Plant Pathology (2011) Doi: 10.1111/j.1365-3059.2010.02404.x

Host suitability of *Vitis* rootstocks to root-knot nematodes (*Meloidogyne* spp.) and the dagger nematode *Xiphinema index*, and plant damage caused by infections

C. Gutiérrez-Gutiérrez[a], J. E. Palomares-Rius[a], R. M. Jiménez-Díaz[a,b] and P. Castillo[a]*

[a]*Instituto de Agricultura Sostenible (IAS), Consejo Superior de Investigaciones Científicas (CSIC), Apdo. 4084, 14080 Córdoba; and* [b]*Escuela Técnica Superior de Ingenieros Agrónomos y Montes, Universidad de Córdoba, Edificio C-4 'Celestino Mutis', Campus de Rabanales, Ctra. Madrid-Cádiz, km 396, 14071 Córdoba, Spain*

The host suitability of commercial *Vitis* rootstocks commonly used in Spain (161-49C, 41B, 1103P, 110R, 140Ru and SO4) to root-knot nematodes (*Meloidogyne arenaria*, *M. incognita*, *M. javanica*) and *Xiphinema index*, and damage caused by nematode infection were determined under controlled conditions. The three root-knot nematodes reproduced with a rate higher than one in all rootstocks, indicating that they are suitable hosts for these nematodes. Growth of rootstocks infected with the root-knot nematodes was less vigorous than that of nematode-uninfected controls in the majority of the rootstocks studied. Root infection resulted in moderate to severe root galling in all rootstocks. The shoot and main stem diameters appeared to be the most sensitive variables of damage caused by infection by *Meloidogyne* spp., with reduction rates from 36% and 53% in 161-49C to 57% and 66% in 140Ru, respectively. The shoot height was not significantly affected by the root-knot nematodes and the root fresh weight generally increased as a consequence of intensive galling. The nematode *X. index* caused significant root damage with a reproduction factor higher than one in all rootstocks. However, reproduction factor was significantly influenced by the rootstock and significantly decreased by about 12-fold (5·7 to 18·1-fold) with the increase in inoculum density from 100 to 1000 nematodes per plant. The root dry weight was reduced by *X. index* infections, and was the plant growth variable most affected by the nematode infection in all rootstocks at both inoculum densities. *Meloidogyne arenaria*, *M. incognita*, *M. javanica* and *X. index*, prevalent in many world vineyards, are all shown to have a damaging effect on the six tested rootstocks.

Keywords: dagger nematode, grapevine, nematode reproduction, root-knot nematode, *Vitis* spp.

Introduction

Grapevine (*Vitis vinifera*) cultivation for wine and table-grape production is one of the most extensive fruit-crop systems grown under temperate and Mediterranean climates worldwide. Grapevine is host to a large variety of plant-parasitic nematodes, the most common of which include *Meloidogyne* spp. (root-knot nematodes), *Xiphinema* spp. (dagger nematodes), *Pratylenchus* spp. (root-lesion nematodes), *Longidorus* spp. (needle nematodes), *Criconemoides* spp. (ring nematodes) and some species in the genera *Rotylenchus* and *Helicotylenchus* (spiral nematodes) (Nicol *et al.*, 1999; Téliz *et al.*, 2007). *Meloidogyne* spp. and *Xiphinema index* are primary nematode pathogens of grapevines. These nematodes can impair the establishment of new grapevine plantations and reduce the vigour, yield and productive life span of already established vineyards as well as enhance infection and damage of their roots by other pathogens (Anwar *et al.*, 2002; Esmenjaud & Bouquet, 2009). *Meloidogyne arenaria*, *M. incognita* and *M. javanica* cause significant economic losses in sandy soil-grown grapevines under the mild temperature conditions that prevail in most grapevine-growing areas of California, the Mediterranean Basin and South Africa (Nicol *et al.*, 1999; Esmenjaud & Bouquet, 2009). Similarly, *X. index* severely damages grapevines both directly (Di Vito *et al.*, 1985) and as a vector of *Grapevine fanleaf virus* (GFLV) (Hewitt *et al.*, 1958). Grapevine fanleaf is a devastating viral disease in many grape-growing areas around the world (Andret-Link *et al.*, 2004), for which the most effective control measure consists of soil fumigation for eradicating or reducing populations of the nematode (Esmenjaud & Bouquet, 2009). However, the phase-out of methyl bromide and

*E-mail: pcastillo@ias.csic.es

sharp restrictions in the use of other nematicide fumigants worldwide, pose an urgent need for development of nematode-resistant *Vitis* spp. rootstocks as the most promising and practical control alternative to the use of soil fumigants (Esmenjaud & Bouquet, 2009).

The suitability of a plant as a host for plant-parasitic nematode species is defined as its capacity to sustain the nematode feeding and reproducing on it. Host suitability may be expressed objectively as the ratio of the number of nematode units recovered at the end of a nematode-infection assay (final nematode population density (Pf)), to the number of nematode units used to inoculate a plant (initial population density (Pi)) (Lewis, 1987). The host suitability of *Vitis* rootstocks to root-knot nematodes and *X. index* can be determined by assessing the severity of root galling and nematode reproduction on rootstocks after artificial inoculations (Nicol *et al.*, 1999; Esmenjaud & Bouquet, 2009). When a compatible host-parasite interaction has been established by a nematode, infection of the plant usually impairs physiological processes leading to pathogenesis (Perry & Moens, 2006). This physiological impairment can be assessed by reduction in plant growth variables. In general, impairment of plant growth caused by *Meloidogyne* spp. is influenced by the nematode species and/or aggressiveness, as well as the initial nematode population density in the soil at crop establishment (Perry *et al.*, 2009). In grapevine, that damage is also related to *Vitis* rootstocks and grapevine cultivars. The relationship between nematode population density and grapevine growth has already been described for *X. index* on *V. vinifera* cv. Aglianico (Di Vito *et al.*, 1985), and more recently for *M. incognita* race 1 on 1103P rootstock (Sasanelli *et al.*, 2006). However, the reaction of *Vitis* rootstocks to those nematodes may be influenced by differences in the methodology used by independent researchers (Nicol *et al.*, 1999), as well as differences in reproductive potential among the nematode population as shown for populations of *X. index* from Italy, California, Israel and France (Coiro *et al.*, 1990).

