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FULL RESEARCH PAPER

Suitability of weed species prevailing in Spanish vineyards as hosts for root-knot nematodes

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Abstract Commercial vineyards in southern Spain were surveyed and sampled during October to December 2004 to determine the extent to which common weeds present were suitable hosts of root-knot nematodes infesting soils of those vineyards. Seven weed species commonly growing in grapevine soils in southern Spain were found infected by either *Meloidogyne incognita* or *M. javanica*: *Amaranthus retroflexus* (redroot pigweed), *Anchusa azurea* (ox-tongue), *Chenopodium album* (goosefoot), *Erodium moschatum* (musk stork's bill), *Malva rotundifolia* (low mallow), *Sinapis alba* (white mustard), and *Solanum nigrum* (black nightshade). The host suitability of the weeds to root-knot nematodes was evaluated on the basis of root galling severity and nematode population densities in soil and roots. Also, the host–parasite relationship in these naturally *Meloidogyne*-infected weeds was examined. All the weed species in the study were considered suitable hosts for *M. incognita* and *M. javanica* because: (a) high *Meloidogyne* spp. populations occurred in roots and surrounding soil of the weed species; (b) the severity of root galling was high, and (c) well-established permanent feeding sites were observed in the histopathological studies of infected root tissues. In addition, this study presents the first reports of *S. alba* and *A. azurea* as hosts for *M. incognita*, and of *E. moschatum* as a new host for *M. javanica*, thus increasing the list of reported weed hosts for *Meloidogyne* spp. These results indicate that noticeable population densities of *M. incognita* and *M. javanica* can be maintained or increased in these weeds, at population levels higher than those previously reported for the same nematodes infecting grapevine roots. The weeds infesting vineyards thus represent an important source of inoculum of *Meloidogyne* spp., and furthermore may act as reservoirs of these nematodes which can be disseminated within or among vineyards by agricultural operations.

Keywords Alternative host · Grapevine · Histopathology · Host-parasite relationships · *Meloidogyne incognita* · *Meloidogyne javanica* · Nematode reproduction · Reservoir host

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Introduction

Sedentary endoparasitic root-knot nematodes of the genus *Meloidogyne* are among nature's most successful

parasites. These nematodes infect thousands of different herbaceous and woody plants and are major constraints in most agricultural cropping systems, causing both yield reductions and quality loss (Karssen and Moens 2006). Root-knot nematodes, especially *Meloidogyne arenaria*, *M. incognita*, and *M. javanica*, cause serious root damage to grapevines growing in sandy soils under the mild temperatures which prevail in most grapevine-growing areas of Australia, California, the Mediterranean Basin, and South Africa (Brown et al. 1993; Nicol et al. 1999; Téliz et al. 2007). Grapevine roots infected by root-knot nematodes show malformations (galls) leading to impairment of physiologically active roots, reducing water and nutrient uptake, and making the plant more prone to infection by other fungal pathogens (Sasanelli et al. 2006).

Weeds infesting agricultural soils can play several roles in agricultural ecosystems, including reducing crop yields through competition and allelopathy (Mohler 2001) and serving as reservoirs of arthropod pests and plant pathogens such as plant-parasitic nematodes (Bendixen 1986; Anwar et al. 1992). Several studies worldwide indicate that many weeds and non-cultivated plants constitute natural, alternative hosts for crop-infecting plant-parasitic nematodes, enabling nematode survival and populations increase, and giving rise to severe crop infestation in the following season (Sikora and Fernandez 2005). Similarly, the spatial distribution of host weeds may alter the patterns of distribution of nematode populations normally associated with a given crop plant (Upchurch et al. 1970). In fact, host range studies have shown that many weed species are highly susceptible to root-knot nematodes and can serve as alternative hosts in a number of different crop systems (Edwards and Jones 1984; Bendixen 1986; Anwar et al. 1992; Bélair and Benoit 1996). However, most of these reports did not identify the infecting nematode species, nor did they examine the host–parasite relationships between the weeds and nematode species, particularly the nematodes' capacity to multiply on the roots of these weeds that would allow an estimation of their potential as a source of nematode inoculum.

