Plant-Parasitic Nematodes Attacking Olive Trees and their Management

Importance of Olives and Their Associated Nematodes

Olive trees (*Olea europaea* (L.) subsp. *europaea* (L.)) were among the first fruit trees cultivated and are among the most ancient crops in the Mediterranean Basin (103). Cultivated olive trees can be distinguished from their wild relatives by larger fruits and higher oil content of the fleshy oil-containing mesocarp. The edible oil and fruit products of cultivated olive trees are prized worldwide. Botanical studies of pollen grains, stones, and wood from archaeological contexts indicate that the East Mediterranean (Israel) was the first area in which olive was domesticated (54). In the Mediterranean Basin, olive constitutes a complex of wild forms, weedy types, and cultivated varieties. Wild oleasters (*Olea europaea* (L.) subsp. *sylvestris* (Brot.)) occupy primary niches and occur in disturbed areas and in areas of abandoned cultivation (33). Cultivated olive is also grown extensively in the subtropical regions of Australia, southern Africa, and North and South America. More than 80% of 8 million hectares of olive grown worldwide in 2007 were located in Mediterranean countries, including North Africa. An additional 17% occur in the Middle East (Iraq, Iran, Jordan, and Syria), and less than 2% are found in the Americas (Argentina, Chile, Mexico, Peru, and the United States) (34). Olive crops are one of the most important sources of edible oil, and

olive varieties are cultivated for oil extraction. Olive oil has many favorable nutritional properties; as a result, consumption of olive oil is increasing worldwide, extending the consumption area from the traditional one in the Mediterranean Basin (i.e., 77% of the world production area) to Australia, Canada, Japan, and the United States. Some olive varieties are specifically cultivated for direct consumption as table olives.

Olive trees serve as hosts to a large number of plant-parasitic nematodes, of which root-knot nematodes (*Meloidogyne* spp.), root-lesion nematodes (*Pratylenchus* spp.), spiral nematodes (*Helicotylenchus* spp.), and *Mesocriconema xenoplax* (Raski) Loof & De Grisse, 1989 are widely distributed (Table 1) (2,42,51,65,70,74). Conversely, limited distribution on olive has been reported for the citrus (*Tylenchulus semipenetrans* Cobb, 1913) and the cyst-forming (*Heterodera mediterranea* Vovlas, Inserra & Stone, 1981) nematodes (19,21,59). Of the endoparasitic nematodes (including *Meloidogyne*, *Pratylenchus*, *Tylenchulus*, and *Heterodera*), many are recognized as pathogenic to olive. The effects of parasitism by some nematodes on plant growth and yield result from disruption of the normal process of plant root growth and function (40). Water stress and nutrient deficiencies occur as the plant root system fails to adequately explore and exploit the soil due to plant parasitic nematode attacks. Damage to established olive orchards by nematode parasitism may not be clearly perceived because olive is an extremely vigorous plant able to thrive in relatively dry areas, which may obscure expression of symptoms from nematode attacks. However, modern olive production is based largely on the establishment of new high-input orchards intended to in-

crease yields while reducing the time for investment recovery. This model, broadly adopted in new olive-producing areas of the Southern Hemisphere and the Mediterranean Basin, creates an environment conducive to diseases caused by or involving nematodes. In Argentina, for example, *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 was one of the main pathogens associated with a high incidence of “drying syndrome” in newly established olive orchards (75). Similarly, estimates of olive losses in the United States due to *Meloidogyne* spp. and *T. semipenetrans* ranged from 5 to 10% (44). Nematode damage to olive is particularly evident in nurseries, where optimum irrigation practices favor root proliferation and increased nematode population growth that subsequently impacts adult trees (29,70).

The accurate and timely identification of plant-parasitic nematodes infecting crops is a prerequisite for designing effective management strategies. This is particularly relevant for olive trees because of the diversity of pathogenic nematode genera and species in several growing areas (Table 1). A reliable identification allows distinction between pathogenic and nonpathogenic nematodes and exclusion of species under quarantine or regulatory strategies. Identification of most nematodes is based on morphology and morphometric traits of adult females (20,40), but the task is hindered by the need for experienced diagnosticians to assess the intraspecific variability of morphological traits. To illustrate this point, the main comparative diagnostic traits of *Meloidogyne* spp. attacking olives are summarized (Table 2). The last few years have seen the development of reliable diagnostic methods based on nucleic acid analyses, which have significantly improved and facilitated the routine identi-

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fication of *Meloidogyne* and *Pratylenchus* species (16). Polyphasic taxonomy, which assembles and assimilates all available information, may be a better strategy for delimiting taxa at all levels (e.g., combined use of internal transcribed spacer region

sequences [ITS] of ribosomal DNA [rDNA], esterase phenotypes, and female perineal patterns; Fig. 1). This is especially valid for those *Meloidogyne* spp. that cannot be well resolved by phylogenetic analysis of rDNA sequences only.

Here, we focus on the geographical distribution and pathogenicity of the major nematode species that infect olive trees, along with the symptoms, epidemiology, and management of the diseases they cause. In addition, we address the complex