In previous studies, some *Vitis* rootstocks (e.g. 1103P, 110R, 140Ru, Rupestris du Lot, SO4) that were previously considered moderately resistant to *M. arenaria*, *M. incognita* and *M. javanica*, or to *X. index* (Nicol *et al.*, 1999), were shown to be susceptible to these root-knot nematodes under field conditions in Spain (Téliz *et al.*, 2007). Other *Vitis* rootstocks were susceptible to these nematodes, i.e. 161-49C to *Meloidogyne* spp. (Nicol *et al.*, 1999), 41-B to *X. index* (Coiro *et al.*, 1985). However, no information was available concerning the reaction of those rootstocks to populations of local nematode populations from Spain. Attacks by *Meloidogyne* spp. and *X. index* can reduce plant growth and promote early senescence of grapevine thus increasing yield loss (Nicol *et al.*, 1999). However, there are few reports on the host suitability of *Vitis* rootstocks to *Meloidogyne* spp. or *X. index* and the resulting plant impairment after infection by the nematode (Anwar & Van Gundy, 1989; Sasanelli *et al.*, 2006). Therefore, the objectives of this research were to: (i) determine the host suitability of six commercial *Vitis* rootstocks commonly used in France, Italy and Spain to isolates of *M. arenaria*, *M. incognita*, *M. javanica* and *X. index* infecting grapevine in Spain, under controlled conditions; and (ii) to assess plant damage caused by infection with these nematodes. The *Vitis* rootstocks selected for the study were: Couderc 161-49 (161-49C; *V. riparia* × *V. berlandieri*), 41 B Millardet et De Grasset (41B; *V. vinifera* Chasselas × *V. berlandieri*), Paulsen 1103 (1103P; *V. berlandieri* × *V. rupestris*), Richter110 (110R; *V. berlandieri* × *V. rupestris*), Ruggeri 140 (140Ru; *V. berlandieri* × *V. rupestris*), and Selection Oppenheim 4 (SO4; *V. berlandieri* × *V. riparia*). They were selected because of their known agronomic traits (i.e. reportedly resistant or tolerant to plant-parasitic nematodes and phylloxera, and tolerant to calcium excess in soil, drought, salinity and grafting affinity). *Vitis vinifera* cv. Cabernet Sauvignon was used as a susceptible control to all nematode species.

Materials and methods

Nematode inocula

Isolates of *M. arenaria*, *M. incognita*, *M. javanica* and *X. index* used in this study were obtained from roots of naturally-infected *Vitis* rootstocks or their rhizosphere in commercial vineyards in Andalusia, southern Spain (Téliz *et al.*, 2007) (Table 1).

Inocula of *Meloidogyne* spp. were increased on tomato plants (*Solanum lycopersicum* cv. Tres Cantos) grown in clay pots filled with an autoclaved (120°C, 2 h) sandy soil mixture, starting from a single egg mass for each species. Plants were kept in a growth chamber adjusted to 25 ± 1°C, 60–90% relative humidity, and a 16 h photoperiod of fluorescent light at 360 ± 25 μE m^{-2} s^{-1} for 2 months. Isolates of root-knot nematodes were identified to species level and race based on features of the female perineal pattern and response of differential hosts to inoculation and reproduction of the nematode (Hartman & Sasser, 1985). Morphological identification of

Table 1 Root-knot and dagger nematode isolates used in this study, with reference to source of *Vitis* rootstocks and geographic origin in southern Spain

Nematode species	Rootstock source	Geographic origin
Meloidogyne arenaria race 2	Rupestris du Lot[a]	Almonte, Huelva province
Meloidogyne incognita race 1	Richter 110[a]	Bollullos par del Condado, Huelva province
Meloidogyne javanica	Richter 110[a]	Montemayor, Córdoba province
Xiphinema index	Couderc 161-49[b]	Moriles, Córdoba province

[a]Rootstock from which nematodes were isolated.
[b]Nematodes were extracted from the rhizosphere of the rootstock.

Meloidogyne spp. was also confirmed by analysis of isozyme esterases (Esbenshade & Triantaphyllou, 1985) and sequence characterized amplified region (SCAR)-based PCR assays (Zijlstra *et al.*, 2000). Inocula consisted of eggs and second-stage juveniles (J2) extracted from 2-month-old tomato cultures using 1% sodium hypochlorite (Hussey & Barker, 1973) followed by centrifugal flotation (Coolen, 1979).

The single *X. index* isolate in the study was reared on fig *(Ficus carica)* plants grown as above and inoculated by pouring 100 nematode females or juveniles suspended in sterile water on the root ball of a plant. Inoculated plants were grown in a growth chamber adjusted as above for more than 18 months to eliminate its potential as vector of GFLV. After that period, nematodes were confirmed virus-free by reverse transcription (RT)-PCR assays (Demangeat *et al.*, 2004). The virus-free nematode population was then increased on healthy, virus-free certified Cabernet Sauvignon grapevines grown in clay pots filled with an autoclaved sandy soil. The virus-free status of the grapevines used for rearing *X. index* was also confirmed by RT-PCR assay (Demangeat *et al.*, 2004).

Plant material

Vitis rootstocks selected for the study were self-propagated by rooting hardwood cuttings under a mist system in a growth chamber adjusted to 25 ± 1°C in the dark. Briefly, cuttings of each rootstock were selected from well-matured dormant canes of the preceding year's growth (0·5–1·0 cm in diameter, with 3–4 internodes) in a grapevine nursery producing virus-free certified planting stock located at Villarrobledo (Albacete province, central Spain). Stem cuttings were surface-disinfested in 10% NaOCl for 5 min and washed four times in sterile distilled water to prevent fungal contamination. Thereafter, the proximal extremity of cuttings was dipped in 1% indole butyric acid powder (Rootone® F, Compo) to promote rhizogenesis. The treated stem cuttings were planted in 30 L plastic pots containing pasteurized (70°C, 1 h) commercial peat (Torfwerke Ahrens GmbH & Co. KG) and grown for 2 months in a growth chamber adjusted to 25 ± 1°C, 60–90% relative humidity, and a 16 h photoperiod of fluorescent light at 360 ± 25 μE m^{-2} s^{-1}. Cuttings were kept moistened by a 30 s mist every 60 min for the first month, and thereafter they were watered as needed and fertilized with 100 mL of a 0·1% solution of a 20-5-32 + micronutrients hydro-sol fertilizer (Haifa Chemicals) every week. This procedure facilitates the rapid production of homogeneous plants suitable for the experiments.

Plants of uniform root system and shoot size were selected and transplanted into 75 × 77 × 180 mm plastic pots (one plant per pot) filled with an autoclaved (120°C, 1 h, twice) soil mixture (sand/clay loam, 2:1, vol/vol). Plants were watered on alternate days with 100 mL of sterilized tap water, fertilized with 100 mL of a 0·1% solution of a 20-5-32 + micronutrients hydro-sol fertilizer, and pruned to maintain a single shoot every week. Plants were allowed 7 days to recover from transplanting before inoculation with the nematodes.

Growth chamber experiments

Two experiments (I and II) were conducted in a growth chamber adjusted to the same conditions as indicated above. These environmental conditions are optimal for the development and reproduction of *Meloidogyne* spp. and *X. index* (Trudgill & Perry, 1994). For experiment I, plants were inoculated individually by adding 10 000 eggs + J2 of *Meloidogyne* spp. (*M. arenaria* race 2, *M. incognita* race 1 or *M. javanica*) in 10 mL of sterile distilled water. The nematode suspension was added in four holes in the soil of a pot around the base of the plant, which corresponds to a theoretical inoculum density of 9·62 nematode cm^{-3} soil. For experiment II, plants were inoculated by adding 100 or 1000 specimens (all stages) of *X. index* in 10 mL of sterile distilled water. The nematode suspension was added to soil as before, which corresponds to a theoretical inoculum density of 0·1 or 1 nematode cm^{-3} soil. In both experiments, the nematode inoculum density of the water suspension was determined by counting nematode specimens in ten 1-mL aliquots.

Plants were watered with 100 mL of water on alternate days and fertilized weekly with 100 mL of the previously mentioned nutrient solution. The experiments consisted of a factorial treatment design with eight replicated plants per treatment in a completely randomized design. The experiments ended 120 days after inoculation of each nematode species. The experiments were repeated once.