Consequent to their activity as alternative hosts, the weeds may counteract control strategies for *Meloidogyne* species infesting vineyards, such as the use of resistant rootstocks, the application of chemicals, fallow, organic amendments and biofumigant crops (Brown et al. 1993; McLeod and Steel 1999; Bello et al. 2004; Rahman and Somers 2005). Other cultivation strategies, such as the low tillage used to minimise soil disturbance and reduce soil erosion commonly practiced in organic viticulture systems, may favour maintenance or even further development and multiplication of root-knot nematodes in some host weeds. Furthermore, the long-term use of grapevine-resistant rootstocks to manage *Meloidogyne* spp. can impose strong selection pressure on the nematode population, giving rise to new pathotypes which make that rootstock useless for nematode control (McKenry 1987; McKenry et al. 2001). Therefore, if nematodes are able to reproduce and maintain high population levels in nearby host weeds, a large selection base for resistant genotypes would be available.

Many studies have described the giant cells and feeding sites induced by *Meloidogyne* spp. in numerous cultivated woody and herbaceous host plants. However, these studies are lacking for *Meloidogyne*-infected weeds, except for the report of the susceptible host response of *Melilotus alba* (white sweet clover) to infection by *Meloidogyne javanica* in Argentina (Lorenzo et al. 2004). Studies on the host-suitability and host–parasite relationships of *Meloidogyne* spp. on weeds infesting vineyards are of particular interest for weeds prevailing during the autumn, whose infected roots constitute a recently multiplied source of inoculum to be dispersed in the next growing season when environmental conditions (particularly temperature) become favourable for nematode development. Histopathological studies in naturally-infected weed roots can indicate the compatible or incompatible nature of the nematode-plant interactions, providing information about the degree to which *Meloidogyne* spp. are able to thrive and carry out reproductive processes. This knowledge, together with data on nematode population densities in soil and roots of weeds, would be useful to grapevine farmers worldwide and of importance for the understanding and management of root-knot nematodes.

Hence, the main objectives of the study reported in this paper were to determine: (1) if infection by *Meloidogyne* spp. occurs in the most common and abundant weeds species which infest commercial vineyards in Andalusia (southern Spain) during the autumn; and (2) the host–parasite relationships that take place between these infected weeds and the parasitic nematode, allowing assessment of the potential significance of weeds as reservoirs and sources of inocula for the root-knot nematodes in vineyards.

Materials and methods

Field survey and nematode assessment

A field survey was undertaken during October to December 2004, when root-knot nematodes have completed their life cycle and mature females and egg-masses are detected in infected root tissues. Weeds were collected from 25 commercial vineyards in southern Spain known to bear high infestations of *Meloidogyne* spp. (Téliz et al. 2007). These *Meloidogyne*-infested vineyards were selected from a total of sixty-four vineyards of the three major wine production areas, corresponding with the marketing-operating 'wine denomination of origin (D.O.) zones' Condado de Huelva DO (Huelva province), Montilla-Moriles DO (Córdoba province), and Jerez-Xérès-Sherry y Manzanilla-Sanlúcar de Barrameda DO (Cádiz province; Téliz et al. 2007). In each field, the degree of infestation of each weed species was determined following a density scale or infestation index from 1 to 5, in which 1=<1 plant m^{-2}, 2=1–5 plants m^{-2}, 3=6–25 plants m^{-2}, 4=26–50 plants m^{-2}, and 5=>50 plants m^{-2} (Saavedra et al. 1989; Hidalgo et al. 1990). The most common and abundant weed species (i.e., those showing an infestation index ≥2) were selected for studying their suitability as hosts for root-knot nematodes. Weed species with limited extension or occasional incidence (showing an infestation index <2) in the inspected vineyards were not included in this study. These latter weed species included *Convulvulus arvensis* (field bindweed), *Lamium amplexicaule* (common henbit), *Lolium rigidum* (annual ryegrass), *Picris echioides* (bristly oxtongue), *Poa annua* (annual bluegrass), and *Stellaria media* (chickweed).