Table 1. Plant-parasitic nematodes associated with olive trees^a

Nematode group and species	Threshold level (nematodes/cm ³ soil or g ⁻¹ soil)	Country/region
Root-knot nematodes		
<i>Meloidogyne</i> spp.		
<i>M. arenaria</i>	—	Spain
<i>M. baetica</i>	—	Spain
<i>M. hapla</i>	—	Chile, Israel, Portugal
<i>M. incognita</i>	—	Argentina, Brazil, Egypt, India, Italy, Jordan, Lebanon, Spain
<i>M. javanica</i>	0.49–0.91	Chile, China, Egypt, Greece, Iran, Italy, Jordan, Libya, Pakistan, Portugal, Spain, USA
<i>M. lusitanica</i>	—	Portugal, Spain
Root-lesion nematodes		
<i>Pratylenchoides</i> spp.		
<i>P. erzurumensis</i>	—	Turkey
<i>P. ritteri</i>	—	Spain, Turkey
<i>Pratylenchus</i> spp.		
<i>P. crenatus</i>	—	Italy
<i>P. coffeae</i>	—	Australia
<i>P. fallax</i>	—	Spain
<i>P. mediterraneus</i>	—	Turkey
<i>P. neglectus</i>	—	Spain
<i>P. penetrans</i>	—	Italy, Spain, Turkey
<i>P. thornei</i>	—	Spain
<i>P. vulnus</i>	—	Algeria, Italy, Spain, USA
<i>P. zaeae</i>	—	Turkey
<i>Zygotylenchus guevarai</i>	—	Spain, Turkey
Cyst nematodes		
<i>Heterodera mediterranea</i>	—	Italy, Spain
Reniform and related nematodes		
<i>Rotylenchulus</i> spp.		
<i>R. macrodoratus</i>	—	Greece, Italy
<i>R. macrosoma</i>	0.5–1.0	Israel, Spain, Turkey
<i>R. reniformis</i>	—	Egypt, Greece
<i>Trophytylenchulus saltensis</i>	—	Jordan
<i>Tylenchulus semipenetrans</i>	—	Australia, Chile, Greece, Italy, USA
Stunt, awl, and spiral nematodes		
<i>Amplimerlinius</i> spp.		
<i>A. amplus</i>	—	Portugal
<i>A. dubius</i>	—	Turkey
<i>A. macrurus</i>	—	Jordan
<i>A. paraglobigerus</i>	—	Spain
<i>Aorolaimus perscitus</i>	—	Spain
<i>Bitylenchus goffarti</i>	—	Turkey
<i>Dolichodorus heterocephalus</i>	—	Italy
<i>Helicotylenchus</i> spp.		
<i>H. digonicus</i>	—	Cyprus, Jordan, Spain, Turkey
<i>H. dihystra</i>	—	Cyprus, Egypt, Jordan, Spain, Zimbabwe
<i>H. erithrinae</i>	—	Italy
<i>H. neopaxili</i>	—	Italy
<i>H. oleae</i>	—	Italy
<i>H. pseudorobustus</i>	—	Greece, Italy, Spain
<i>H. tunisiensis</i>	—	Israel, Jordan, Spain, Turkey
<i>H. vulgaris</i>	—	Portugal, Spain, Turkey
<i>Hoplolaimus aorolaimoides</i>	—	Portugal
<i>Hoplolaimus galeatus</i>	—	Turkey
<i>Paratrophurus loofi</i>	—	Spain
<i>Plesiorotylenchus striaticeps</i>	—	Turkey
<i>Rotylenchus</i> spp.		
<i>R. buxophilus</i>	—	Turkey
<i>R. cypriensis</i>	—	Turkey
<i>R. robustus</i>	—	Portugal
<i>Quinisulcius acutus</i>	—	Turkey
<i>Scutylenechus lenorus</i>	—	Turkey

(continued on next page)

^a Data from the following references: 2,11,12,20–22,26,28,36,37,39,42,50,51,59,64,65,70,73,74,79,80,93,95,96.

interactions that occur between some nematodes and *Verticillium* wilt of olive caused by the soilborne fungus *Verticillium dahliae* Kleb. Finally, we propose effective measures for nematode management in olive nurseries, which are of paramount

importance for the production of nematode-free olive planting stock.

Root-Knot Nematodes

Root-knot nematodes, *Meloidogyne* spp., are among the most damaging plant

pathogens worldwide (40). *Meloidogyne* species known to damage olive trees are well adapted to temperate and subtropical areas and include *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949; *Meloidogyne baetica* Castillo, Vovlas, Subbotin

Table 1. (continued from previous page)

Nematode group and species	Threshold level (nematodes/cm ³ soil or g ⁻¹ soil)	Country/region
<i>Tylenchorhynchus</i> spp.		
<i>T. aduncus</i>	—	Spain
<i>T. claytoni</i>	—	Cyprus, Turkey
<i>T. clarus</i>	—	Jordan, Spain
<i>T. dubius</i>	—	Greece, Spain
<i>T. cylindricus</i>	—	Turkey
<i>T. goffarti</i>	—	Jordan
<i>T. huesingi</i>	—	Spain
<i>T. mamillatus</i>	—	Spain
<i>T. penniseti</i>	—	Turkey
<i>T. striatus</i>	—	Greece
<i>T. tenuis</i>	—	Jordan
<i>T. tritici</i>	—	Turkey
Ring, shethoid, and pin nematodes		
<i>Criconea princeps</i>	—	Portugal
<i>Criconemoides informis</i>	—	Jordan, Spain
<i>Gracilacus peratica</i>	—	Italy, Portugal, Spain
<i>Gracilacus teres</i>	—	Spain
<i>Hemicriconemoides gaddi</i>	—	Turkey
<i>Hemicyclophora sturhani</i>	—	Turkey
<i>Mesocriconea</i> spp.		
<i>M. curvatum</i>	—	Spain
<i>M. olearum</i>	—	Jordan
<i>M. onoense</i>	—	Brazil
<i>M. siculum</i>	—	Italy
<i>M. spaherocephalum</i>	—	Spain
<i>M. xenoplax</i>	—	Spain
<i>Ogma civellae</i>	—	Zimbabwe
<i>Ogma rhombosquamatum</i>	—	Italy, Portugal, Spain
<i>Paratylenchus</i> spp.		
<i>P. arcuatus</i>	—	Spain, Turkey
<i>P. baldacii</i>	—	Spain
<i>P. microdorus</i>	—	Spain
<i>P. nanus</i>	—	Spain
<i>P. sheri</i>	—	Spain
<i>P. vandenbrandei</i>	—	Italy
Dagger, needle, and stubby-root nematodes		
<i>Xiphinema</i> spp.		
<i>X. aequum</i>	—	Italy
<i>X. barese</i>	—	Italy
<i>X. californicum</i>	—	USA
<i>X. elongatum</i>	—	Egypt
<i>X. index</i>	—	Greece
<i>X. ingens</i>	—	Italy, Jordan
<i>X. italiae</i>	—	Italy
<i>X. macroacanthum</i>	—	Italy
<i>X. pachtaicum</i>	—	Italy, Jordan, Spain
<i>X. sahelense</i>	—	Spain
<i>X. turcicum</i>	—	Spain
<i>X. vuittenezi</i>	—	Spain
<i>Longidorus</i> spp.		
<i>L. africanus</i>	—	Egypt
<i>L. belloi</i>	—	Portugal
<i>L. closelongatus</i>	—	Greece
<i>L. cretensis</i>	—	Greece
<i>L. macrosoma</i>	—	Spain
<i>L. siddiqii</i>	—	Jordan
<i>L. vinearum</i>	—	Portugal
<i>Paratrichodorus</i> spp.		
<i>P. minor</i>	—	Spain
<i>P. teres</i>	—	Spain
<i>Trichodorus</i> spp.		
<i>T. giennensis</i>	—	Spain
<i>T. primitivus</i>	—	Portugal
<i>T. taylori</i>	—	Italy

