Plant-Parasitic Nematodes Infecting Grapevine in Southern Spain and Susceptible Reaction to Root-Knot Nematodes of Rootstocks Reported as Moderately Resistant

Daniel Téliz, Instituto de Fitosanidad, Colegio de Postgraduados, 56230 Montecillo, Texcoco, Mexico; **Blanca B. Landa**, Instituto de Agricultura Sostenible (IAS), Consejo Superior de Investigaciones Científicas (CSIC), Apdo. 4084, 14080 Córdoba, Spain, and Escuela Técnica Superior de Ingenieros Agrónomos y de Montes (ETSIAM), Universidad de Córdoba (UCO), Edificio C4- “Celestino Mutis”, Carretera de Madrid Km 396, Campus de Rabanales, 14071 Córdoba, Spain; **Hava F. Rapoport**, IAS-CSIC; **Fernando Pérez Camacho**, ETSIAM-UCO; **Rafael M. Jiménez-Díaz**, ETSIAM-UCO and IAS-CSIC; and **Pablo Castillo**, IAS-CSIC

ABSTRACT

Téliz, D., Landa, B. B., Rapoport, H. F., Pérez Camacho, F., Jiménez-Díaz, R. M., and 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 Dis.* 91:1147-1154.

Incidence and nematode population densities of plant-parasitic nematodes were determined in 64 samples of soil and grapevine roots collected from commercial vineyards in southern Spain between October 2003 and May 2005. In addition, a histopathological study was done of rootstock roots naturally infected by root-knot nematodes (*Meloidogyne* spp.). Nematodes infecting the rootstocks were identified according to conventional procedures, and the *Meloidogyne* spp. were furthermore identified by sequence characterized amplified region-polymerase chain reaction (SCAR-PCR) and isozyme esterase analyses. The most important plant-parasitic nematodes detected, in order of decreasing frequency of total soil infestation and root infection (percentage of samples), were *Mesocriconema xenoplax* (34.4%), *Meloidogyne incognita* (26.6%), *Meloidogyne javanica* (14.1%), *Xiphinema index* (12.5%), *Xiphinema italiae* (10.9%), *Pratylenchus vulnus* (6.3%), and *Meloidogyne arenaria* (1.6%). No disease symptoms were observed on aboveground plant parts of the infected grapevines, except for plants in some fields where soil was infested with the virus-vector nematodes *X. index* and *X. italiae*. Those grapevines showed a yellow mosaic pattern in leaves early in the growing season and the internode shortening characteristic of infections by *Grapevine fanleaf virus*. Rootstocks infected by root-knot nematodes (*Meloidogyne* spp.) showed distorted feeder roots and large- to moderate-sized root galls, present either singly or in clusters. Histopathology of galled roots showed a typical susceptible response to infection by root-knot nematodes: cellular alterations were induced in the cortex, endodermis, pericycle, and vascular system, including giant-cell formation and severe distortion of vascular tissues. Most *Meloidogyne* egg masses occurred on the surface of the galled root tissues, a position that could facilitate dispersion of the nematode eggs and juveniles and the occurrence of secondary infections. Some of the grapevine rootstocks surveyed in this study (Paulsen 1103, Richter 110, Rupestris du Lot, and SO4) had previously been reported to be resistant to *Meloidogyne* spp.; however, the population densities of these nematodes found in soil and roots sampled in the present study, as well as the compatible host-parasite relationship revealed by histopathology, indicate a susceptible response to *Meloidogyne* spp. from southern Spain.

Additional keywords: dagger nematodes, pin nematodes, ring nematodes, root-lesion nematodes, *Vitis* spp.

Grapevine (*Vitis vinifera*) production for wine and table grapes is one of the most extensive fruit crop agricultural systems worldwide. *V. vinifera* is native to Asia Minor and the Caucasus region, was dis-

tributed throughout Europe, and is now extensively grown in the Mediterranean Basin, the subtropical regions of Australia, Southern Africa, and North and South America (6). Approximately 50% of the more than 7.5×10^6 ha of grapevines grown in the world in 2004 are in Mediterranean countries (13).

Plant-parasitic nematodes have often been found in soils where grapevines showed reduced vigor. Currently, one of the most serious disease problems affecting grapevine production is the *Grapevine fanleaf virus* (GFLV) transmitted by the dagger nematodes *Xiphinema index* and *Xiphinema italiae* (6). In addition, grapevine roots can be infected and damaged by

root-knot (*Meloidogyne* spp.), root-lesion (*Pratylenchus* spp.), citrus (*Tylenchulus semipenetrans*), and ring (*Mesocriconema* spp.) nematodes (6,14,30,31,34). Root-knot nematodes, especially *Meloidogyne arenaria*, *M. incognita*, and *M. javanica*, cause serious root damage in sandy soils under the mild temperatures that prevail in most grapevine-growing areas in California, the Mediterranean Basin, and South Africa (3,6,28,32). *M. hapla* predominates in the cooler areas of Southern Australia and Hungary (20,38), *M. ethiopica* parasitizes grapevines in Chile (7), and *M. hispanica* and *M. nataliei* infect grapevines in South Africa (22) and Michigan (5), respectively.

Pathogenicity tests under controlled conditions have demonstrated the potential of most of these nematodes to damage grapevines (6). The extent of yield losses caused by plant-parasitic nematodes is both variable and difficult to determine. Stirling et al. (39) estimated a 7% yield loss by root-knot nematodes in Australia, while Brown et al. (6) found a 20% production loss in California (6). Knowledge of the distribution and extent of damage due to plant-parasitic nematodes in grapevine fields in southern Spain is limited mainly to studies of nematode vectors of plant viruses (35,43).