In each of the inspected vineyards, three whole plants of each weed species were selected randomly, but choosing only individuals that were growing in spots isolated from other plants so as to prevent mixing root systems. The surveyed weed plants were carefully dug out of the soil in such a way that the whole root system was collected intact, bagged, labelled, and brought to the laboratory for the immediate analysis of infection by *Meloidogyne* spp. The host suitability of the sampled weed plants to root-knot nematodes was evaluated by assessing the root galling severity and nematode population densities in both roots and the rhizosphere soil. The complete root system of each weed plant was washed free of soil, weighed, and the root gall severity (RGS) was rated on a 0–10 scale: in which 0=no galls on roots; 1=few small galls, difficult to find; 2=small galls only, but clearly visible, main roots clean; 3=some larger galls visible, main roots clean; 4=larger galls predominate but main roots clean; 5=50% of roots infected, galling on some main roots, reduced root system; 6=galling on main roots; 7=majority of main roots galled; 8=all main roots, including tap root, galled, few clean roots visible; 9=all roots severely galled, plant usually dying; and 10=all roots severely galled, no root system, plant usually dead (Bridge and Page 1980).

Nematode populations in both root and soil were assessed for each sample. To determine the population (eggs and second-stage juveniles) of *Meloidogyne* spp. in roots, 5 g samples were extracted from galled root zones (one per plant) using 1% sodium hypochlorite (Hussey and Barker 1973) followed by centrifugal flotation (Coolen 1979). Similarly, for the nematode population in soil, 100 cm^3 soil samples were extracted by centrifugal-flotation (Coolen 1979). The 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 1,100×*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 1,100×*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).

Meloidogyne species were identified by means of microscopic examinations of at least 20 perineal patterns of mature females, as well as with species-specific molecular markers amplified in polymerase chain reaction (PCR) assays based on sequence-characterized amplified regions (Zijlstra et al. 2000).

Histopathology

Infected roots from the sampled weed plants were gently washed free of adhering soil and debris. Individual galls and healthy root pieces were selected and fixed in FAE (formalin/acetic acid/60% ethanol=2:1:17 *v*/*v*/*v*) 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 56–58°C (Merck, Darmstadt, Germany). Embedded tissues were sectioned transversely at 10–12 μm with a rotary microtome, mounted on glass slides, stained with tannic acid, iron chloride, safranin and fast-green (modified from Jensen 1962), mounted permanently and observed with an optical microscope. Images were captured with a Leica QW5001 image processing system.

Statistical analysis

Data of root gall severity (RGS) and nematode population densities in soil and roots were first tested for equality of variance in order to check for normality and transformed to $\log_{10}$ (X+1; Gomez and Gomez 1984). Analyses of variance were carried out using Statistix 8.0 (NH Analytical Software, Roseville, MN, USA). Means of RGS and nematode populations in soil and roots were compared using Fisher's protected least significant difference test (LSD) at P=0.05.

Results

The most common weed species infesting the surveyed vineyards at fall season in southern Spain (Andalusia) belong to seven plant families (Table 1) and were identified as: *Amaranthus retroflexus* (redroot pigweed), *Anchusa azurea* (ox-tongue), *Chenopodium album* (goosefoot), *Erodium moschatum* (musk stork's bill), *Malva rotundifolia* (low mallow), *Sinapis alba* (white mustard), and *Solanum nigrum* (black nightshade). All weed species were consistently infected by *Meloidogyne* spp. in each of the surveyed vineyards where they were found. Black nightshade was the most widespread weed, being detected in seven vineyards, goosefoot in five, low mallow in four, redroot pigweed and white mustard in three, ox-tongue in two and musk stork's bill in one vineyard.