Assessment of plant growth variables and data analysis

Plant growth, root galling and nematode reproduction were rated at the end of experiments I and II. The effects of treatments on plant growth were assessed by the root fresh (experiment I) or dry weight (experiment II), shoot dry weight, shoot height, and main stem and shoot diameters of individual plants in inoculated and control plants. Shoot height was measured from ground level, stem diameter was recorded 6–8 cm from soil level and shoot diameter was measured using the principal shoot located in the second internode. The three latter growth variables were measured both at the time of nematode inoculation and at the end of the experiments. The variation in those growth variables was referred to as net increase during the experiments (expressed as percentage of increase relative to initial measurements). For dry weights, plant parts were dried in the oven at 80ºC for 72 h. Before assessment of root weight, the root system of a plant was gently washed free of adhering soil and debris, and the root galling was assessed. For experiment I, the severity of root symptoms by *Meloidogyne* spp. were rated according to a 0–6 scale, where 0 = no galls; 1 = 1–10; 2 = 11–20; 3 = 21–40; 4 = 41–70; 5 = 71–90; and 6 ≥ 91 galls. For experiment II, the severity of root damage by *X. index* was assessed according to a 0–3 rating scale, where

0 = healthy root system; 1 = few localized swollen or curved root tips; 2 = enlarged swellings of root tips very evident throughout root system, and 3 = segments of roots greatly enlarged, little or no lateral root formation, attacks very intensive throughout root system.

Final soil and root populations of nematodes were determined by flotation-centrifugation and maceration-centrifugation, respectively (Coolen, 1979). For extraction of nematodes from soil samples by centrifugal flotation (Coolen, 1979), soil was washed thoroughly with tap water through a 710-μm mesh sieve and the filtered water was collected in a beaker and thoroughly mixed with 4% kaolin (v/v). This mixture was centrifuged at 1100 ***g*** for 4 min, then the supernatants were discarded, pellets were resuspended in 250 mL $MgSO_4$ (δ = 1·16) and the new suspensions were centrifuged at 1100 ***g*** for 3 min. Supernatants were sieved through 5 μm mesh, and nematodes collected on the sieve were washed with tap water, transferred to Petri dishes and counted under a stereomicroscope (Coolen, 1979). To assess nematode populations in *Meloidogyne*-infected roots, the complete root system of a plant was washed free of soil and cut into 1–2 cm segments, and nematodes (eggs and J2s) were extracted by maceration followed by centrifugation. Root tissues were homogenized in 250 mL of a 1% solution of NaOCl using a Waring blender at 1800 ***g*** for 1 min, and homogenates were centrifuged and extracted as described above (Hussey & Barker, 1973; Coolen, 1979). Population densities were used to calculate the reproduction index (Rf = final population density in soil and roots (Pf) divided by initial population density (Pi)). All data on severity of root symptoms, nematode reproduction and relative plant growth (X) were transformed into $\log_{10}(X + 1)$ and to arcsine-square root, respectively, before analysis (Gómez & Gómez, 1984). Similarity between the experiments was tested by preliminary analysis of variance using experimental runs as factors. The experiment × treatment interaction was determined as not significant ($P \geq 0{\cdot}05$) and thus the data of both experimental runs were combined for further analysis. Analyses of variance were carried out using Statistix 9.0 (NH Analytical Software). Significant differences among means of plant growth variables, severity of root symptoms and nematode reproduction were estimated using the LSD multiple range test ($P = 0{\cdot}05$). Data from uninoculated control treatments were not included in analysis of severity of root symptoms and nematode reproduction, to avoid the use of zero in analysis of variance.

Results

Suitability of *Vitis* rootstocks as hosts of *Meloidogyne* spp. and *Xiphinema index*

Symptoms on above ground plant parts did not appear on either nematode-inoculated or nematode-free control plants at the end of experiments I and II, 4 months after inoculation. However, either a significant ($P < 0{\cdot}05$) plant growth increase or decrease, or no significant ($P \geq 0{\cdot}05$) growth response occurred, depending upon the rootstock genotype-nematode combination (Tables 2 & 3).

In experiment I, all plants of the tested *Vitis* rootstocks were infected by each of the *M. arenaria*, *M. incognita* and *M. javanica* isolates and showed distorted feeder roots and root galls of large (5–9 mm) to moderate (2–3 mm) size. Galls occurred singly or in clusters (Fig. 1). Overall, the *Meloidogyne* isolates induced moderate to severe root galling in the tested rootstocks, the galling severity rating averaging 2·2, 2·8 and 2·5 for *M. arenaria*, *M. incognita* and *M. javanica*, respectively. The severity of root galling was significantly ($P < 0{\cdot}001$) influenced by the rootstock, the isolate of *Meloidogyne* species, and their interaction. Consequently, comparisons were carried out within rootstocks and within each *Meloidogyne* species (Table 2). Root galling induced by *M. arenaria* was significantly higher in Cabernet Sauvignon (41–70 galls per root system), intermediate in rootstocks 41B, 161-49C and 1103P, and had a lower severity in SO4, 140Ru and 110R (Table 2). Similarly, infection by *M. incognita* induced a severe root galling in Cabernet Sauvignon, followed by 41B (more than 40 galls per root system), and to a lesser extent in the other rootstocks (averaging 11–30 galls per root system) (Table 2). Infection by *M. javanica* also caused severe root galling in Cabernet Sauvignon; the severity of root symptoms was significantly different in *Vitis* rootstocks, with 110R and SO4 showing the lowest root galling (averaging < 20 galls per root system) (Table 2).

The three *Meloidogyne* species reproduced in all the tested *Vitis* rootstocks and Cabernet Sauvignon to a variable extent. However, the nematode reproduction rate (Rf) was significantly ($P < 0{\cdot}001$) influenced by the rootstock, the *Meloidogyne* species and their interaction (Table 2). Overall, the average Rf value for *M. arenaria* and *M. incognita* was significantly higher ($P < 0{\cdot}001$) in Cabernet Sauvignon than in the rootstocks. The rootstocks were classified according to Rf in decreasing order as follows: 41B, 161-49C, 140Ru, 1103P, 110R and SO4 (Table 2). The Rf value for *M. javanica* was significantly higher ($P < 0{\cdot}001$) in Cabernet Sauvignon, 161-49C and 41B than in the others rootstocks, with SO4 sustaining the lowest ($P < 0{\cdot}001$) reproduction rate (Table 2).

In experiment II, all plants of the tested *Vitis* rootstocks were infected by *X. index* irrespective of the initial inoculum density level used. Most of the nematode-infected plants had enlarged swellings of root tips extended throughout the root system (Fig. 2) with little or no lateral root formation (i.e. rating score ≥ 2). A minority of plants (9·1%) showed a few localized swollen or curved root tips (rating score 1; Fig. 2). The severity of root tip galling was significantly ($P < 0{\cdot}001$) influenced by the rootstock, initial inoculum density of *X. index* ($P < 0{\cdot}001$), and their interaction ($P = 0{\cdot}011$) (Table 3). Overall, root tip galling of plants inoculated with 100 *X. index* per plant was quite similar among rootstocks, averaging a rating of about 2 (enlarged swellings of root

Table 2 Host suitability of grapevine rootstock genotypes to root-knot nematodes (*Meloidogyne* spp.) and effects of infection by the nematodes on plant growth under controlled conditions[a]