Plant-parasitic nematodes, as well as soilborne fungi and phylloxera root pest (*Daktulosphaira vitifoliae*), are managed primarily by the use of resistant rootstocks. Resistant grapevine rootstocks are used to limit damage caused by root-knot nematodes in grapevines grown on sandy soil worldwide (6). However, the long-term use of the resistant rootstocks can impose strong selection pressure on the root-knot nematode population. Thus, nematodes able to reproduce and adapt to the roots of a resistant or moderately resistant rootstock will give rise to new pathotypes, making that rootstock useless for control of the nematode (24).

Information is scarce concerning the host-parasite relationships in natural infections by root-knot nematodes of grapevine rootstocks commonly reported to have some resistance to plant-parasitic nematode species, especially to *Meloidogyne*

Corresponding author: P. Castillo
E-mail: ag1cascp@uco.es

*The e-Xtra logo stands for “electronic extra” and indicates that Figures 2 and 3 appear in color in the online edition.

Accepted for publication 6 April 2007.

doi:10.1094/PDIS-91-9-1147

© 2007 The American Phytopathological Society

spp. Histopathological studies in naturally infected rootstock roots reveal important details about the capacity of the nematode to enter, develop, and reproduce in the root tissues and thus indicate the compatible or incompatible nature of the nematode-plant interactions. Such knowledge, together with data on nematode population densities in soil and roots of cultivated grapevines, would be useful to viticulturalists worldwide and important to manage diseases and to lessen the reliance on nematocides. In consequence, the objectives of this study were: (i) to determine the identity, frequency, and population density of plant-parasitic nematodes infecting grapevine rootstocks growing in southern Spain, and (ii) to evaluate the host-parasite relationships in grapevine rootstocks in the case of naturally occurring infections by root-knot nematodes. To our knowledge, this is the first histopathological study of grapevine rootstocks naturally infected by root-knot nematodes.

MATERIALS AND METHODS

Nematode survey. Disease surveys were undertaken in commercial vineyards in Andalusia, Southern Spain between 2003 and 2005. For that purpose, the region was divided into three geographically separated zones that include the three major wine production areas corresponding to marketing-operating “wine denomination of origin (DO) zones” Condado de Huelva DO (Huelva Province), Montilla-Moriles DO (Córdoba Province), and Jerez-Xérès-Sherry y Manzanilla-Sanlúcar de Barrameda DO (Cádiz Province). Sixty-four vineyards were selected, and grapevine feeder roots together with the rhizosphere and bulk soils were sampled. Samples were collected with a shovel from the upper 40 cm of soil of three to four plants chosen arbitrarily in each vineyard, from October 2003 to May 2005 (Table 1). The surveyed vineyards were representative of grapevines grown in southern Spain; they were selected on the basis of parameters

indicative of their commercial importance, including the total growing area and geographical distribution of cultivars, rootstock, and crop age. The 64 root samples included several rootstocks (Table 1), as well as unknown accessions of *Vitis berlandieri* (1 sample) and *Vitis riparia* (1 sample).

Frequency of infestation and population density of plant-parasitic nematodes were determined. The frequency of infestation was calculated as the percentage of samples in which a nematode species was diagnosed. Nematode population densities in root and soil were assessed for each sample and calculated as the average of the soil and root counts. A 5-g root subsample was washed free of soil, weighed, and used to extract nematodes by the maceration-centrifugation procedure (9). When galled roots were found, representative roots were selected for histopathological studies and to determine the number of *Meloidogyne* eggs present. Eggs were collected from the galled roots by blending in a 0.5% NaOCl solution for 4 min (17). Nematodes from the soil were extracted from a 100-cm³ subsample using the centrifugal-flotation method (9). The nematodes were then fixed in 4% formaldehyde, counted, and identified by morphological traits to genus. Selected adult specimens of separate genera were processed to glycerin (37) and mounted on microscope slides for species identification.

Meloidogyne species were identified by perineal pattern morphology. For each *Meloidogyne* population, at least 20 perineal patterns were prepared and examined (16). The root tissues were teased apart with forceps and half-spear to remove adult females. The head and neck region of the nematode was excised, and the posterior part was placed in a 45% lactic acid solution to remove all body tissues. Then, the perineal pattern was trimmed, transferred to a drop of glycerin, and processed as described by Hartman and Sasser (16). Frequency of infestation was calculated as

the percentage of samples in which a nematode species was identified.

DNA extraction, polymerase chain reaction (PCR) assays, and isozyme esterase analyses. Morphological identification of *Meloidogyne* species is difficult because of the need of training and experience by operators as well as intra-specific variability of morphological traits of taxonomic use (21). Consequently, the *Meloidogyne* spp. from this study were also identified using sequence characterized amplified region (SCAR)-PCR (44) and analysis of isozyme esterases (12). For PCR assays, total genomic DNA was extracted from at least five to eight adult females per sample according to Castillo et al. (8). PCR assays were carried out using the species-specific SCAR primer pairs Far/Rar (*M. arenaria*), Finc/Rinc (*M. incognita*), and Fjav/Rjav (*M. javanica*) and the reaction conditions described by Zijlstra et al. (44). Amplifications were performed with a PTC 200 thermocycler (MJ Research, Bio-Rad Laboratories, Madrid, Spain). Amplification products were separated using the FlashGel system (Cambrex Bio Science Rockland, Inc., Rockland, ME, USA) by electrophoresis at 250 V for 5 to 7 min. The AmpliSize 50–2,000 bp ladder (Bio-Rad) was used for electrophoresis. The assays were repeated at least twice, and always included negative controls (no DNA) and positive DNA controls from *M. arenaria* and *M. incognita* adult females from olive nurseries (27), and *M. javanica* adult females from potato (41), whose identity was certain.