Based on both morphological traits and molecular analysis, the root-knot nematodes infecting the sampled weed plants were identified as *Meloidogyne incognita* and *M. javanica*. Infections of plants by either of those two nematode species gave rise to extensive galling of the root system (Fig. 1; Table 1). In all weed species, the root galls occurred individually or in clusters and resulted in noticeable distortions of the entire root morphology (Fig. 1). Numerous galls varying in size and shape occurred on both the main and lateral roots

Table 1 Populations of eggs and second-stage juveniles of *Meloidogyne* spp. detected in soil and roots of weeds species infesting commercial vineyards in southern Spain (Andalusia) during autumn[a]

Family Weed species (common name)	RGS[b]	Nematodes 100 cm^{-3} soil	Nematodes g^{-1} root
M. incognita			
Amaranthaceae			
Amaranthus retroflexus (redroot pigweed) (3)	6.6 b	94.0 ab	3,152.3 b
Boraginaceae			
Anchusa azurea (ox-tongue) (2)	6.2 b	61.3 c	337.4 d
Chenopodiaceae			
Chenopodium album (goosefoot) (5)	7.6 a	109.6 a	4,402.5 a
Malvaceae			
Malva rotundifolia (low mallow) (4)	7.7 a	88.2 b	2053.8 c
Brassicaceae			
Sinapis alba (white mustard) (3)	5.3 c	44.0 c	70.1 d
Solanaceae			
Solanum nigrum (black nightshade) (6)	7.7 a	104.3 ab	3,299.5 b
LSD 5%v	0.8	22.8	511.0
M. javanica			
Geraniaceae			
Erodium moschatum (musk stork's bill) (1)	6.5 a	74.7 a	361.4 b
Solanaceae			
Solanum nigrum (black nightshade) (1)	7.7 a	95.0 a	3,163.3 a
LSD 5%	1.7	23.8	1,557.8

[a] Data are the mean of three replicate plants per weed species in each *Meloidogyne*-infested vineyard in which that weed species was found (shown in parentheses following the weed common name). Means within each *Meloidogyne* species followed by common letters in a column are not significantly different ($P \geq 0.05$) according to Fisher's protected LSD test. Analyses were carried out using $\log_{10}$ (X+1) transformed data, untransformed means are tabulated.

[b] *RGS*=Root gall severity, assessed on a 0 to 10 scale, in which 0=no galls on roots; 1=few small galls, difficult to find; 2=small galls only, but clearly visible, main roots clean; 3=some larger galls visible, main roots clean; 4=larger galls predominate but main roots clean; 5=50% of roots infected, galling on some main roots, reduced root system; 6=galling on main roots; 7=majority of main roots galled; 8=all main roots, including tap root, galled, few clean roots visible; 9=all roots severely galled, plant usually dying; and 10=all roots severely galled, no root system, plant usually dead.