			Increase during the experiment (%)[b]				
Rootstock genotype	Inoculation treatment	Root fresh weight (g)	Shoot height	Main stem diameter	Shoot diameter	Root symptoms[c]	Rf[d]
Cabernet Sauvignon	Uninoculated control	11·2 c	58·7 a	15·1 a	18·5 a	0	–
	M. arenaria race 2	16·7 ab	50·5 a	7·9 b	11·5 b	4·2 aA	14·2 bA
	M. incognita race 1	19·0 a	59·9 a	7·7 b	9·9 b	4·7 aA	29·2 aA
	M. javanica	13·3 bc	57·4 a	8·2 b	11·6 b	4·1 aA	6·6 aA
161-49C	Uninoculated control	12·4 a	57·7 a	9·6 a	22·1 a	0	–
	M. arenaria race 2	13·9 a	42·3 a	7·2 b	13·9 b	2·3 aB	4·1 bB
	M. incognita race 1	14·6 a	42·7 a	6·1 b	11·8 b	2·5 aC	8·6 aC
	M. javanica	13·6 a	57·3 a	7·5 b	12·8 b	2·3 aCD	6·3 aA
41B	Uninoculated control	14·0 a	45·6 a	16·7 a	16·9 a	0	–
	M. arenaria race 2	11·6 a	40·7 a	14·5 a	15·4 a	2·4 bB	2·7 cC
	M. incognita race 1	13·7 a	44·7 a	9·7 b	12·2 b	3·5 aB	12·5 aB
	M. javanica	13·8 a	41·6 a	9·3 b	11·4 b	3·0 aB	6·2 bA
1103P	Uninoculated control	10·7 c	50·4 a	16·3 a	17·9 a	0	–
	M. arenaria race 2	14·2 b	45·3 a	10·8 b	13·1 b	2·0 bBC	2·4 bC
	M. incognita race 1	19·3 a	40·6 a	9·3 b	10·9 b	2·3 abC	7·1 aC
	M. javanica	13·9 b	42·3 a	8·9 b	12·1 b	2·6 aBC	4·5 aBC
110R	Uninoculated control	15·0 a	50·6 a	11·9 a	16·0 a	0	–
	M. arenaria race 2	13·5 a	45·8 a	10·6 a	16·3 a	1·2 cE	1·5 cD
	M. incognita race 1	16·9 a	49·4 a	11·2 a	14·3 a	2·3 aC	6·8 aC
	M. javanica	15·4 a	38·8 a	12·4 a	15·9 a	1·8 bE	3·1 bCD
140Ru	Uninoculated control	11·4 b	52·6 a	15·3 a	14·6 a	0	–
	M. arenaria race 2	11·9 b	47·4 a	13·2 a	13·5 ab	1·5 cD	2·5 bC
	M. incognita race 1	15·5 a	45·9 a	6·8 b	9·6 c	2·5 aC	7·3 aC
	M. javanica	9·3 b	47·6 a	8·7 b	11·2 bc	2·0 bDE	5·3 aAB
SO4	Uninoculated control	10·8 a	49·7 a	13·0 a	15·4 a	0·0	–
	M. arenaria race 2	12·5 a	44·5 a	12·3 a	15·3 a	1·8 aC	2·3 aC
	M. incognita race 1	11·5 a	49·2 a	11·7 a	14·6 a	2·2 aC	2·3 aD
	M. javanica	11·1 a	41·9 a	11·0 a	15·2 a	1·4 bF	1·7 bD

–: not tested.

[a]Data are mean of 16 replicated plants per treatment combination. Plants were inoculated with 10 000 eggs + J2 (Pi) of *Meloidogyne* spp. For each grapevine rootstock, means followed by the same lowercase letter do not differ significantly ($P \geq 0{\cdot}05$) according to Fisher's protected LSD test. Upper-case letters refer to mean comparisons among rootstocks within each nematode species. Actual data are presented for each grapevine rootstock but severity of root symptoms, nematode population density and relative plant growth were transformed into $\log_{10}(X + 1)$ and to arcsine-square root, respectively, before analysis.

[b]Average percentage growth of each variable during the experiment.

[c]Assessed on a 0–6 rating scale according to the number of root galls developed, where 0 = no galls; 1 = 1–10; 2 = 11–20; 3 = 21–40; 4 = 41–70; 5 = 71–90; and 6 ≥ 91 galls.

[d]Nematode reproduction factor (Rf) = final nematode numbers per plant (Pf)/initial nematode inoculum per plant (Pi).

tips very evident throughout root system), 161-49C showing the lowest root tip galling (Table 3). Similarly, root tip galling of plants inoculated with 1000 *X. index* per plant was also similar among rootstocks, averaging more than 2 except for 161-49C, that showed the lowest root tip galling (Table 3). The increase in the initial inoculum density from 100 to 1000 nematodes per plant increased significantly ($P < 0{\cdot}05$) the root tip galling in Cabernet Sauvignon as well as in 161-49C and 110R rootstocks but not in the other rootstock genotypes (Table 3).

All *Vitis* rootstocks sustained the reproduction of *X. index* irrespective of initial inoculum density level (Table 3). The nematode reproduction rate (Rf) was significantly ($P = 0{\cdot}002$) influenced by the rootstock and significantly ($P < 0{\cdot}05$) decreased by about 12-fold (5·7 to 18·1-fold) with the increase in inoculum density of the nematode from 100 to 1000 nematodes per plant ($P < 0{\cdot}001$) (Table 3). The average Rf in 41B, 1103P, 110R and SO4 inoculated with 100 nematodes per plant did not differ from that in Cabernet Sauvignon and it was significantly higher ($P = 0{\cdot}002$) than the average Rf in 161-49C and 140Ru rootstocks sustaining the lowest reproduction rate (Table 3). Similarly, the average Rf in 1103P and SO4 inoculated with 1000 nematodes per plant

Table 3 Host suitability of grapevine rootstock genotypes to the dagger nematode, *Xiphinema index*, and effects of infection by the nematode on plant growth under controlled conditions[a]

			Increase during the experiment (%)[b]				
Rootstock genotype	Inoculation treatments	Root dry weight (g)	Shoot height	Main stem diameter	Shoot diameter	Root symptoms[c]	Rf[d]
Cabernet Sauvignon	Uninoculated control	4·2 a	48·1 a	16·3 a	21·1 a	0	–
	X. index 100	4·6 b	37·3 ab	7·2 b	13·1 b	2·2 bAB	74·2 aA
	X. index 1000	3·4 c	25·1 b	5·1 b	10·4 b	2·7 aA	9·1 bA
161-49C	Uninoculated control	4·3 a	37·5 a	10·0 a	23·0 a	0	–
	X. index 100	3·5 b	32·9 a	9·4 a	17·4 b	1·6 bC	39·7 aB
	X. index 1000	2·5 c	36·1 a	7·8 a	18·0 b	1·7 aC	2·2 bC
41B	Uninoculated control	3·8 a	30·1 a	14·5 a	16·2 a	0	–
	X. index 100	2·7 b	26·7 a	11·2 a	10·9 b	2·5 aA	70·1 aA
	X. index 1000	2·3 c	15·9 b	6·1 b	9·3 b	2·3 aAB	3·9 bB
1103P	Uninoculated control	3·9 a	54·9 a	24·1 a	22·3 a	0	–
	X. index 100	3·5 b	43·5 a	10·5 b	7·1 b	2·0 aBC	57·0 aAB
	X. index 1000	3·4 c	24·1 b	6·7 b	8·9 b	2·3 aAB	10·0 bA
110R	Uninoculated control	4·7 a	51·8 a	13·8 a	19·4 a	0	–
	X. index 100	4·5 b	62·1 a	11·6 a	14·0 b	1·8 bBC	49·5 aAB
	X. index 1000	2·8 c	23·4 b	8·5 b	11·7b	2·2 aB	5·0 bB
140Ru	Uninoculated control	2·5 a	33·2 a	15·2 a	16·7 a	0	–
	X. index 100	2·2 b	32·4 a	12·7 a	9·7 b	2·0 aBC	35·0 aB
	X. index 1000	1·6 c	35·9 a	5·8 b	6·8 b	2·1 aBC	2·4 bC
SO4	Uninoculated control	3·1 a	31·9 a	13·1 a	14·6 a	0	–
	X. index 100	2·7 b	27·0 ab	10·0 ab	10·2 b	2·1 aAB	76·0 aA
	X. index 1000	2·2 c	16·0 b	7·0 b	7·3 b	2·3 aAB	8·5 bA

–: not tested.