For esterase analyses, one young egg-laying female representative of those found in the samples and two egg-laying females of an *M. javanica* reference population from potato (41) were placed in microtubes containing 5 µl of 20% sucrose and 1% Triton X-100 and macerated. Electrophoresis was carried out in 7 × 8 cm homogeneous polyacrylamide gels, 0.75 mm thick, in a Mini Protean II electrophoresis unit (Bio-Rad). Gels were stained for esterases with the substrate α-naphthyl acetate as described before (12).

Histopathology. Galled roots from naturally infected rootstocks sampled from vineyards in all three provinces were selected for histopathological studies. The roots were carefully washed free of adhering soil and debris, and individual galls and healthy root pieces were fixed in FAE (formalin:acetic acid:60% ethanol = 2:1:17 vol/vol/vol) for a minimum of 48 h, dehydrated in a tertiary butyl alcohol series (70–85–90–100%), and embedded in Histosec embedding paraffin melting point 56 to 58°C (Merck, Darmstadt, Germany). Embedded tissues were sectioned transversely at 10 to 12 µm with a rotary microtome, mounted on glass slides, stained with tannic acid, iron chloride, safranin, and fast-green (19), and examined under a light

Table 1. Localities and grapevine rootstocks sampled in southern Spain

Province	Locality	No. of samples	Grapevine rootstocks
Cádiz	Chipiona	4	Couderc 161-49; Paulsen 1103; Richter 110; SO4
	Jerez de la Frontera	12	Couderc 161-49; 333 École Montpellier; Millardet & Grasset 41-B
	Sanlúcar de Barrameda	4	Couderc 161-49; Moscatel
	Trebujena	3	Couderc 161-49
Córdoba	Aguilar de la Frontera	8	Couderc 161-49; Richter 110
	Montemayor	10	Millardet & Grasset 41-B; Richter 110; Ruggieri 140; <i>Vitis berlandieri</i>
	Montilla	6	Couderc 161-49; Millardet & Grasset 41-B; Richter 110, Ruggieri 140
	Monturque	3	Couderc 161-49; Richter 110
Huelva	Puente Genil	3	Couderc 161-49; Richter 110
	Almonte	4	Richter 110; Rupestris du Lot
	Bollullos del Condado	3	Couderc 161-49; Richter 110
	Bonares	1	Castel 196-17
	Niebla	2	Richter 110
	Rociana	1	<i>Vitis riparia</i>

microscope. Images were captured with a Leica QW5001 image processing system.

RESULTS

Nematode survey. Eighteen genera and 36 species of plant-parasitic nematodes were found associated with grapevine rootstocks in southern Spain (Table 2). Potentially damaging species included the root-knot nematodes *Meloidogyne arenaria*, *M. incognita*, *M. javanica*, ring nematode (*Mesocriconema xenoplax*), root-lesion nematode (*Pratylenchus vulnus*), and the vector-virus nematodes *Xiphinema index* and *X. italiae* (Table 2). Overall, the frequency of these nematode species in soil and root samples ranged from 34.4% (*Mesocriconema xenoplax*) to 1.6% (*Meloidogyne arenaria*) (Table 2).

Morphological studies of the perineal patterns showed typical traits for each species as described in the literature. Isozyme analysis of *M. arenaria* from

Almonte (Huelva Province) showed a characteristic double band, *M. incognita* from several localities of all three provinces studied, such as Chipiona and Sanlúcar de Barrameda (Cádiz Province), Aguilar de la Frontera, Montemayor, Montilla, Monturque, and Puente Genil (Córdoba Province), and Bonares and Niebla (Huelva Province) shared a characteristic single band, and *M. javanica* from Montemayor (Córdoba Province), and Almonte, Bollullos par del Condado and Rociana (Huelva Province) shared three characteristics bands. PCR assays using the SCAR primer pairs specific for *Meloidogyne* species amplified the predicted products from DNA of the reference isolates (a band of 420 bp for *M. arenaria*, primer pair Far/Rar; 1,200 bp for *M. incognita*, primer pair Finc/Rinc; and 720 bp for *M. javanica*, primer pair Fjav/Rjav) (44) (Fig. 1A, lane 1; Fig. 1B, lane 1; Fig. 1C, lane 1). Results of assays of the single sampled

egg-laying females from Almonte (Huelva Province) confirmed the identity of *M. arenaria* (Fig. 1A, lanes 2 and 3), *M. incognita* for the single female samples originating from grapevine fields in several localities of the three provinces surveyed (Fig. 1B, lanes 2 to 6), and *M. javanica* for the single females sampled from different grapevine rootstocks from Montemayor (Córdoba Province), and Almonte, Bollullos par del Condado, and Rociana (Huelva Province) (Fig. 1C, lanes 2 to 5). No amplifications occurred when samples of the reference *Meloidogyne* species were assayed using heterologous primer pairs, and no cross amplification was observed when a given unknown sample was assayed with several primer pairs (Fig. 1A, lanes 4 to 11; Fig. 1B, lanes 7 to 11; Fig. 1C, lanes 6 to 11).

Generally, no disease symptoms were observed aboveground, except in some vineyards in Huelva Province that were

Table 2. Frequency and population densities of plant-parasitic nematodes in soil and roots of grapevine rootstock sampling in different localities and provinces in southern Spain^a