& Troccoli, 2003; *Meloidogyne hapla* Chitwood, 1949; *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949; *M. javanica*; and *Meloidogyne lusitanica* Abrantes & Santos, 1991 (Table 1, Fig.1). They occur in sporadic distributions in wild olive, olive nurseries, and established orchards, and cause heavy root galling (Fig. 2) and plant growth retardation in pathogenicity tests (22,45,70,85,86). The economic importance of these nematodes in olive cultivation has increased in recent years because most chemical agents for the control of plant-parasitic nematodes have been banned due to environmental and health concerns.

Meloidogyne species are obligate biotrophic pathogens with evolved strategies for infecting thousands of plant species in a similar manner, probably by manipulating fundamental elements of plant cell development (40). Infections by root-knot nematodes induce formation of permanent feeding cells, the giant cells, and stelar destruction in olive roots (Fig. 3). Hyperplasia of the surrounding cells leads to the formation of typical root galls, the primary visible symptom of infection (Figs. 2 and 3). Preferred feeding sites are primary phloem or adjacent undifferentiated parenchyma cells (70). Low to intermediate levels of olive root infections by root-knot nematodes result in moderate root galling

in most olive cultivars tested under controlled conditions, as was previously observed in naturally infested rootstocks from olive nurseries in Andalusia, southern Spain (67,70,85,86).

Pathogenicity assays under controlled environmental conditions demonstrated the potential of *M. arenaria* race 2, *M. incognita* race 1, and *M. javanica* to damage olive rootstocks (FS 17, DA 12 I) (85,86) and self-rooted olive cultivars Arbequina and Picual (67). Planting stocks of Picual (one of the olive cultivars most extensively grown in the Mediterranean Basin) infected by *Meloidogyne* spp. showed a distinct yellowing affecting the uppermost leaves, followed by partial defoliation (Fig. 4). Symptoms were more severe on *M. arenaria*- and *M. javanica*-infected plants than on *M. incognita*-infected plants. Tolerance limits for inoculum (87) of *M. javanica* on cultivars FS 17 and DA 12 I grown under greenhouse conditions were established at 0.49 and 0.61 second-stage juveniles (J₂)/g soil, respectively (Fig. 5); and minimum yields near 50% were estimated for seedling growth (85,86). Studies on the pathogenicity of *Meloidogyne* spp. to olives suggest that main stem diameter is a highly sensitive parameter for assessing damage caused by *M. arenaria* and *M. javanica* (67,85). A new species, *M. baetica*, was recently described from wild

olives in Spain (22). Although the economic importance of this nematode on cultivated olive is still unknown, the histopathological response of Arbequina and Picual olives was similar to that reported for other root-knot species. This may indicate a potential risk for olive growing if this nematode species is widely distributed (22). Long-term experiments under field or microplot conditions are needed to further assess the damaging potential of these nematodes to growth and yield of the most extensively grown olive cultivars.

Root-Lesion Nematodes

Root-lesion nematodes are obligate migratory endoparasites that damage olive roots by feeding in the cortical parenchyma, causing extensive necrosis of cortical and endodermal cells (20). Infection of olive roots by *Pratylenchus* spp. causes cortical lesions and reduces root number and size. Both root penetration and migration of the nematode within root tissues are facilitated by a combination of stylet thrusting and enzymatic softening of the host cell walls (104). Although a large number of root-lesion nematode species were found associated with olive in disease surveys (Table 1), artificial inoculations of self-rooted olive plants demonstrated that most of these species do not reproduce on olive (67) and should be considered non-

Table 2. Differential morphological traits among *Meloidogyne* species infecting olive trees^a

	<i>M. arenaria</i>	<i>M. baetica</i>	<i>M. hapla</i>	<i>M. incognita</i>	<i>M. javanica</i>	<i>M. lusitanica</i>
Female						
Stylet length	15.5 (14.4-15.8)	17.5 (17-19)	15 (13-17)	14 (13-16)	17.0 (14.0-18.0)	17 (16-19)
Knobs shape	Rounded and backwardly directed	Ovoid, sloping backwards	Relatively small, rounded and set off	Rounded or drawn out laterally	Rounded	Ovoid and backwardly directed
EP/ST ^b ratio	2.4	0.7 (0.5-0.8)	3.0	1.4	2.8	2.6 (1.6-3.8)
Perineal pattern	Round to oval with dorsal arch low, striae fairly widely separated and lateral field usually marked by some forked and broken striae	Distinct inner striae forming two distinct longitudinal bands, extending throughout the perineum to just below the vulva	Rounded with low dorsal arch, with punctations in tail terminus area	Striae closely spaced, very wavy to zig-zag, dorsal arch high	Striae interrupted laterally by conspicuous double incisures edging lateral ridges	Striking, trapezoid with coarse striae and medium to high dorsal arch
Isozyme ^c phenotype	Est A1,A2,A3 Mdh N1	VS1 N1c	H1 H1	II N1	J3 N1	A1 P3
Infective second-stage juvenile (J ₂)						
Body length	738 (510-1012)	403 (394 to 422)	337 (360-500)	371 (337-403)	417 (387-459)	450 (390-515)
Stylet length	11.6 (11.1-12.6)	13.5 (13-14)	9.7 (7.9-10.9)	10.5 (9.6-11.7)	10.4 (9.4-11.4)	14.2 (13-16)
Hemizonid position	Anterior, not adjacent to EP	2-3 annuli anterior to EP	Anterior, not adjacent to EP	Just anterior to EP	Immediately anterior to EP	One or two annuli anterior to EP
Tail length	55.8 (52.2-59.9)	50 (47-54)	(48-70)	48.7 (45-52)	49(36-56)	44 (39-50)
Tail hyaline portion	14.8 (10.8-19.8)	12 (10-13)	(12-19)	8.9 (6.3-13.5)	13.7 (9.8-18.0)	12 (10-14)
Male						
Stylet length	23.5 (20.7-25.0)	21.6 (20.7-23.4)	20.5 (19-22)	24.8 (24.3-26.1)	20.3 (18.9-23.4)	24.5 (21-27)
Knobs shape	Ovoid and backwardly sloping	Smoothly rounded to stylet	Relatively small, rounded, set off	Rounded with flat, concave or toothed anterior margins	Low and wide, often anteriorly indented, and set off from the shaft	Elongate, pear shaped, slightly set off from shaft
Spicules	36.5 (35.4-37.9)	27 (24-36)	25.7 (21.6-28.1)	35 (29-40)	29.5 (22.9-35.3)	37.9 (32.0-44.5)
Gubernaculum	9.8 (9.5-10.7)	8.2 (7.2-9.4)	8.2 (7.2-9.4)	11.2 (9.4-13.7)	8.0 (5.7-10.1)	10.2 (8.5-12.0)