[a]Data are mean of 16 replicated plants per treatment combination. Plants were inoculated with 100 or 1000 specimens (all migratory stages) of *Xiphinema index*. For each grapevine rootstock, means followed by the same lowercase letter do not differ significantly ($P \geq 0{\cdot}05$) according to Fisher's protected LSD test. Upper-case letters refer to mean comparisons among rootstocks within each nematode inoculum level. Actual data are presented for each grapevine rootstock but severity of root symptoms, nematode population density and relative plant growth were transformed into $\log_{10}(X + 1)$ and to arcsine-square root, respectively, before analysis.

[b]Average percentage growth of each variable during the experiment.

[c]Assessed on a 0–3 rating scale where 0 = healthy root system; 1 = few localized swollen or curved root tips; 2 = enlarged swellings of root tips very evident throughout root system, and 3 = segments of roots greatly enlarged, little or no lateral root formation, attacks very intensive throughout root system.

[d]Nematode reproduction factor (Rf) = final nematode numbers per plant (Pf)/initial nematode inoculum per plant (Pi).

did not differ from that in Cabernet Sauvignon and it was significantly higher ($P < 0{\cdot}001$) than the average Rf in 41B, 110R, 161-49C and 140Ru rootstocks, the latter two sustaining, as previously, the lowest reproduction rate (Table 3).

Growth of *Vitis* rootstocks infected with *Meloidogyne* spp. and *Xiphinema index*

Both the *Vitis* rootstock and the isolate of the *Meloidogyne* species, as well as their interaction, significantly ($P < 0{\cdot}001$) influenced the root fresh weight, and relative growth of the main stem and shoot diameters (Table 2). Conversely, the relative increase in shoot height varied significantly ($P = 0{\cdot}030$) among the rootstocks but it was not influenced by nematode infection, irrespective of the rootstock (Table 2).

Infection by root-knot nematodes significantly ($P < 0{\cdot}001$) increased the root fresh weight of some rootstocks: Cabernet Sauvignon infected by *M. arenaria* and *M. incognita*; 1103P infected by *M. arenaria*, *M. incognita* and *M. javanica*; and 140Ru infected by *M. incognita*. Conversely, infection by *Meloidogyne* spp. did not influence the root fresh weight in 161-49C, 41-B, 110R and SO4 (Table 2). The main stem and shoot diameters were significantly ($P < 0{\cdot}05$) reduced in Cabernet Sauvignon as well as in 161-49C and 1103P rootstocks by infection with the three *Meloidogyne* species, and in 41B and 140Ru rootstocks by infection with *M. incognita* and *M. javanica*. Infection with the nematodes had no significant effect on main stem and shoot diameters of 110R and SO4 rootstocks (Table 2).

Infection by *X. index* significantly ($P < 0{\cdot}001$) reduced the plant root dry weight. The degree of the reduction was significantly ($P < 0{\cdot}001$) influenced by the rootstock genotype and increased with the initial inoculum density (from 100 to 1000 nematodes per root system) (Table 3). Similarly, the relative shoot height as well as the main

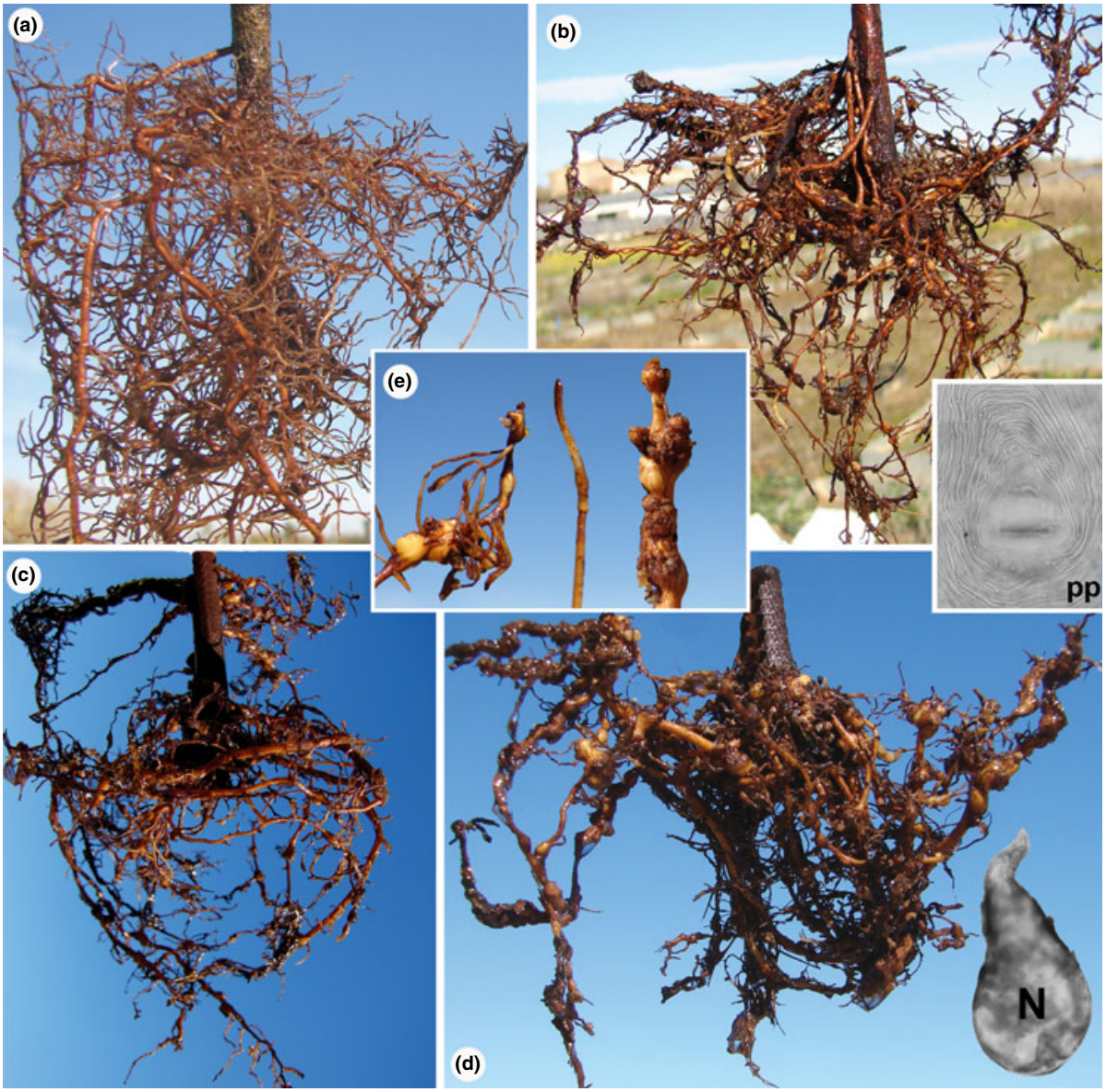

Figure 1 Root system of 41 B Millardet et De Grasset (*Vitis vinifera* Chasselas × *V. berlandieri*) rootstock uninfected (a), or infected by the root-knot nematodes *Meloidogyne arenaria* race 2 (b), *Meloidogyne incognita* race 1 (c), and *Meloidogyne javanica* (d) at high inoculum densities (10 000 eggs + J2 per plant), showing typical galls. (e) Details of roots severely affected (left and right) by nematode infection as compared with healthy root (central). Note the perineal pattern (pp) morphology of *M. incognita* and the complete female (N).