Nematode species	Overall frequency ^b	Cádiz Province		Córdoba Province		Huelva Province	
		Nematodes/100 cm ³ soil	Nematodes/g root	Nematodes/100 cm ³ soil	Nematodes/g root	Nematodes/100 cm ³ soil	Nematodes/g root
		Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
<i>Amplimerlinius</i> sp.	3.1	- ^c	-	4.0 ^d	-	2.0	-
<i>Aorolaimus perscitus</i>	4.7	-	-	58.0 ± 76.4	-	116.0	-
<i>Criconeoides informis</i>	14.1	5.3 ± 1.5	-	9.5 ± 6.2	-	-	-
<i>Gracilacus peratica</i>	15.6	24.0 ± 18.6	0.3 ± 0.7	6.7 ± 4.7	0.7 ± 0.6	3.0	-
<i>Helicotylenchus digonicus</i>	53.1	39.5 ± 39.7	0.18 ± 0.5	47.0 ± 41.4	0.14 ± 0.4	7.0	-
<i>H. pseudorobustus</i>	7.8	19.5 ± 2.1	0.3 ± 0.5	128.0	-	14.0 ± 2.8	7.1 ± 8.1
<i>H. vulgaris</i>	6.3	-	-	4.0	-	20.0 ± 9.2	-
<i>Longidorus macrosoma</i>	14.1	-	-	7.0 ± 7.1	-	-	-
<i>Meloidogyne arenaria</i>	1.6	-	-	-	-	239.0	76.7
<i>M. incognita</i>	26.6	169.6 ± 142.1	46.9 ± 96.8 ^e	28.9 ± 53.1	359.1 ± 894.0	7.7 ± 13.3	136.5 ± 211.5
<i>M. javanica</i>	14.1	-	-	365.0	33,311	51.4 ± 77.6	69.6 ± 106.0
<i>Merlinius brevidens</i>	53.1	11.0 ± 6.6	-	29.4 ± 35.8	-	12.0 ± 5.6	-
<i>M. joctus</i>	3.1	-	-	9.0 ± 7.1	-	-	-
<i>Mesocriconema annulifer</i>	1.6	3.0	-	-	-	-	-
<i>M. kirjanovae</i>	1.6	-	-	-	-	54.0	-
<i>M. sphaerocephala</i>	7.8	99.0 ± 158.6	-	-	-	25.0 ± 32.5	7.1 ± 8.1
<i>M. xenoplax</i>	34.4	201.8 ± 157.4	1.3 ± 2.7	19.1 ± 41.2	-	44.6 ± 46.2	1.4 ± 1.6
<i>Ogma rhombosquamatum</i>	1.6	-	-	-	-	-	-
<i>Paratrophurus loofi</i>	1.6	-	-	68.0	-	-	-
<i>Paratylenchus arcuatus</i>	6.5	23.3 ± 9.1	1.0 ± 1.4	-	-	-	-
<i>P. ciccaronei</i>	28.1	35.5 ± 55.2	1.3 ± 1.3	19.3 ± 16.9	0.3 ± 0.5	19.0 ± 12.6	0.7 ± 1.4
<i>P. microdorus</i>	28.1	24.0 ± 24.0	1.1 ± 1.1	20.1 ± 21.7	1.7 ± 3.3	-	-
<i>Pratylenchus neglectus</i>	14.1	-	-	11.0 ± 14.5	1.3 ± 3.5	1.0	0.2
<i>P. penetrans</i>	1.6	-	-	7.0	-	-	-
<i>P. thornei</i>	10.9	24.0	-	7.4 ± 6.7	-	-	-
<i>P. vulnus</i>	6.3	-	-	50.0 ± 41.0	0.4 ± 0.6	8.5 ± 3.5	7.1 ± 8.1
<i>Rotylenchus goodeyi</i>	14.1	19.0	-	-	-	78.8 ± 54.7	-
<i>R. incultus</i>	1.6	-	-	5.0	-	-	-
<i>Trichodorus</i> sp.	6.3	-	-	2.0	-	19.0 ± 21.9	-
<i>Tylenchorhynchus clarus</i>	7.8	-	-	11.3 ± 4.7	-	12.0	-
<i>Tylenchorhynchus</i> sp.	15.6	7.6 ± 2.1	-	-	-	-	-
<i>Xiphinema index</i>	12.5	8.8 ± 5.6	-	6.0	-	2.3 ± 0.6	-
<i>X. italiae</i>	10.9	6.0	-	-	-	4.8 ± 2.8	-
<i>X. pachtaicum</i>	79.7	17.5 ± 21.9	-	8.1 ± 8.0	-	9.6 ± 5.2	-
<i>X. turcicum</i>	7.8	3.0	-	13.5 ± 15.3	-	-	-
<i>Zygotylenchus guevarai</i>	20.3	7.0	-	16.2 ± 14.4	-	52.0 ± 69.3	-

^a Data are the mean ± standard error (SE) of nematodes per 100 cm³ soil and nematodes per g root in 64 soil and root samples collected in vineyards in Cádiz, Córdoba, and Huelva provinces (southern Spain). Species are listed alphabetically according to genus.

^b Frequency = percentage of samples in which a nematode species was found.

^c - = absence of nematode population of that species.

^d SE not provided because nematode species was found in a single sample.

^e Numbers indicate eggs + second-stage juveniles collected from galled roots blended in a 0.5% NaOCl solution for 4 min.

infested with the virus-vector nematodes *X. index* and *X. italiae*. Plants from those vineyards showed typical GFLV symptoms consisting of a yellow mosaic pattern on leaves early in the growing season and shortening of vine internodes (Fig. 2A). However, all plants infected by root-knot nematodes showed distorted feeder roots and root galls, ranging from 2 to 6 mm (Fig. 2B to F). The galls developed either singly or in clusters (Fig. 2C to E).

Meloidogyne spp. were found in both soil and rootstocks from all three surveyed provinces, and they were associated mainly with sandy soils. Nematode population densities ranged from 8 to 365 eggs and second-stage juveniles (J2) per 100 cm³ of soil, and from 47 to 33 eggs and J2 per gram of root (Table 2). *M.*

incognita was found infecting all main rootstocks used in the three provinces, including Castel 196-17, Couderc 161-49, Millardet & Grasset 41-B, Moscatel, Paulsen 1103, Richter 110, Ruggeri 140, Rupestris du Lot, and *V. riparia*. Conversely, *M. javanica* occurred only in Córdoba and Huelva provinces, infecting Couderc 161-49 and Richter 110 rootstocks; and *M. arenaria* was detected only in Huelva infecting Richter 110 rootstock. Nematode soil population densities were in the range of 8 to 167 J2 per 100 cm³ for *M. incognita* and 51 to 365 J2 for *M. javanica*.