^a All measurements are in micrometers unless otherwise stated.

^b EP/ST = excretory pore to female anterior end/female stylet length.

^c Isozyme phenotypes used are defined by Esbenshade and Triantaphyllou (32).

pathogenic to olive. In fact, research in our laboratory showed that the reproduction rates of *Pratylenchus fallax* Seinhorst, 1968; *Pratylenchus thornei* Sher & Allen, 1953; and *Zygotylenchus guevarai* (Tobar Jiménez, 1963) Braun & Loof, 1966 on Arbequina and Picual olive were less than 1.0, suggesting that these cultivars are not suitable hosts for those species. These results agree with field observations, which indicate that these lesion nematodes reproduce predominantly on herbaceous hosts and their presence in fruit tree nurseries (including olive) does not imply a major problem for olive nursery production (67,76). Although *Pratylenchus coffeae* (Zimmermann, 1898) Filipjev & Schuurmans-Stekhoven, 1941 has been reported on olive in Australia (51), the damage it might cause to olive growth or yield is still unknown. Consequently, the two major species most frequently diagnosed as damaging to olive worldwide are *Pratylenchus penetrans* (Cobb, 1917) Filipjev & Schuurmans-Stekhoven, 1941 and *Pratylenchus vulnus* Allen & Jensen, 1951 (Fig. 6), which reduced seedling growth in bioassays (46,67).

P. penetrans and *P. vulnus* can be differentiated by phylogenetic analysis of sequences of the D2-D3 expansion regions of 28S rRNA and 18S rRNA (92) as well as several differential morphological traits (20) such as (i) rounded spermatheca, post-vulval uterine sac 1 to 1.5 vulval body diameter long, and subcylindrical female tail in *P. penetrans*, compared with (ii) oval spermatheca, postvulval uterine sac 2 vulval body diameter long, and conoid

female tail with subacute smooth tip in *P. vulnus*. In spite of the abilities of both *P. penetrans* and *P. vulnus* to infect and reproduce in olive planting stocks, usually they do not induce any macroscopic symptoms on either roots or foliage (67). However, 'Manzanilla' olive planting stocks infected with *P. vulnus* were partially defoliated, and weighed 42% less than noninfected plants (46). Isolates of *P. vulnus* of different geographical origin and host source may vary in pathogenicity to olive. Pinochet et al. (77) showed that a population from rose plants in northeastern Spain failed to reproduce on Arbequina olive. In contrast, Nico et al. (67,70) found that a population of *P. vulnus* from olive nurseries in Andalusia reproduced well on Arbequina and Picual. Pathogenicity assays under controlled conditions showed that infection by *P. vulnus* and *P. penetrans* significantly reduced the main stem diameter in both cultivars.

Since mixed populations of root-knot and root-lesion nematodes commonly co-infect root systems of olive planting stocks under natural conditions (70), competition between and among nematode species within roots may influence host-parasite interactions. Lamberti et al. (47) reported that the reproduction of *P. vulnus* in 'Lecicino' and 'Pendolino' olive plants was significantly reduced by co-infection with *M. incognita*.

Cyst-Forming Nematodes

These nematodes are characterized by a cyst stage that allows them to survive in soil for several years in the absence of a

host plant. Few cyst nematode species have been reported parasitizing woody plants (8). The cyst-forming nematode, *H. mediterranea*, was found in commercial Manzanilla olive orchards established in sandy soils in southern Spain (21). Artificial inoculations had previously demonstrated the ability of *H. mediterranea* to infect olive plants, thus enlarging the otherwise narrow host range (lentisc, pistachio) known until then (98). Host-parasite studies revealed that sedentary juvenile stages and adult females of *H. mediterranea* have a semi-endoparasitic feeding habit and do not completely penetrate olive roots (Fig. 7) (21). Those studies also showed that the nematode's feeding in olive roots induces syncytia, involving cellular alterations in the cortex, endodermis, pericycle, and vascular parenchyma. For most *Heterodera* spp., egg hatching as well as orientation toward host tissues is stimulated by host root exudates (8); however, no difference in egg hatching was found between sterile distilled water and dilutions of root exudates from Arbequina and Picual olive planting stocks (Fig. 8) (19). Poor or complete lack of stimulation of egg hatch by root exudates has been suggested as a component of host tolerance to some cyst-forming nematodes (8).

The large populations of *H. mediterranea* found in orchard soils as well as the anatomical alterations in infected olive roots suggest that parasitism by this nematode may contribute to decline of olive seedlings in new plantations or olive nurseries (19,21). Although olive trees infected by *H. mediterranea* do not show any

Fig. 1. Polyphasic approach to differentiate the six *Meloidogyne* species known to infect olive trees. The analysis combines the use of maximum parsimony (MP) analysis of internal transcribed spacer (ITS) regions of ribosomal DNA (rDNA), esterase (Est.) phenotypes compared to that of *M. javanica*, and perineal patterns (PP) (see Table 1 for further details). DNA sequences were not available for *M. lusitanica*.

symptoms on aboveground plant parts, artificial inoculation of self-rooted olive seedlings demonstrated that *H. mediterranea* reproduced on Arbequina (19,21). Pathogenicity assays also showed that plant growth is severely affected by infection with *H. mediterranea* (10,000 eggs + second-stage juveniles per plant) with significant reduction of shoot and root dry and fresh weights, as well as shoot height, trunk diameter, and number of nodes in Arbequina but not in Picual (19). The final nematode populations in soil and roots, as well as the reproduction rate and total number of cysts per plant, were significantly higher in Arbequina than in Picual, which confirmed that Arbequina is a better host for *H. mediterranea*.