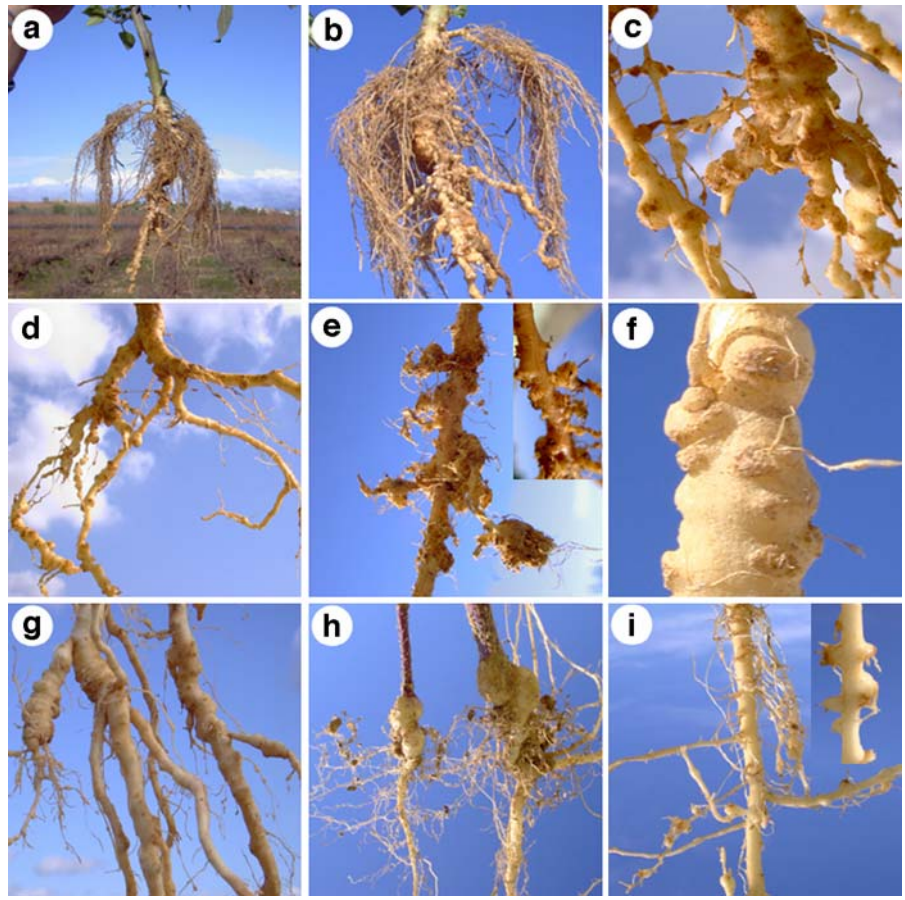

Fig. 1 Severe galling in root systems of several *Meloidogyne* spp.-infected weeds infesting commercial vineyards in southern Spain (Andalusia). Nodules, which varied somewhat in form, were numerous and occurred on both the main and lateral roots. The infected weed species are: **a**, **b** *Solanum nigrum*, **c** *Malva rotundifolia*, **d** *Chenopodium album*, **e**, **f** *Amaranthus retroflexus*, **g** *Anchusa azurea*, **h** *Sinapis alba*, **i** *Erodium moschatum*

(Fig. 1). Root galling severity in the *M. incognita*-infected weeds varied significantly ($P<0.05$) among weed species, with black nightshade, low mallow, and goosefoot showing the highest galling severity (RGS>7) and white mustard the lowest (5.3; Fig. 1; Table 1). Similarly, the *M. incognita* population densities in both the soil samples and root systems differed significantly ($P<0.05$) among weed species, with the highest population occurring in goosefoot and the lowest in ox-tongue and white mustard (Table 1). Root galling severity in weeds infected by *M. javanica*, however, was similar ($P\geq0.05$) between the two infected weed species; and for black nightshade was also similar in severity to that induced by *M. incognita* in the same species (Table 1). Whereas the root population densities of *M. javanica* differed between the weed species, with the value for black nightshade significantly ($P<0.05$) higher than that of musk stork's bill (Table 1), the soil rhizosphere population densities for that nematode did not differ significantly ($P\geq0.05$) between them (Table 1).

In most cases, the nematode-induced permanent feeding sites consisted of groups of three to four large, multinucleate giant cells, but two to six giant cells per feeding site were also commonly observed in all weed hosts in the study (Fig. 2). Histological observations of *Meloidogyne*-infected weed roots confirmed that the nematodes successfully established permanent feeding sites which caused cellular alterations in the root cortex, endodermis, pericycle, and vascular parenchyma (Fig. 2). In all weed species, abundant permanent feeding sites were usually induced adjacent to the vascular tissues. The feeding sites were established either in the secondary phloem and cortical parenchyma (e.g. roots of black nightshade and goosefoot; Fig. 2a,d); the primary and secondary xylem, closely associated with differentiated but distorted xylem elements (e.g., roots of redroot pigweed and ox-tongue; Fig. 2e,f); or in the cortex and stelar parenchyma (e.g., white mustard; Fig. 2h). Often, these feeding sites gave rise to severe distortion and crushing of xylematic tissues (Fig. 2e–g).