Mesocriconema xenoplax was found in soil and root samples from all three provinces (34.4%). The population density of this species ranged from 19 to 202 nema-

todes per 100 cm³ of soil, and from 1 to 2 nematodes per gram of root. *X. index* was found in 12.5% of soil samples in all three surveyed provinces; whereas *X. italiae* was detected in Cádiz and Huelva provinces with a frequency of 10.9% (Table 2). Finally, low population densities of the root-lesion nematode *P. vulnus* were found in 6.3% of soil samples, and in roots of Ruggeri 140 rootstock in Córdoba, and Richter 110 and Rupestris du Lot rootstocks in Huelva (Table 2). Other root-lesion nematodes (*P. neglectus*, *P. penetrans*, *P. thornei*, and *Z. guevarai*) were detected in moderate (20.3%) to low (1.6%) frequencies in soil and root samples (Table 2).

Other plant-parasitic nematodes that were detected in soil and rootstocks included needle (*Longidorus macrosoma*), dagger (*X. pachtaicum* and *X. turcicum*), spiral (*Helicotylenchus* spp., *Rotylenchus* spp.), ring (*Criconeimoides informis*, *Mesocriconema* spp.), stunt (*Tylenchorynchus* spp., *Amplimerlinius* spp.), and pin (*Gracilacus peratica*, *Paratylenchus* spp.) nematodes, but population levels were generally low (Table 2).

Histopathology. Root galls caused by root-knot nematodes occurred individually or in clusters, causing severe distortion of the entire root perimeter (Fig. 2B to E). Swellings along the root axis were spheroid in shape with a diameter as large as one and a half to twofold that of healthy roots. Usually each gall contained one female, but occasionally two to three were observed (Fig. 3B and C). Egg masses with 200 to 450 eggs mainly protruded from the roots (Fig. 2G), but sometimes were located within the root cortex (Fig. 3C). *Meloidogyne* infections caused alterations in cells of the root cortex, endodermis, pericycle, and vascular cylinder (Fig. 3). In early stages of infections, J2 were found migrating through the root cortex apoplast, eventually reaching the vascular cylinder (Fig. 3D) where giant cells were induced to form.

In all *Meloidogyne*-infected rootstocks, the giant cells were induced adjacent to vascular tissue (Fig. 3B to I). In the typical susceptible reaction, the formation of giant cells led to extensive distortion and crushing of the vascular cylinder, as well as hyperplastic disorganization of the root cortex (Figs. 3E to I). In young Paulsen 1103 rootstocks, the giant-cell feeding site induced by *M. incognita* invaded nearly the entire stele, almost completely preventing the formation of vascular tissues (Fig. 3I). Commonly, the nematode feeding sites comprised three to five giant cells surrounding the nematode's head, but in some cases up to 11 giant cells were induced by a single female (Fig. 3I). The giant cells exhibited the characteristic cytological features of granulated cytoplasm, thickened cell walls, and variable numbers (4 to 22) of hypertrophied nuclei and nucleoli

Fig. 1. A, Polymerase chain reaction (PCR) amplification product using primer pair Far/Rar and 1 to 10 ng of template DNA of representative samples of *Meloidogyne* isolates: *M. arenaria* reference isolate from olive nurseries, lane 1; *M. arenaria* from grapevine rootstock Richter 110 from Almonte, Huelva Province, lanes 2-3; *M. incognita* from olive nurseries in Spain, lanes 4-7; *M. javanica* from potato in Malta, lanes 8-10; control (no template DNA), lane 11. B, PCR amplification product using primer pair Finc/Rinc and 1 to 10 ng of template DNA of *Meloidogyne* isolates: *M. incognita* reference isolate from olive nurseries, lane 1; *M. incognita* from grapevine rootstocks sampled at Sanlúcar de Barrameda (Cádiz Province), Montilla and Montemayor (Córdoba Province), and Niebla and Bonares (Huelva Province), lanes 2-6, respectively; *M. arenaria* from olive nurseries in Southern Spain, lanes 7-8; *M. javanica* from potato in Malta, lanes 9-10; control (no template DNA), lane 11. C, PCR amplification product using primer pair Fjav/Rjav and 1 to 10 ng of template DNA of *Meloidogyne* isolates: *M. javanica* reference isolate from potato in Malta, lane 1; *M. javanica* from grapevine rootstocks sampled at Montemayor (Córdoba Province), and Almonte, Bollullos par del Condado, and Rociana (Huelva Province), lanes 2-5, respectively; *M. arenaria* from olive nurseries in Spain, lanes 6-7; *M. incognita* from olive nurseries in southern Spain, lanes 8-10; control (no template DNA), lane 10.

(Fig. 3H and I). In some cases, necrotic areas were evident in the root cortical parenchyma near the body of the nematode (Fig. 3B, C, and F).

DISCUSSION

The primary goal of this study was to determine the extent of soil infestation and rootstock infection by plant-parasitic

nematodes in commercial vineyards of key grapevine-growing areas of southern Spain. Among the 36 species of plant-parasitic nematodes diagnosed in the study,

Fig. 2. **A**, Typical brilliant yellow mosaic pattern shown in spring on leaves of a grapevine infected by a chromogenic virus strain in a commercial vineyard at Trebujena, Cádiz Province. **B**, Root system of grapevine rootstock Millardet & Grasset 41B severely infected by *Meloidogyne incognita* showing nodulation and abundant egg masses (em). **C**, Apical gall of grapevine rootstock Richter 110 induced by *M. javanica*. **D and E**, Details of galls induced by *M. incognita* in intermediate root zones of several grapevine rootstocks showing adult females (glossy white masses). **F**, Entire female of *M. incognita* and detail of dissected median bulb (mb) and stylet (st).