Reniform and Other Ectoparasitic Nematodes

Reniform nematodes (*Rotylenchulus* spp.) are semiendoparasites of roots of herbaceous and woody plants, mainly in tropical and subtropical regions worldwide. Currently, three *Rotylenchulus* species (*R. macrodorus* Dasgupta, Raski & Sher, 1968; *R. macrosoma* Dasgupta, Raski & Sher, 1968; and *R. reniformis* Linford & Oliveira, 1940) are reported as parasites of olive (23,96). Artificial inoculation of both wild olives and olive cultivars Arbequina and Picual with *R. macrosoma* revealed reproduction factors of 2.5, 2.2, and 1.9 times the initial population density (P_i), respectively (23). The number of free-living stages of the nematode (juveniles, males, and immature females) and sedentary mature females in soil and roots, as well as the final population density and reproduction rate, did not differ significantly between wild and cultivated olives; however, there was no clear evidence of actual growth impairment in infected plants.

R. macrosoma survives in soil in the juvenile and adult male stages. Immature females penetrate the root and become established in the endodermis (23). The feeding site induced by *R. macrosoma* in olive roots consists of a stellar syncytium, which originates from an endodermal cell enlarging near a curved sheet of pericycle cells formed by hypertrophy of pericycle cells (Fig. 9). Clear anatomical differences in the feeding site distinguish infection by *R. macrodorus* from that by *R. macrosoma*. *R. macrodorus* causes the formation of an enlarged uninucleate stellar or cortical "nurse cell" with dense cytoplasm and hypertrophic nucleolus (uninucleate giant cell) (96), whereas *R. macrosoma* forms a syncytium (Fig. 9). For both species, the kidney-shaped females produce a gelatinous matrix that covers the female body in which about 50 eggs are laid. Usually, soil adhering to this matrix blocks detection of the female on the root surface.

In addition to *Rotylenchulus* spp., a number of rather specialized sedentary

ectoparasitic nematodes attack olive: the citrus biotype of the citrus nematode *Tylenchulus semipenetrans* infects olive trees in California, Chile, and Italy (52,59);

Gracilacus peratica (Fig. 10) and *Ogma rhombosquamatum* (Mehta & Raski, 1971) Andrssy, 1979 were observed feeding on olive roots (280 to 360 nematodes/g fresh

Fig. 2. A, Olive root system severely infected with root-knot nematodes; note small galls. B, Root galls caused by *Meloidogyne javanica*. C, Details of adult *Meloidogyne incognita* female (N) and egg mass (em). D and E, Details of typical galled olive root tip due to infection by *Meloidogyne* spp. F, Detail of *Meloidogyne* spp. egg mass stained with phloxine B.

Fig. 3. Cross section of a young olive root (cv. Arbequina) infected by *Meloidogyne arenaria*. A, Detail of the lip region of an adult female (N) showing the stylet (st), median bulb (mb), and induced giant cells (gc) with hypertrophied nuclei (hn). B, Detail of a feeding site showing xylem (xy) and giant cells with hypertrophied nuclei.

root) in Italy and Spain (36,70,97); and *Trophotylenchulus saltensis* Hashim, 1984 was found parasitizing olives in Jordan, but damage to the plant was not studied (51). The cortical root cells on which *Gracilacus peratica* Raski, 1962 fed showed thickened and lignified walls near the site of stylet penetration as well as a feeding tube associated with nematode salivation (Fig. 10) (36). Females of *O. rhombosquamatum* remained attached to the olive root by the stylet, which penetrated the epidermis and two to three layers of cortical cells. Nematode feeding activity induced thickening of cortical cell walls and hypertrophy of the nucleus and nucleolus, which appeared 4 to 5 times larger than those of normal cortical cells (97). However, convincing evidence of pathogenicity by the above nematodes to olive has only been demonstrated for high infection levels of *T. semipenetans* (52,59).

Other plant-parasitic nematodes associated with olive comprise a long list of migratory ectoparasites, including spiral, dagger, needle, stunt, and stubby-root nematode species (Table 1). Several species of ectoparasitic spiral nematodes of the genus *Helicotylenchus* have been associated with olive roots (Table 1), some of which were associated with necrosis in olive roots and were considered capable of affecting olive tree growth under particular growing conditions (37), including *H. digonicus* Perry, in Perry, Darling & Thorne, 1959; *H. dihystra* (Cobb, 1893) Sher, 1961; *H. erythrinae* (Zimmermann, 1904) Golden, 1956; *H. pseudorobustus* (Steiner, 1914) Golden, 1956; and *H. oleae* Inserra, Vovlas & Golden, 1979. In general, *Helicotylenchus* spp. can adopt semi-endoparasitic feeding positions on olive feeder roots (Fig. 11).

Xiphinema and *Longidorus* nematodes cause damage to olive by feeding on unmodified plant root cells. However, some species are also capable of transmitting viruses, leading to diseases in a wide range of fruit and vegetable crops (58). Olive is reported as a natural host of nepoviruses

transmitted by *Xiphinema diversicaudatum* (Micoletzky, 1927) Thorne, 1939 and *Xiphinema vuittenzei* Luc, Lima, Weischer & Flegg, 1964 (58) viz., *Arabis mosaic virus* (ArMV), *Strawberry latent ringspot virus* (SLRV), and *Cherry leaf roll virus* (CLRV). However, none of these viruses cause a specific syndrome in olive except for SLRV, which has been found associated with striking malformations of leaves and fruits of cv. Ascolana tenera (57). *Xiphinema elongatum* Schuurmans Stekhoven & Teunissen, 1938 has been shown to reduce top weight of olive plantlets by 65% in Egypt (51). *Xiphinema index* Thorne & Allen, 1950, which often occurs in the rhizosphere of grapevine plants and fig trees, has also been found in olive nurseries and olive orchards in Italy (83). Several olive cultivars were demonstrated to be suitable hosts for *X. index*, but olive cv. FS 17 and rootstock DA 12 I were found to be resistant to the nematode (83). Resistance of olive to *X. index* was correlated with phenol content and peroxidase activity in leaves and roots (78).