stem and shoot diameters were significantly ($P < 0{\cdot}001$) influenced by the rootstock genotype and inoculum density of *X. index*, and also by their interaction, except for the shoot diameter (Table 3). The shoot height was not affected by infection with *X. index* in 161-49C and 140Ru rootstocks at any inoculum density of *X. index*, but the higher inoculum density significantly ($P < 0{\cdot}001$) reduced this variable in the other rootstocks (Table 3). Infection by the nematode significantly ($P < 0{\cdot}001$) reduced the shoot diameter in Cabernet Sauvignon and all *Vitis* rootstocks irrespective of the initial inoculum density (Table 3). Conversely, infection by *X. index* significantly ($P < 0{\cdot}001$) reduced main stem diameter in all rootstocks genotypes except in 161-49C, but in 41B, 110R, 140Ru, and SO4 rootstocks, such effect was statistically significant only at the higher nematode inoculum (Table 3).

Discussion

Grapevine production in Mediterranean areas is affected mainly by drought and high levels of active lime occurring in some regions. These harsh environmental conditions as well as the incidence of biotic stresses

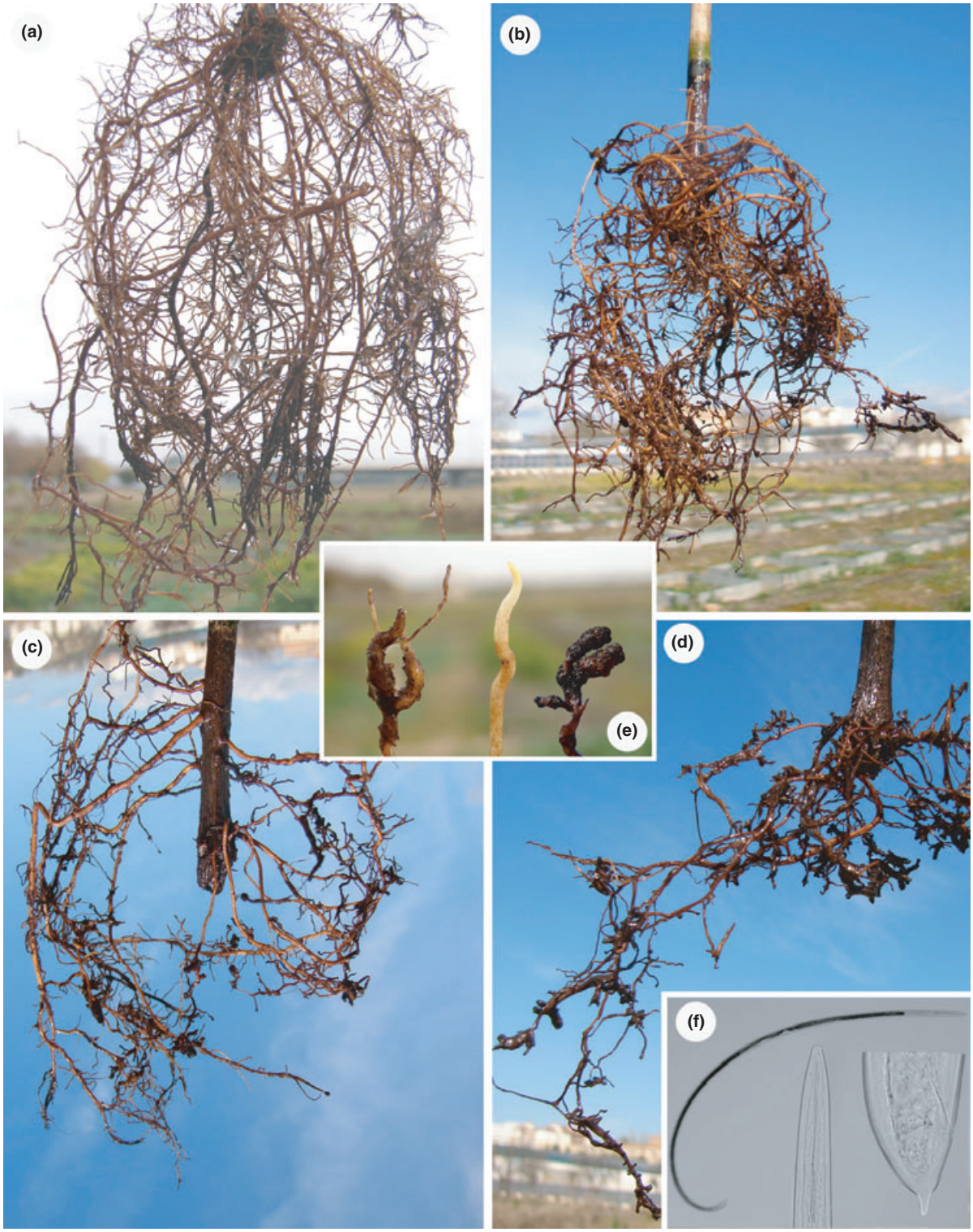

Figure 2 Root system of Paulssen 1103 (*Vitis berlandieri* × *V. rupestris*) rootstock uninfected (a) or infected with the dagger nematode *Xiphinema index* at low (100 nematodes per plant) (b) or high (1000 nematodes per plant) (c, d) inoculum densities showing typical swellings of root tips. (e) Details of root tips severely affected (left and right) by nematode infection as compared with healthy root tip (central). (f) Detail of *Xiphinema index* morphology.

(e.g. plant-parasitic nematodes, phylloxera and grafting) have generated the need to select special rootstocks. The main objective of this research was to determine the reaction of *Vitis* rootstocks commonly used in Mediterranean viticulture (1103P, 110R, 161-49C, 41B, 140Ru and SO4) to infection by nematodes to which they were previously considered moderately resistant, including *M. arenaria*, *M. incognita*, *M. javanica* and to *X. index* (Edwards, 1989; Nicol *et al.*, 1999; Mor *et al.*, 2003). Previous field studies in Andalusia, southern Spain, indicated that infections of these rootstocks by the root-knot nematodes occurred with incidence and severity indicative of a susceptible reaction under the field conditions prevailing in that region (Téliz *et al.*, 2007). Consequently, there was a need to confirm those field observations and to study the effects of those root-knot nematodes and the dagger nematode *X. index* on the rootstocks commonly used in Mediterranean viticulture under carefully controlled experimental conditions.