Fig. 3. **A**, Transverse section of a nonsymptomatic area of a young grapevine root of rootstock Richter 110, showing regular distribution and size of xylem and cortical parenchyma. **B and C**, Transverse sections of roots of grapevine rootstocks Richter 110 and Couderc 161-49, respectively, infected by *Meloidogyne incognita*. Feeding by the nematode female (N) has stimulated the formation of giant cells (GC) and disrupted the cortex and vascular tissue. **D**, Transverse section of grapevine root of rootstock Millardet & Grasset 41B showing an early infection by *M. incognita*. The second-stage juvenile (J2) was observed migrating in the apoplast at the junction between the cortex and the vascular cylinder. **E**, Transverse sections of grapevine roots of rootstock Rupes-tris du Lot showing mature infections by adult females (N) of *M. incognita*. **F**, Transverse sections of grapevine roots of rootstock Richter 110 showing mature infections by adult nematode females (N) of *M. arenaria*. **G**, Transverse section of grapevine root of rootstock Richter 110 showing mature infection by adult female (N) of *M. javanica*. **H and I**, Higher magnifications of feeding sites on root of grapevine rootstocks Moscatel and Paulsen 1103, respectively, induced by *M. incognita*. All scale bars 20 μ m.

six species, *Mesocriconema xenoplax*, *Meloidogyne arenaria*, *M. incognita*, *M. javanica*, *X. index*, *X. italiae*, and *Pratylenchus vulnus* have been reported to cause significant economic losses in other grapevine areas of the world (6). Moreover, for three of these species, *Mesocriconema xenoplax*, *Meloidogyne incognita*, and *M. javanica*, the population densities ranged from moderate to high. Particularly, *Mesocriconema xenoplax* has been found widely distributed in southern Spain, often occurring at population densities exceeding the levels reported to damage grapevine (36). Feeding of *M. xenoplax* on grapevine was reported to cause rapid darkening and destruction of root tissues, thus resulting in stunted root systems with a few feeder roots (36). The threshold population density of *Meloidogyne* spp. reported to cause yield losses in grapevine is 50 eggs and J2/100 cm³ of soil (4). Therefore, the soil population densities of *Meloidogyne* spp. found in the surveyed vineyards are large enough (mean population of 144 eggs and J2/100 cm³ of soil) to severely affect grapevine production under field conditions of southern Spain.

No estimates have been reported of direct grapevine yield losses due to infection by the dagger nematodes, *X. index* and *X. italiae*, under field conditions. Those nematodes, however, can transmit GFLV (5), reducing the lifespan of vineyards by about 12 to 20 years (33). Consequently, they should be considered a serious threat to grapevine production in southern Spain, especially as the presence of GFLV was clearly evidenced by the presence of plants exhibiting symptoms. Accordingly, these data indicate that further surveys should be carried out to determine the importance of infections by GFLV or other viruses in grapevines in southern Spain. This would allow establishing quarantine measures to assure that grapevine rootstocks are free of virus. Other nematodes found in most of the surveyed vineyards in southern Spain, such as spiral, stunt, ring, and pin nematodes, as well as the root-lesion nematodes *P. neglectus*, *P. thornei*, and *Z. guevarai*, are common parasites of monocotyledonous and dicotyledonous weeds and are not expected to cause important economic damage to grapevines.

The absence of infestations by the citrus nematode (*T. semipenetrans*) in the surveyed vineyards could be related to these vineyards being far away from the nearest citrus production area. The absence could also be due, at least in part, to resistance of some grapevine rootstocks (such as Richter 110, SO4) to that nematode (1,25) since *T. semipenetrans* has been reported infesting citrus orchards in southern Spain (29) and the three biotypes of this nematode can reproduce in grapevines (40).

Histopathological observations of *Meloidogyne*-infected rootstocks revealed typical alterations in root cell structure and

morphology which would negatively affect the efficacy of nutrient and water uptake and transport by infected roots (18). Generally, the high numbers of eggs and J2 extracted from those rootstocks indicate that they are good to excellent hosts of the nematodes. The development and parasitic habit of *Meloidogyne* spp. that we observed in naturally infected grapevine rootstocks in southern Spain were similar to those found in Moscatel rootstocks artificially infected with *M. javanica* (10,23), and *Vitis vinifera* cv. Thompson seedless artificially infected with *M. incognita* (15). Also, the occurrence of the nematode egg masses with numerous eggs formed within root tissues was similar to infections in olive planting stocks in southern Spain (27). The majority of the nematode egg masses, however, protruded from the root tissues, a situation which could further facilitate secondary infections by the nematodes by favoring their dispersal within and between vineyards by agricultural operations and movement of machinery. The histopathological responses, and the numerous egg masses and J2 stages observed in roots and rhizosphere, indicate that the nematodes developed and reproduced extensively in the major grapevine rootstocks grown in southern Spain. Consequently, those rootstocks should be considered good or very good hosts for *Meloidogyne* spp., evidence which, furthermore, should be taken into account for future field replants.

Overall, these results emphasize the importance of determining the presence of *Meloidogyne* spp. infestations in the soil, as well as using resistant rootstocks in southern Spain. The present study shows that rootstocks (such as Paulsen 1103, Richter 110, Rupestris du Lot, SO4) that were previously considered moderately resistant to *M. arenaria*, *M. incognita*, and *M. javanica* (11,26,28) should be considered susceptible under Spanish field conditions. Therefore, these reportedly resistant rootstocks cannot be considered effective against the *Meloidogyne* spp. populations occurring in southern Spain. Also, care should be taken with respect to *Meloidogyne* spp. populations present in other geographical areas. Whether these populations are newly damaging and/or resistance-breaking ones selected from the indigenous populations (2) needs to be ascertained through experiments under controlled conditions. Variation in virulence of populations of *M. incognita* on grapevine rootstocks has also been reported previously (3). Moreover, the susceptible response of the uncharacterized accessions of *V. berlandieri* and *V. riparia* rootstocks to infection by *M. incognita* and *M. javanica* agrees with results reported by Walker et al. (42), who found these rootstocks susceptible or only partially resistant to *M. incognita*.