Interactions Between Nematodes and Fungi in Olive

Nematode damage to olive roots may also predispose olive trees to other pathogens. The best-known interaction between plant-parasitic nematodes and other olive pathogens is that of root-knot nematodes and *Verticillium dahliae*, the causal agent of Verticillium wilt. *M. javanica* and *V. dahliae* caused considerable damage to olive orchards in the northern part of Iran, and the growth of olive cv. Zard seedlings

inoculated with these two pathogens was significantly reduced compared with that of seedlings inoculated with each pathogen alone (82). Similarly, the proportion of chlorotic, wilted, and necrotic leaves of seedlings was highest in plants simultaneously inoculated with both pathogens. In contrast, studies by Lamberti et al. (49) of olive cultivars Leccino and Pendolino failed to show any significant interaction between infections by *V. dahliae* and *M. incognita* and between *V. dahliae* and *P. vulnus*, but the authors suggested that a longer duration of the experiments might be needed to demonstrate interactions between the pathogens. The mechanism of interaction and the precise role of each component in a disease complex have to be further investigated. These results suggest that interactions between plant-parasitic nematodes and fungi can vary considerably over plant genotypes, cultivars, or lines. In Argentina, the etiology of a generalized olive drying syndrome (designated "seca") was attributed to a disease complex involving *M. javanica* and several species of the soilborne fungus *Fusarium*, although a causal relationship has not been established (15,75).

Control of Nematode Diseases on Olive

Exclusion. Exclusion is the first control strategy to be considered for management of plant-parasitic nematodes on olive. Heavy infestations of plant pathogens in agroecosystems can often be traced back to accidental introductions at the initial stages of crop development (91). Preventing the

Fig. 4. Aboveground symptoms of olive plant cv. Arbequina infected by *Meloidogyne arenaria* (right) showing a distinct yellowing in uppermost leaves. Uninoculated control to the left.

Fig. 5. Relationships between relative shoot length olive cv. FS 17 and rootstock DA 12 I and initial population density (P_i) of *Meloidogyne javanica*. Abbreviations: m = minimum value of relative shoot length (relative length at very large P_i values; T = tolerance limit).

entry of plant-parasitic nematodes into the agroecosystem must be implemented first by nursery managers. Modern propagation procedures in olive nurseries include an initial stage in which soft-wood cuttings are rooted in standard growing media such as peat or perlite, followed by a second phase in which rooted cuttings are transplanted to soil-filled containers. Nematode infection is unlikely to occur in the first stage, since growing media are reasonably free from pathogens, and aboveground olive plant parts, such as cuttings, do not contain parasitic nematodes. Consequently, later propagation stages require the use of uninfested soil as an essential measure of nematode exclusion. In fact, control measures for plant-parasitic nematodes attacking olive have been addressed mainly to nurseries. Planting stocks, especially those containerized such as olive, may act as effective "Trojan horses" that deliver previously absent plant pathogens to the planting site. The potential for future damage to olive production has been taken into account by legislation in Spain (9,10) and the European Union (EU) (30) through mandates for pathogen-free certification programs for olive planting stocks and rootstocks (30). Unfortunately, adoption of certification programs by nurseries is not compulsory in Spain, and therefore its practice is very limited. Under these circumstances, selecting a well-reputed nursery as supplier of planting material may serve as a rational exclusion practice (5). Growers should also choose new plantation sites free of plant-parasitic nematodes in which to establish planting material (56).

The appropriate application of exclusion principles should lead to nematode-free nursery stock. Although this is theoretically possible with the use of nematode-free potting mixtures, many times nursery managers must resort to nematode-infested soil for economic reasons. Disinfestation of soil by any means cannot achieve total nematode control. The use of potting mixtures that are not fully disinfested reduces nematological risk, but could mask the presence of pathogen nematodes in seemingly healthy stock. The need for a tradeoff suggests the need for tolerance thresholds. No such thresholds are standardized for olive, but we suggest adopting the criteria presented by Whitehead (101), who recommended control of at least 95% of root lesion nematodes and 99.9% of root-knot nematodes. Although these standards were proposed for annual crops and may therefore be considered unsuitable for long-lived perennials like olive, nematode reproduction rates are much lower in woody-perennial than in annual-herbaceous hosts.

Exclusion of olive-parasitic nematodes on a regional basis includes surveillance measures. Three species of fruit crop-infecting nematodes potentially damaging to olive are absent in Europe: *Longidorus*

diadecturus Eveleigh & Allen, 1982; *Xiphinema americanum* Cobb, 1913; and *X. californicum* Lamberti & Blevé-Zacheo, 1979. Consequently, these species have been declared quarantine pests in the EU (31). Other olive-growing countries that have imposed quarantine measures against plant-parasitic nematodes potentially pathogenic to olives include Chile, where *H. mediterranea* and *Xiphinema italiae* Meyl, 1953 are considered quarantine pests (60).

Chemical control. Chemical measures aim to control nematodes present in the rhizosphere soil or in infected plant tissues, or both. Chemical control may be hampered by uncertain efficacy, environmental hazards, and/or deleterious impact on plants. Available nematicides are classified as fumigants (i.e., gaseous or highly volatile nonspecific biocides) and nonfumigant nematicides (NFNs).

Soil disinfestation with fumigants prior to planting is a nematode control strategy broadly used in some fruit crops. For particular crops, soil disinfestation may even

be publicly subsidized, as is the case of grapevine in some Spanish production areas (27). Nevertheless, fumigation is unusual in olive production. A lack of awareness of the damage potential of nematodes probably underlies this fact. Chemical disinfestation of nursery substrates is a suitable approach for production of nematode-free nursery stock. A large number of fumigants or fumigant-like products are available, in spite of an increasing number of failures in re-registration. In Spain, the list currently includes dazomet, metham sodium, metham potassium, and sodium tetrathiocarbonate (61).