The three root-knot nematodes, *M. arenaria*, *M. incognita* and *M. javanica*, and the dagger nematode *X. index*, had a reproduction rate higher than one in all *Vitis* rootstocks, indicating that these rootstocks are suitable hosts for the nematodes. The growth of *Vitis* rootstocks infected with the root-knot nematodes was less vigorous than that of nematode-uninfected controls in the majority of the rootstocks studied. Root infection after artificial inoculations with the three root-knot nematodes resulted in moderate to severe root galling in all *Vitis* rootstocks, thus confirming previous observations in naturally-infested rootstocks in Andalusia (Téliz *et al.*, 2007). However, the results for some of the *Vitis* rootstocks (i.e. 161-49C, 41B, 110R and SO4) failed to reveal a significant correlation between the three *Meloidogyne* spp. numbers and either the shoot height or the root fresh weight. In contrast, shoot and main stem diameters appeared to be highly sensitive variables for assessing root-knot nematode damage on *Vitis* rootstocks, which agree with previous studies (Sasanelli *et al.*, 2006). In fact, some *Meloidogyne-Vitis* rootstock combinations gave rise to a tolerant reaction to the nematode, whereby a high reproduction rate of the nematode does not cause a significant impairment on plant growth (Shaner *et al.*, 1992). This was the case in 110R and SO4 rootstocks, which grew comparable to uninfected controls despite successful infection by each of the three root-knot nematode species. Consequently, these rootstocks globally express tolerance characteristics to *Meloidogyne* spp., even though differences in reproduction rates among nematode species as well as the interactions between nematodes and *Vitis* rootstocks may occur.

These findings also agree well with previous results under field conditions in southern Spain, where high soil nematode populations and compatible reactions were observed for *M. incognita*, *M. javanica* and *M. arenaria* and the rootstocks 1103P, 110R, Rupestris du Lot, 161-49C, 41B, 140Ru and SO4 (Téliz *et al.*, 2007). However, in other studies, root growth of 110R was reduced by 35–40% after inoculation with different Australian populations of *M. arenaria*, *M. hapla*, *M. incognita* and *M. javanica*, while SO4 inoculated with the same nematodes remained undamaged (Stirling & Cirami, 1984). Recent studies have also revealed that SO4 is tolerant to high inoculum levels of *M. arenaria*, *M. incognita* and *M. javanica*, to the extent that plant growth was stimulated by nematode infections (McKenry & Anwar, 2006). This shows that significant differences may be observed both in plant growth of rootstocks and suitability, depending on the geographical origin of the nematode populations and the methods and standards of rating used to determine these responses (Stirling & Cirami, 1984; Nicol *et al.*, 1999). In addition, the inoculum density used in the present study (approximately 10 eggs and J2 cm^{-3} soil) was clearly higher than that of threshold damage density of *Meloidogyne* spp. reported to cause yield losses in grapevine (0·5 eggs and J2 cm^{-3} of soil; Anwar & Van Gundy, 1989), or tolerance limits of 1·28 and 0·78 eggs and J2 cm^{-3} soil for 1103P rootstock and Italia cultivar, respectively (Sasanelli *et al.*, 2006). Therefore, although parasitism by root-knot nematodes can impair *Vitis* rootstock growth under controlled conditions, long term experiments under field or microplot conditions would be needed to determine the potential of these nematodes to cause significant damage to growth of *Vitis* rootstocks in vineyards and to reduce yield of grafted grapevines. Previously, under greenhouse conditions, Anwar & Van Gundy (1993) observed a significant reduction of shoot length in grapevine plants (*V. vinifera* cv. Colombard) infected by *M. incognita* only when plants were harvested 350 days after inoculation, but not after shorter periods (100 or 250 days after inoculation).

All *Vitis* rootstocks tested in this study were susceptible to infections by *X. index* that also caused significant root damage. The most affected plant growth variable in all rootstocks was the root dry weight, at the two tested nematode initial inoculum densities, indicating an important disturbance in root growth due to root tip deformation and parasitism. In this case, none of the *Vitis* rootstocks showed tolerance to infection by *X. index* as occurred in rootstocks 110R and SO4 infected by *Meloidogyne* spp. Nevertheless, contradictory results have been previously reported for SO4 rootstock, which was found susceptible and sustaining high levels of root damage (Malan & Meyer, 1993) or moderately resistant (Harris, 1983) to *X. index*. Similarly to *Meloidogyne* spp., pathogenic variability has also been reported for *X. index* depending on the geographical origin of the tested population (Coiro *et al.*, 1990).

Shoot height was significantly reduced by 1000 *X. index* nematodes per plant in most of the *Vitis* rootstocks tested, except in 161-49C and 140Ru, which showed the lowest *X. index* reproduction rates in spite of the severe root damage caused. Interestingly, this high disruption of root tissues with high inoculum levels of *X. index* had adverse effects on final nematode populations in all rootstocks, probably due to the competition among nematodes for infection sites and root spaces. Tolerance limits for *X. index* in grapevine (cv. Aglianico)

were estimated as 1·7 nematodes cm^{-3} by Di Vito *et al.* (1985), whereas more than 100 nematodes per 500 g of soil were needed to cause high damage in grapevine according to McKenry (1992). Compared with the present results, the latter data suggests that the Spanish population of *X. index* tested here is highly aggressive to *Vitis* rootstocks since low inoculum density (0·1 nematode cm^{-3} soil) caused significant root damage and reduction of shoot diameter in all *Vitis* rootstocks.

Consequently, the data suggest that low inoculum levels (e.g. 100 nematodes per plant) are more adequate for further grapevine rootstock evaluation to *X. index*, since high inoculum levels leads to saturation by the competition for infection sites and root spaces. In addition, the results of pathogenicity tests suggest that long-term experiments (around 1 year) should be considered for evaluation of grapevine rootstocks against *X. index* under growth chamber or greenhouse conditions, which is in agreement with other authors (Anwar & Van Gundy, 1993).

The importance of assessing the performance of the rootstocks used in southern Spain viticulture to local nematode populations is reinforced by the moderate to high incidence of infections by *Meloidogyne* spp. and *X. index* found in commercial vineyards in this region (Téliz *et al.*, 2007; Gutiérrez-Gutiérrez *et al.*, 2011). The results of the present and other studies indicate that these nematodes pose a serious risk for grapevine production worldwide as a consequence of the nematode prevalence and vulnerability of some rootstocks to *Meloidogyne* spp., and all of them to *X. index*. Consequently, control measures such as assessment of nematode population in soil and soil disinfestations should be taken into account before establishing new grapevine plantations. In addition, the role of weed hosts of *Meloidogyne* spp. in the maintenance and dissemination of the nematode within and across vineyards (Castillo *et al.*, 2008), as well as the presence of other nematode species, such as the recently reported *M. hispanica* (Castillo *et al.*, 2009), should also be considered. Consequently, accurate identification of *Meloidogyne* spp. and *Xiphinema* spp. infecting grapevine growing areas is a prerequisite for the efficient use of *Vitis* rootstocks and effective management of these nematodes on grapevine.

Acknowledgements

This research is part of a PhD study by CGG and was supported by grant P06-AGR-01360 from Consejería de Economía, Innovación y Ciencia (CEIC), Junta de Andalucía of Spain. The authors thank M. Montes Borrego, J. Martin Barbarroja, F. J. Durán Gutiérrez, C. Cantalapiedra-Navarrete and G. León Ropero (IAS-CSIC) for technical assistance, F. López Collado (Acciones Hortícolas S.A., Villarrobledo, Albacete) for providing grapevine rootstocks, and B. B. Landa and J. A. Navas-Cortés (IAS-CSIC) for critically reading the manuscript prior to submission.