In conclusion, these results demonstrate the importance of studying natural infections by root-knot nematodes in grapevine rootstocks in infested areas. Also, the information obtained highlights the need for proper nematode identification coupled with analysis of rootstock responses to nematode infection, to facilitate the selection of tolerant or resistant cultivars and help to lessen dependence on nematicides. Finally, the performance of rootstocks previously reported to possess a degree of resistance to some species of *Meloidogyne* must be evaluated under the growing conditions prevailing in southern Spain, and in response to locally occurring nematode species or isolates, before they can be considered resistant and are planted extensively.

ACKNOWLEDGMENTS

This research was supported in part by grant P06-AGR1360 from Consejería de Innovación, Ciencia y Empresa (CICE), Junta de Andalucía. The research stay in Spain of D. Téliz was supported by a grant from the University of Córdoba, Spain. The authors thank C. Weiland for his help in the survey of Huelva Province, J. A. Navas-Cortés for reviewing the manuscript prior to submission, and J. Martín-Barbarroja and E. García-Cuevas Agredano for technical assistance. B. B. Landa was recipient of a Ramón y Cajal grant from Ministerio de Educación y Ciencia of Spain.

LITERATURE CITED

1. Aballay, E., and Navarro, A. 2005. Tolerance of some grapevine rootstocks to *Tylenchulus semipenetrans* Cobb 1914 in Chile. *Agric. Técnica* 65:319-322.
2. Anwar, S. A., and McKenry, M. V. 2002. Developmental response of a resistance-breaking population of *Meloidogyne arenaria* on *Vitis* spp. *J. Nematol.* 34:28-33.
3. Anwar, S. A., McKenry, M. V., and Faddoul, J. 2000. Reproductive variability of field populations of *Meloidogyne* spp. on grape rootstocks. *J. Nematol.* 32:265-270.
4. Anwar, S. A., and Van Gundy, S. D. 1989. Influence of four nematodes on root and shoot growth parameters in grape. *J. Nematol.* 21:276-283.
5. Bird, G., Diamond, C., Warner, F., and Davenport, J. 1994. Distribution and regulation of *Meloidogyne nataliei*. *J. Nematol.* 26:727-730.
6. Brown, D. J., Dalmasso, A., and Trudgill, D. L. 1993. Nematode pests of soft fruits and vines. Pages 427-462 in: *Plant-Parasitic Nematodes in Temperate Agriculture*. K. Evans, D. L. Trudgill, and J. M. Webster, eds. CAB International, Wallingford, UK.
7. Carneiro, R. M. D. G., Randig, O., Almeida, M. R. A., and Gomes, A. C. M. M. 2004. Additional information on *Meloidogyne ethiopica* Whitehead, 1968 (*Tylenchida*: *Meloidogynidae*), a root-knot nematode parasitising kiwi fruit and grape-vine from Brazil and Chile. *Nematology* 6:109-123.
8. Castillo, P., Vovlas, N., Subbotin, S., and Troccoli, A. 2003. A new root-knot nematode, *Meloidogyne baetica* n. sp. (Nematoda: *Heteroderidae*), parasitizing wild olive in Southern Spain. *Phytopathology* 93:1093-1102.
9. Coolen, W. A. 1979. Methods for extraction of *Meloidogyne* spp. and other nematodes from roots and soil. Pages 317-329 in: *Root-Knot Nematodes (Meloidogyne species)*. Systematics, Biology, and Control. F. Lamberti and C. E. Taylor, eds. Academic Press, New York.
10. Doucet, M., Ponce de León, E. J., and Bustos, E. 1984. Alteraciones histológicas en *Vitis* vi-