The list of NFNs includes a series of organophosphate and carbamate products that display a certain degree of insecticidal and acaricidal effects as well. In Spain, the official list of authorized NFNs pesticides includes ethoprophos, fosthiazate, fenamiphos, and oxamyl, but none are registered for use in olive (61). A similar situation, in which a certain number of specific nematicides are registered but none of them for

Fig. 6. Micrographs of the root-lesion nematode *Pratylenchus vulnus*. **A**, Whole body of female and male. **B**, Female anterior region showing stylet (st), dorsal pharyngeal gland outlet (dgo), and excretory pore (pe). **C**, Detail of lip region. **D**, Female vulval region showing postvulval uterine sac (PUS). **E** and **F**, Female tails. **G**, Male tail. (Scale bars: **A** = 100 μ m; **B** to **G** = 10 to μ m)

Fig. 7. Natural infections of olive trees cv. Manzanilla by *Heterodera mediterranea*. A to C, Partially embedded second- (J₂) and third/fourth- (J₃₋₄) stage juveniles and young cyst (arrow) in young olive roots. D, A mature female showing large gelatinous matrix with eggs. E and F, Cross sections of olive roots infected by *H. mediterranea*, showing a syncytium involving endodermal and pericycle cells (deeper stain). G, Magnification of a stelar syncytium showing fused syncytial cells and stelar distortion due to their expansion.

Fig. 8. Cumulative egg hatch of *Heterodera mediterranea* incubated at 20°C in olive root exudates for 22 days. Abbreviations: SDDW = sterile deionized distilled water; P-Un = undiluted root exudates from olive planting stock cv. Picual; P-1:1, P-1:2 = root exudates from olive planting stock cv. Picual diluted, respectively, 1:1 or 1:2 with SDDW; Ar-Un = undiluted root exudates from olive planting stock cv. Arbequina; Ar-1:1, Ar-1:2 = root exudates from olive planting stock cv. Arbequina diluted, respectively, 1:1 or 1:2 with SDDW.

use in olive, occurs in other olive growing countries such as Australia (6), Argentina (14), Italy (62), and Portugal (89). A misperception of nematological risk discourages pesticide companies from facing registration expenses for new nematicides. If allowed by regulation, NFNs can be used in a wide variety of situations. Experience in other fruit crops supports their application either at pre- or postplanting. In the nursery, NFNs can be applied as dips of bare-root stocks, additions to substrates, or foliar sprays. Dipping was successfully used in *Citrus* spp. for control of *T. semipenetrans* and *Radopholus similis* (71,72). Addition of phenamiphos to substrate reduced root-knot nematode infections in olive (48). Because of their systemic nature, spraying of some NFNs onto foliage may also protect roots from infections by soilborne nematodes (1). Vovlas et al. (99) compared the three strategies of application in fruit crops and found that efficacy of NFNs depends mainly on the crop and the nematode target species rather than the application method.

NFNs can be applied postplanting either by chemigation with emulsifiable concentrates, or by mixing granular formulations into the soil. The opportunity for using the latter mode must be checked in each particular country, because the use of granular pesticides is being progressively prohibited by many regulatory agencies due to avian risk (90). There is a scarcity of data concerning postplanting treatment with NFNs in olive orchards. A field trial in Chile (38) compared the effects of fenamiphos chemigation on root growth and development of olive trees naturally infected by *T. semipenetrans*, compared with treatments with a biocontrol formulation and an amendment with organic manure. No significant differences in effects on root mass were found, suggesting that the biocontrol and amendment treatments were as efficient as a chemigation with fenamiphos. In Egypt, Shawky et al. (88) compared the efficacy of recommended rates of five NFNs (fenamiphos, ethoprophos, carbofuran, oxamyl, and aldicarb) for the control of *M. incognita* on olive trees. All nematicides were effective in reducing the number of nematodes in both soil and roots, but fenamiphos gave the greatest reduction in nematodes in soil and the number of galls, females, and egg masses, whereas carbofuran gave the least reduction.

Nonchemical control. Concerns about the eventual environmental impact of the use of pesticides have promoted the development of a number of nonchemical strategies for disinfecting agricultural substrates. One of the most successful is soil solarization, a hydrothermal process whereby moist soil is heated under transparent plastic tarps to temperatures detrimental to soilborne pathogens (41). Olive production takes place at medium-latitude areas of

Fig. 9. Reniform nematodes infecting olive trees. **A** to **E**, *Rotylenchulus macrosoma*. **A**, Micrograph of whole body of female. **B**, Nematode female attached to a root of wild olive. **C** to **E**, Cross sections of olive roots infected by *R. macrosoma* showing feeding cells (fc) and the stelar syncytium (S) with hypertrophied nuclei (hn), stelar distortion and compressed xylem elements (xy). **F** to **I**, *Rotylenchulus macrodoratus*. **F**, Micrograph of whole body of female. **G**, Scanning electron micrograph of a nematode female parasitizing an olive root (v = vulva). **H**, Detail of vulval region showing an egg outside. **I**, Cross section of an olive root showing enlarged uninucleate stelar or cortical "nurse cell" (gc).

Mediterranean climate where summer weather offers optimal conditions for soil solarization. Solarization of small piles of nursery substrates used as potting mixtures for glasshouse-grown plants is a novel approach for the management of soilborne plant pathogens (68). Solarization of piled soil (Fig. 12) provided a significant reduction of *M. incognita* eggs and egg masses in soil at a maximum depth of 40 cm after 10 days of treatment (68). Furthermore, the thermal threshold reached by solarization for inactivating free eggs and egg masses of *M. incognita* may also control other plant-parasitic nematodes potentially hazardous to olive plants, e.g., *P. vulnus* and *P. penetrans* (67).

Solarization might also be used in established orchards to control nematode infestations as it has been done to control other olive soilborne plant pathogens. Tjamos (94) and López-Escudero and Blanco-López (55) attained a significant reduction in Verticillium wilt incidence when solarization was applied to individual *V. dahliae*-infected olive trees of different ages.