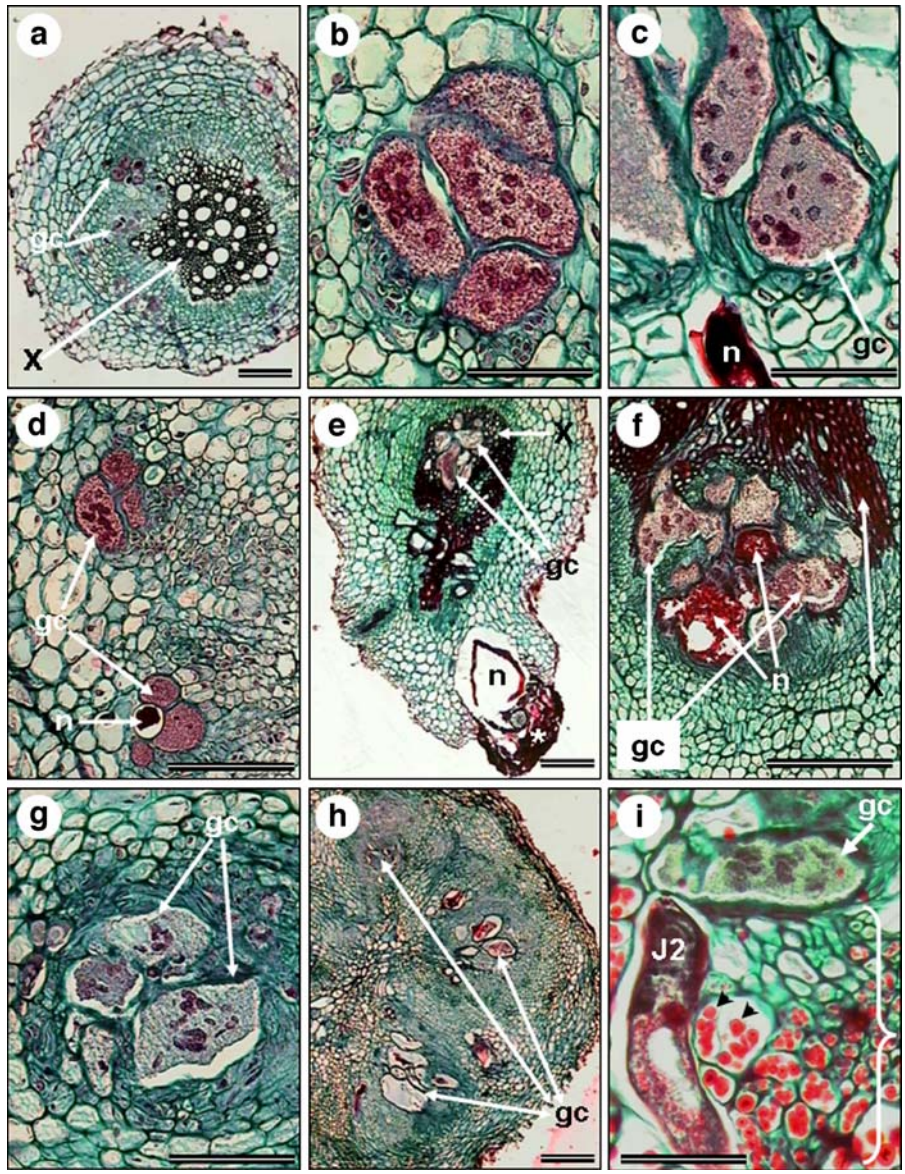

Fig. 2 Transverse sections of weed roots of different plant species infected by *Meloidogyne* spp. The infections were characterized by the presence of giant cells (*gc*) and mature female nematodes (*n*). **a** *Solanum nigrum* infected by *M. incognita*, showing two groups of giant cells (gc) in the secondary phloem. **b** *Solanum nigrum* infected by *M. javanica*. Detail of feeding site composed of four giant cells in the cortex. The giant cells are distinguished by highly dense cytoplasm and contain dark, hypertrophied nuclei. **c** *Malva rotundifolia* infected by *M. incognita*. Mature female nematode (n) close to three giant cells, two of which show dark, hypertrophied nuclei. **d** *Chenopodium album* infected by *M. incognita*. Two groups (feeding sites) of three giant cells each have formed between the cortex (*left part* of photo) and secondary phloem (*right part* of photo). **e** *Amaranthus retroflexus* infected by *M. incognita* showing the mature female (n) and egg mass (*) within a protrusion of the root cortex, and giant cells (gc) in the primary and secondary xylem, closely associated with differentiated but distorted xylem elements (*x*). **f** Detail of *Amaranthus retroflexus* infected by *M. incognita* (*E*), showing the close association of mature females (n), giant cells and host plant xylem cells (x), the latter highly distorted in their structure and arrangement. **g** *Anchusa azurea* infected by *M. incognita*, with four giant cells in the cortex containing irregular hypertrophied nuclei. **h** *Sinapis alba* infected by *M. incognita* showing groups of giant cells (gc) in the cortex and stelar parenchyma. **i** *Erodium moschatum* infected by *M. javanica*. Juvenile (*J2*) which has migrated apoplastically between cortical cells (*bracket*) and giant cell (gc) which it has induced. The cortical cells contain numerous starch grains (*arrows*) and the giant cell nuclei are hypertrophied. Scale bars **a**, **d**, **e**, **f**, **h**= 20 μm; **b**, **c**, **g**, **i**=10 μm

Early during infections of musk stork's bill by *M. javanica*, the second-stage juveniles of the nematode were observed to migrate in the apoplast between cortical cells, and induce giant cells at or within the vascular cylinder (Fig. 2i). The giant cells formed in all observed nematode-weed species combinations showed characteristic dense cytoplasm and 4 to 18 hypertrophied nuclei and nucleoli (Fig. 2b,c,g,i). Additionally, hyperplasia of tissues adjacent to the giant cells contributed to root tissue expansion, leading to the formation