- nifera* variedad Moscatel por *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949. Rev. Invest. Agropec. INTA 14:107-113.
11. Edwards, M. 1989. Resistance and tolerance of grapevine rootstocks to plant parasitic nematodes in vineyards in north-east Victoria. Aust. J. Exp. Agric. 29:129-131.
 12. Esbenshade, P. R., and Triantaphyllou, A. C. 1985. Identification of *Meloidogyne* species employing enzyme phenotypes as differentiating characters. Pages 135-140 in: An Advanced Treatise on *Meloidogyne*. Vol. 2. Methodology. K. R. Barker, C. C. Carter, and J. N. Sasser, eds. North Carolina State University Graphics, Raleigh.
 13. FAO, 2005. Faostat Database Collections. FAO. Online publication.
 14. Ferris, H., and McKenry, M. V. 1975. Relationship of grapevine yield and growth to nematode densities. J. Nematol. 7:295-304.
 15. Hafez, S. L., and Sundararaj, P. 2000. Host parasite interaction of *Meloidogyne incognita* and *Vitis vinifera* under green house conditions. Int. J. Nematol. 10:71-74.
 16. Hartman, K. M., and Sasser, J. N. 1985. Identification of *Meloidogyne* species on the basis of differential host test and perineal pattern morphology. Pages 69-77 in: An Advanced Treatise on *Meloidogyne*. Vol. 2. Methodology. K. R. Barker, C. C. Carter, and J. N. Sasser, eds. North Carolina State University Graphics, Raleigh.
 17. Hussey, R. S., and Barker, K. R. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Dis. Rep. 57:1025-1028.
 18. Hussey, R. S., and Williamson, V. M. 1997. Physiological and molecular aspects of nematode parasitism. Pages 87-108 in: Plant and Nematode Interactions. K. R. Barker, G. A. Pederson, and G. L. Windham, eds. American Society of Agronomy, Madison, WI.
 19. Jensen, W. A. 1962. Botanical Histochemistry. W.H. Freeman and Co., San Francisco, CA.
 20. Jenser, G., Debaj, K. H., and Lehoczy, J. 1991. The effect of the northern root-knot nematode (*Meloidogyne hapla* Chitwood) as a possible cause of unsuccessful replanting of vineyards on sandy soil. Novenyvedelem 27:62-65.
 21. Jepson, S. B. 1987. Identification of root-knot nematodes. CAB International, Wallingford, UK.
 22. Kleynhans, K. P. N. 1993. *Meloidogyne hispanica* Hirschmann, 1986 and *M. ethiopica* Whitehead, 1968 in South Africa (Nemata: Heteroderidae). Phytophylactica 25:283-288.
 23. Lamberti, F., Vovlas, N., and Hugo, H. J. 1990. Damage by *Meloidogyne javanica* on vines in south Africa. Nematol. Mediterr. 18:1-3.
 24. McKenry, M. V. 1987. Control strategies in high-value crops. Pages 329-349 in: Principles and Practice of Nematode Control in Crops. R. H. Brown and B. R. Kerry, eds. Academic Press, Orlando, FL.
 25. McKenry, M. V., and Anwar, S. A. 2006. Nematode and grape rootstock interactions including an improved understanding of tolerance. J. Nematol. 38:312-318.
 26. Mor, M., Bar-Eyal, M., Gottlieb, Y., and Harcavi, E. 2003. Grape rootstocks resistant or tolerant to the root-knot nematode species *Meloidogyne javanica* and *M. incognita*. Phytoparasitica 31:414.
 27. Nico, A. I., Rapoport, H. F., Jiménez-Díaz, R. M., and Castillo, P. 2002. Incidence and population density of plant-parasitic nematodes associated with olive planting stocks at nurseries in Southern Spain. Plant Dis. 86:1075-1079.
 28. Nicol, J. M., Stirling, G. R., Rose, B. J., May, P., and Van Heeswijk, R. 1999. Impact of nematodes on grapevine growth and productivity: Current knowledge and future directions, with special reference to Australian viticulture. Aust. J. Grape Wine Res. 5:109-127.
 29. Peña Santiago, R., Castillo, P., Escuer, M., Guerrero, P., Talavera, M., and Vieira, P. 2004. Tylenchid species (Nematoda, Tylenchida) recorded in the Iberian Peninsula and the Balearic Islands: A Compendium. Collection "Monographic Papers on Nematology", Servicio de Publicaciones, Universidad de Jaén, Jaén, Spain.
 30. Pinkerton, J. N., Schreiner, R. P., Ivors, K. L., and Vasconcelos, M. C. 2004. Effects of *Mesocriconema xenoplax* on *Vitis vinifera* and Associated Mycorrhizal Fungi. J. Nematol. 36:193-201.
 31. Pinochet, J., and Cisneros, T. 1986. Seasonal fluctuations of nematode populations in three Spanish vineyards. Rev. Nematol. 9:391-398.
 32. Quader, M., Riley, I. T., and Walker, G. E. 2001. Distribution pattern of root-knot nematodes (*Meloidogyne* spp.) in South Australian vineyards. Australas. Plant Pathol. 30:357-360.
 33. Raski, D. J. 1988. Parasites of grapes. Pages 55-59 in: Compendium of Grape Diseases. R. C. Pearson and A. C. Goheen, eds. American Phytopathological Society, St. Paul, MN.
 34. Raski, D. J., Hart, W. H., and Kasimatis, A. N. 1973. Nematodes and their control in vineyards. Calif. Agric. Exp. Stn. Circ. 533.
 35. Salmerón Parra, T., Andreu López, M., and Tobar Jiménez, A. 1986. Observaciones sobre el "amerinado" de los viñedos del condado de Huelva. Bol. Serv. Plagas 12:143-146.
 36. Santo, G. S., and Bolander, W. J. 1977. Effects of *Macroposthonia xenoplax* on the growth of concord grape. J. Nematol. 9:215-217.
 37. Seinhorst, J. W. 1962. On the killing, fixation and transferring to glycerine of the nematodes. Nematologica 8:29-32.
 38. Stirling, G. R., and Cirami, R. M. 1984. Resistance and tolerance of grape rootstocks to South Australian populations of root-knot nematode. Aust. J. Exp. Agric. Anim. Husbandry 24:277-282.
 39. Stirling, G. R., Stanton, J. M., and Marshall, J. 1992. The importance of plant-parasitic nematodes to Australian and New Zealand agriculture. Australas. Plant Pathol. 24:104-115.
 40. Verdejo Lucas, S., Sorribas, F. J., Pons, J., Forner, J. B., and Alcaide, A. 1997. The mediterranean biotypes of *Tylenchus semipenetrans* in Spanish citrus orchards. Fund. Appl. Nematol. 20:399-404.
 41. Vovlas, N., Midsuf, D., Landa, B. B., and Castillo, P. 2005. Pathogenicity of the root-knot nematode *Meloidogyne javanica* on potato. Plant Pathol. 54:657-664.
 42. Walker, M. A., Ferris, H., and Eyre, M. 1994. Resistance in *Vitis* and *Muscadinia* species to *Meloidogyne incognita*. Plant Dis. 78:1055-1058.
 43. Weiland-Ardaiz, C., and Pérez Camacho, F. 1995. Nematodes vectors of virus in the 'Denominación De Origen Condado de Huelva', Spain. Acta Hort. 388:31-35.
 44. Zijlstra, C., Donkers-Venne, D. T. H. M., and Fargette, M. 2000. Identification of *Meloidogyne incognita*, *M. javanica* and *M. arenaria* using sequence characterised amplified region (SCAR) based PCR assays. Nematology 2:847-853.