References

Andret-Link P, Laporte C, Valat L *et al.*, 2004. Grapevine fanleaf virus: still a major threat to the grapevine industry. *Journal of Plant Pathology* **86**, 183–95.

Anwar SA, Van Gundy SD, 1989. Influence of four nematodes on root and shoot growth parameters in grape. *Journal of Nematology* **21**, 276–83.

Anwar SA, Van Gundy SD, 1993. Influence of the interaction of *Meloidogyne incognita* and *Pratylenchus vulnus* on root-shoot growth parameters in grape. *Afro-Asian Journal of Nematology* **3**, 5–11.

Anwar SA, McKenry M, Ramming D, 2002. A search for more durable grape rootstock resistance to root-knot nematode. *American Journal of Enology and Viticulture* **53**, 19–23.

Castillo P, Rapoport H, Palomares-Rius JE, Jiménez-Díaz RM, 2008. Suitability of weed species prevailing in Spanish vineyards as hosts for root-knot nematodes. *European Journal of Plant Pathology* **120**, 43–51.

Castillo P, Gutiérrez-Gutiérrez C, Palomares-Rius JE, Cantalapiedra-Navarrete C, Landa BB, 2009. First report of root-knot nematode *Meloidogyne hispanica* infecting grapevines in Southern Spain. *Plant Disease* **93**, 1353.

Coiro MI, Lamberti F, Borgo M, Egger E, 1985. Reproduction of *Xiphinema index* on different grapevine rootstocks. *Phytopathologia Mediterranea* **24**, 177–9.

Coiro MI, Taylor CE, Borgo M, Lamberti F, 1990. Resistance of grapevine rootstocks to *Xiphinema index*. *Nematologia Mediterranea* **18**, 119–21.

Coolen WA, 1979. Methods for the extraction of *Meloidogyne* spp. and other nematodes from roots and soil. In: Lamberti F, Taylor CE, eds. *Root-knot Nematodes (*Meloidogyne *species): Systematics, Biology and Control*. London, UK: Academic Press, 317–29.

Demangeat G, Komar V, Cornuet P, Esmenjaud D, Fuchs M, 2004. Sensitive and reliable detection of grapevine fanleaf virus in a single *Xiphinema index* nematode vector. *Journal of Virological Methods* **122**, 79–86.

Di Vito M, Ekanayake HM, R K, Savino V, 1985. The effect of initial population densities of *Xiphinema index* on the growth of grapevine. *Nematologia Mediterranea* **13**, 185–9.

Edwards M, 1989. Resistance and tolerance of grapevine rootstocks to plant parasitic nematodes in vineyards in north-east Victoria. *Australian Journal of Experimental Agriculture* **29**, 129–31.

Esbenshade PR, Triantaphyllou AC, 1985. Use of enzyme phenotypes for identification of *Meloidogyne* species. *Journal of Nematology* **17**, 6–20.

Esmenjaud D, Bouquet A, 2009. Selection and application of resistant germplasm for grapevine nematodes management. In: Ciancio A, Mukerji KG, eds. *Integrated Management of Fruit Crops Nematodes*. London, UK: Springer Science, 195–214.

Gómez KA, Gómez AA, 1984. *Statistical Procedures for Agricultural Research*, 2nd edn. New York, NY, USA: John Wiley & Sons, Inc.

Gutiérrez-Gutiérrez C, Palomares-Rius JE, Cantalapiedra-Navarrete C, Landa BB, Castillo P, 2011. Prevalence, polyphasic identification, and molecular phylogeny of dagger and needle nematodes infesting vineyards in southern Spain. *European Journal of Plant Pathology* doi: 10.1007/s10658-010-9705-y.

Harris AR, 1983. Resistance of some *Vitis* rootstocks to *Xiphinema index*. *Journal of Nematology* **15**, 405–9.

Hartman KM, Sasser JN, 1985. Identification of *Meloidogyne* species on the basis of differential host test and perineal pattern morphology. In: Barker KR, Carter CC, Sasser JN, eds. *An Advanced Treatise on Meloidogyne. Vol. II: Methodology*. Raleigh, NC, USA: NCSU Graphics, 69–77.

Hewitt WB, Raski DJ, Goheen AC, 1958. Nematode vector of soil-borne fanleaf virus of grapevines. *Phytopathology* **48**, 586–95.

Hussey RS, Barker KR, 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. *Plant Disease Reporter* **57**, 1025–8.

Lewis SA, 1987. Nematode-plant compatibility. In: Veech JA, Dickson DW, eds. *Vistas on Nematology*. Hyattsville, MD, USA: The Society of Nematologists, 246–52.

Malan A, Meyer AJ, 1993. Interaction between a South African population of *Xiphinema index* and different grapevine rootstocks. *South African Journal of Enology and Viticulture* **14**, 11–5.

McKenry MV, 1992. Nematodes. In: Flaherty DL, Christensen LP, Lanini WT, Marois JJ, Phillips PA, Wilson LT, eds. *Grape Pest Management*, 2nd edn. Oakland, CA, USA: University of California.

McKenry MV, Anwar SA, 2006. Nematode and grape rootstock interactions including an improved understanding of tolerance. *Journal of Nematology* **38**, 312–8.

Mor M, Bar-Eyal M, Gottlieb Y, Harcavi E, 2003. Grape rootstocks resistant or tolerant to the root-knot nematode species *Meloidogyne javanica* and *M. incognita*. *Phytoparasitica* **31**, 414.

Nicol JM, Stirling GR, Rose BJ, May P, Van Heeswijck R, 1999. Impact of nematodes on grapevine growth and productivity: current knowledge and future directions, with special reference to Australian viticulture. *Australian Journal of Grape and Wine Research* **5**, 109–27.

Perry RN, Moens M, 2006. *Plant Nematology*. Wallingford, UK: CABI.

Perry RN, Moens M, Starr JL, 2009. *Root-knot Nematodes*. Wallingford, UK: CABI.

Sasanelli N, D'Addabbo T, Lišková M, 2006. Influence of the root-knot nematode *Meloidogyne incognita* r. 1 on growth of grapevine. *Helminthologia* **43**, 168–70.

Shaner G, Stromberg EL, Lacy GH, Barker KR, Pirone TP, 1992. Nomenclature and concepts of pathogenicity and virulence. *Annual Review of Phytopathology* **30**, 47–66.

Stirling GR, Cirami RM, 1984. Resistance and tolerance of grape rootstocks to South Australian populations of root-knot nematode. *Australian Journal of Experimental Agriculture and Animal Husbandry* **24**, 277–82.

Téliz D, Landa BB, Rapoport HF, Pérez Camacho F, Jiménez-Díaz RM, Castillo P, 2007. Plant-parasitic nematodes infecting grapevine in southern Spain and susceptible reaction to root-knot nematodes of rootstocks reported as moderately resistant. *Plant Disease* **91**, 1147–54.

Trudgill DL, Perry JN, 1994. Thermal time and ecological strategies – a unifying hypothesis. *Annals of Applied Biology* **125**, 521–32.

Zijlstra C, Donkers-Venne DTHM, Fargette M, 2000. Identification of *Meloidogyne incognita*, *M. javanica* and *M. arenaria* using sequence characterised amplified region (SCAR) based PCR assays. *Nematology* **2**, 847–53.