of the observed root galling (Figs. 1 and 2). Nematode egg masses were observed mostly at the root surface, irrespective of the weed species, although occasionally some egg masses were found inside the cortical root tissues (Fig. 2e). The majority of the egg masses contained numerous eggs (Fig. 2e). Furthermore, numerous starch granules were found in the parenchymatic cortical cells of musk stork's bill infected by *M. javanica*, but none or very low numbers of them were observed in adjacent giant cells induced by the nematode (Fig. 2i).

Discussion

High populations of *M. incognita* and *M. javanica* occurred in roots and rhizosphere soil of the seven most common weed species found growing in commercial vineyards of southern Spain (Andalusia) during the fall season. Furthermore, well-established permanent feeding sites of the nematodes together with severe root galling were observed in the infected roots. These observations indicate that the weed species are very suitable hosts for these root-knot nematodes (Hussey 1985), and suggest that they may act as a source of inocula for infection of grapevines. The high infections by the same *Meloidogyne* spp. previously detected by Téliz et al. (2007) on feeder roots of the grapevines of these vineyards confirms this likelihood. Additionally, most of these weeds are widely distributed in southern Spain and are frequently associated with other crops, such as corn, cotton, sunflower, sugar-beet, and wheat (Saavedra et al. 1989; Hidalgo et al. 1990), so their identification as hosts for *Meloidogyne* spp. is also a useful tool for the management of root-knot nematodes in those crops.

Although the ability of *Meloidogyne* spp. to induce root galling and reproduce within the galls varied among the different weed species, the results of this work and previous observations clearly demonstrate that the weeds play a critical role in the increase and maintenance of root-knot nematode populations in vineyards in southern Spain. In our study, the large numbers of egg masses, second-stage juveniles, and eggs recovered from infected weed roots and rhizosphere soil indicate a successful host–parasite relationship between the nematodes and weeds, and qualify the latter as satisfactory or excellent hosts for *M. incognita* and/or *M. javanica*. More specifically from field data, black nightshade, goosefoot, redroot pigweed, and low mallow are very suitable hosts for *M. incognita*, compared with ox-tongue and white mustard which appear to be less suitable. Similarly, musk stork's bill and black nightshade are very suitable hosts for *M. javanica*.

