
Mj EX-S	Grape (<i>Vitis vinifera</i> L.)	Badajoz, Extremadura, Spain
Mj OU-M	Peach	Oualidia, Morocco
Mj NA-MX	Tomato	Nayarit, Mexico

Table 3. Gallings by and reproduction of a mixture of ten isolates of *Meloidogyne javanica* on peach and plum rootstocks at 120 d (Trial 1) or 140 d (Trial 2) after inoculation with 1000 eggs and larvae per plant.

Rootstock ^z	Percentage of galled root system	Final nematode population in root system	Nematodes per gram FW of root	Resistance rating ^y
<i>Trial 1</i>				
Adesoto 101	0 a ^x	0 a	0 a	I
Bruce	0 a	0 a	0 a	I
Ishtara	0 a	0 a	0 a	I
AC-952	0 a	0 a	0 a	I
Cadaman	0 a	0 a	0 a	I
Garnem	0 a	0 a	0 a	I
Orotava	7 b	46 b	17 b	MR
Myrocal	28 c	3,839 c	402 c	S
Montclar	45 c	18,800 d	1,393 cd	S
Adafuel	68 c	47,660 d	3,850 d	S
<i>Trial 2</i>				
Adesoto 101	0 a ^x	0 a	0 a	I
Adara	0 a	0 a	0 a	I
Myro-10	0 a	0 a	0 a	I
Constantí	0 a	0 a	0 a	I
AD-105	0 a	0 a	0 a	I
Cadaman	0 a	30 b	0 a	HR
G x N No. 17	0 a	252 b	24 b	HR
Tetra	5 b	70 b	8 a	R
Laroda F1OP	43 c	46,480 c	5,900 c	S
Myro-Almond	54 c	56,730 c	8,243 c	S
Mayor	61 c	156,310 c	9,800 c	S
Adafuel	80 c	120,939 c	13,290 c	S
Sirio	76 c	187,900 c	11,330 c	S

^zData are means of seven replications. Actual data are presented, but data were transformed to log₁₀ (x+1) for analysis. Percentage of gallings based on arcsin ($\sqrt{x}/100$) transformed values for analysis.

^yI = immune; HR = highly resistant; R = resistant; MR = moderately resistant; S = susceptible.

^xMean separation within columns by Tukey's multiple range test, $P \leq 0.05$.

were susceptible and showed extensive galling and nematode reproduction. The highest population increases were recorded on Montclar and Adafuel.

In the second trial, the rootstocks Adesoto 101, Adara, Myro-10, Constantí, AD 105, Cadaman, and G x N No. 17 showed no galling. These rootstocks differed from Tetra, which showed minor galling equivalent to 5% of its root system affected. The other rootstocks, Laroda F1OP, Myro-almond, Mayor, Adafuel, and Sirio, were extensively galled. Larger-size galls (>5 mm diameter) were observed in Mayor and Adafuel. Significant differences were found in the final nematode population among resistant rootstocks, some of which showed no nematode reproduction (Adesoto 101, Adara, Myro-10, Constantí, and AD 105), whereas Cadaman, G x N No. 17, and Tetra presented some minor nematode reproduction. However, whether *M. javanica* indeed reproduced on Cadaman and G x N No. 17, or if the specimens extracted are remnants of the initial inoculum, is difficult to determine since galling was not found on either rootstock. In contrast, Laroda F1OP, Myro-almond, Mayor, Adafuel, and Sirio showed higher nematode reproduction. Nematodes per gram of root presented a similar pattern of significance as for root galling.

Other than the resistant standards Cadaman and Adesoto 101, 13 of the rootstocks evaluated in this study had never been tested against root-knot nematodes. Also, Bruce, Garnem (previously known as G x N No. 15), and Mayor (previously known as Balones) had been evaluated against another root-knot spe-

cies, *M. incognita* (Fernández et al., 1994; Pinochet et al., 1996). Bruce and Garnem also had a high level of resistance to *M. javanica*, but Mayor appeared to be more affected (considerably higher galling and nematode population buildup) by the mixture of 10 populations of *M. javanica*. This discrepancy is probably due to differences in experimental conditions, or to the larger pathogenic diversity of the *M. javanica* isolates used in our study.

New rootstocks will be one of the most important factors in the success of orchards in the future (Weil, 1998). The incorporation of root-knot nematode resistance into new rootstock material that has also been selected for a wide range of adverse agronomic conditions (calcareous soils, dryland conditions, heavy soils, salinity, and soilborne pathogens), will allow a greater diversification of peach, plum, and apricot (*P. armeniaca* L.) cultivation in Mediterranean production zones, and especially in replant situations (Nyzcepir, 1991; Pinochet, 1997). Also, the gradual introduction of these materials into the market, both in Europe as well as in the United States, will take place in the next few years prior to the banning of methyl bromide fumigant in the year 2005 (Hansen, 1999). This should allow sufficient time for testing so horticultural techniques can be commercially adapted. This would be very important in areas that are currently dependent on the use of fumigants for orchard establishment. The use of these new rootstocks should be considered as the soundest alternative to soil fumigation from both economic and environmental points of view.

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