The levels of root galling and nematode population densities of *M. incognita* and *M. javanica* found in our study are consistent with previous reports concerning redroot pigweed, black nightshade, and goosefoot in Canada (Davidson and Townshend 1967), Italy (Ciancio et al. 1995), Pakistan (Anwar et al. 1992), and Spain (Barceló et al. 1997; Zancada et al. 1998). In particular, the intermediate level of root galling and the short galls that we found in redroot pigweed infected by *M. incognita* were similar to those reported by Davidson and Townshend (1967). Similarly, our results confirm that weed members of the Amaranthaceae, Chenopodiaceae and Solanaceae are particularly good hosts for *Meloidogyne* spp. (Barceló et al. 1997; Quénéhervé et al. 1995).

This study presents the first report of *S. alba* and *A. azurea* as hosts for *M. incognita*, and *E. moschatum* as a host for *M. javanica*, thus increasing the list of weed hosts for *Meloidogyne* spp. previously reported. Although *S. alba* has been indicated as susceptible to *M. incognita* and *M. javanica* in several experimental host-suitability studies carried out under controlled conditions (Gardner and Caswell-Chen 1994; Liébanas and Castillo 2004), to our knowledge natural infections of this species and of ox-tongue by *M. incognita*, and of musk stork's bill by *M. javanica*, have not yet been reported.

The susceptible reaction of black nightshade to *M. incognita* and *M. javanica* in this study, together with several reports of infections by other *Meloidogyne* spp., such us *M. ethiopica* in Ethiopia (Whitehead 1968), *M. hapla* in Hungary (Dabaj and Jenser 1990), *M. incognita* in Canada (Davidson and Townshend

1967), or *M. javanica* in Italy (Ciancio et al. 1995) and Pakistan (Zarina and Abid 1995), confirm that this weed is highly susceptible to infections by root-knot nematodes species infecting grapevines worldwide. Finally, although no detailed study on the concentration of starch granules in the root tissues of musk stork's bill was carried out in this investigation, the low levels of starch granules in adjacent giant cells induced by *M. javanica* may be similar to observations in other plant-nematode interactions, such as *Mesocriconema xenoplax* on peach (Olien et al. 1995), in which nematode parasitism reduces starch accumulation.

Some cruciferous plant species have potential for use in the management of soil-borne pathogens, either as green manure crops or as break crops in rotation (Kirkegaard et al. 1998). Glucosinolates (amino-acid-derived products of secondary metabolism) stored in the vacuoles of crucifers such as white mustard weed have proven to have nematicidal activity against some plant-parasitic nematodes (Potter et al. 1999). However, the release of these toxic compounds requires cellular disruption which perhaps is not triggered by *Meloidogyne* parasitism, since the nematode progresses intercellularly within tissues while migrating through root-tip regions (where cells are young and not vacuolated) to the vascular cylinder where it settles and grows (Wyss et al. 1992). This reasoning and the lack of nematicidal activity of white mustard against *Meloidogyne* spp. observed by Liébanas and Castillo (2004) may be due to the susceptibility of white mustard to infections by *M. incognita* as demonstrated in this study.

Results of this present work indicate that weeds infesting commercial vineyards in southern Spain in the autumn may contribute to the maintenance, re-infestation, multiplication and spread of *Meloidogyne* spp. within a field, and thus to increase the potential for grapevines to be damaged by nematode attacks. In summary, a number of weeds species infesting vineyards are hosts on which populations of *Meloidogyne* spp. can be maintained or even increased to densities higher than those found in grapevines. Additional research is needed under controlled conditions to validate our field findings regarding the infection capacity and reproductive fitness of *Meloidogyne* spp. on these weeds. Therefore, not only does the occurrence of weeds in vineyards represents a potential source of nematode inoculum for infection of grapevine roots during the growing season, but also these weeds should be considered as primary bio-indicators to assess the presence and/or absence of the nematode in grapevine orchards. In addition, the weeds growing in infested areas may aid in the dissemination of *Meloidogyne* spp. within or among vineyards by many agricultural operations, and they can reduce the beneficial effects of the *Meloidogyne*-resistant grapevine rootstocks. Based on our results, it should be emphasized that efficient weed control before replanting a soil with grapevines as well as during vineyard management are necessary and suitable practices for the integrated management of *Meloidogyne* spp. in grapevine soils in southern Spain.

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