Another environmentally friendly approach for substrate disinfestation is the use of organic amendments (3). Many different agricultural and industrial by-products have been assayed for this purpose (25). Composts, extensively recommended as potential suppressors of soilborne fungi, have not been adequate for nematode control. Nico et al. (69) tested amendment of olive potting substrate with dry cork compost at different rates for control of *M. incognita*. Nematode numbers in the amended substrate decreased

Fig. 10. **A**, Colonies of mature females (N) of *Gracilacus peratica* (red-stained with acid fuchsin) infecting olive (cv. Coratina) feeder roots. **B**, Cross section of an olive feeder root showing the feeding tube in a nematode-infected cortical cell (arrow).

exponentially as the rate of compost increased; whereas the lowest rate of amendment provided a sufficient level of control, the highest suppression occurred using pure compost as substrate (Fig. 13).

Suppression of plant pathogens in soil can be also achieved using special amendments in a process known as biofumigation (43), which is based on the release of bio-cidal volatiles during breakdown of tissues of some plants, mainly of *Brassica* and *Sorghum* species. Biofumigation is often practiced with plastic tarping, in order to confine the volatile compounds produced and enhance their biological effect. The effect of biofumigation with leaf tissues of *Sorghum sudanense* (Piper) Stapf. on suppression of *M. incognita* was tested in a laboratory with different rates of artificially infested potting mixture as well as times of exposure and use of tarp (A. Nico, R. M. Jiménez-Díaz, and P. Castillo, unpublished). The final nematode population was significantly reduced by treatment in all cases, but the degree of suppression

was adequate only when biofumigation lasted for 30 to 60 days. Therefore, biofumigation does not appear to be as efficient as soil solarization as a single control measure for disinfesting soil of plant-parasitic nematodes.

Protection of olive planting with mycorrhizae. Establishment of arbuscular mycorrhizal fungi (AMF) in plant root systems can protect plants against soilborne diseases through improvement of P absorption, direct competition with pathogens for infection sites and nutrients, and other mechanisms (7). The effectiveness of AMF in conferring protection against plant-parasitic nematodes was documented in several pathosystems, including *Meloidogyne* spp. and olive (17). Early inoculation of planting stocks of olive cultivars Arbequina and Picual with *Glomus intraradices* Schenck & Smith, *G. mosseae* (Thaxter) Gerd. & Trappe, or *G. viscosum* Nicolson favored plant growth and conferred protection against *M. incognita* race 1 and *M. javanica* by inhibiting nematode

Fig. 11. A, Micrograph of female whole body of *Helicotylenchus oleae*. B to F, Cross sections of olive roots infected by *H. oleae*. B and E, Details of a coiled nematode specimen (N) in the root cortex (CO). C, D, and F, Details of the lip regions of *H. oleae* (N) penetrating cell walls in the cortex of olive feeder roots (st = stylet; hn = hypertrophic cell nucleus).

Fig. 12. Conical piles of soil 80 cm high and with a base diameter of 1 m solarized for 3 weeks in July at Córdoba, southern Spain, for disinfestation from *Meloidogyne incognita* eggs and J₂ placed in nylon bags at a depth of 20 and 40 cm inside a pile.

reproduction, reducing the severity of root galling, and probably, enhancing olive plant nutrition (17). Consequently, the use of AMF-infected planting material during olive plant propagation in nurseries may be a useful practice for minimizing the effects of root-knot nematodes, as well as for protecting these plants against future nematode infections in commercial olive orchards.

Resistance. Host plant resistance probably offers the easiest, safest, and cheapest long-term approach to minimize damage

by plant-parasitic nematodes. The use of resistant rootstocks constitutes a fundamental pillar for root-knot nematode management in some fruit crops, such as *Prunus* spp. (76). In olive, however, less effort has been dedicated to identifying and using host plant resistance against plant-parasitic nematodes. In part, this may be due to the fact that olive, unlike most fruit crops, is rarely propagated by grafting. Nevertheless, development of resistant rootstocks may be a valuable tool for nematode management in olive, as sug-

Fig. 13. Relationship between final nematode population of *Meloidogyne incognita* race 1 on olive planting stocks cv. Picual and the rate of dry cork compost amending a potting mixture (0, 25, 50, 75, and 100%). Each point represents the average of two trials. Lines represent the predicted function calculated by fitting the expanded negative exponential model to data.

gested by the successful results obtained with the clonal rootstock 'Allegra' in California (59).

Host resistance to nematodes may vary to a great extent depending on olive cultivar, usually propagated by self-rooting, and nematode species. In Egypt, six olive cultivars were tested for their reaction to

M. incognita and *R. reniformis* (4). Cultivars Meashon and Tofany were found to be resistant to *M. incognita* but moderately susceptible to *R. reniformis*; while cultivars Manzanillo and Egazi were moderately susceptible to *M. incognita* but tolerant of *R. reniformis*, and cultivars Hamed and Bekwal were susceptible to both nematode species (4). Similarly, screening of olive cultivars most commonly used in Italy for resistance against root-knot nematodes showed that cultivar Coratina is resistant to *M. incognita* and *M. javanica*, whereas cultivars Leccino and Yusti are resistant to *M. javanica* and moderately susceptible to *M. incognita* (86). Other cultivars (Cima di Bitonto, Cellina di Nardo, Frantoio, and FS 17) were shown to be moderately susceptible to *M. incognita* (86), whereas the rootstock DA 12 1

was highly susceptible to *M. javanica* (85,86). The use of in vitro olive explants has proven to be useful in reducing the duration of resistance screening assays of olive germplasm against root-knot nematodes (84).

Miscellaneous. Covering orchard soil with permanent vegetation is becoming one of the most commonly used orchard management systems for preventing soil erosion, improving soil structure and fertility, increasing water infiltration, and reducing surface runoff (16,35). Plant covers are usually natural flora, and their species composition is therefore fortuitous. However, orchard soil can also be covered with specifically selected crop species (24) or the natural flora can be managed by use of adequate herbicide treatment (81). Some plant species used as cover crops or exist-

ing in natural vegetation (Fig. 14) may be suitable hosts for plant-parasitic nematodes such as *Meloidogyne* spp. (18,53). Even plants that are poor hosts for the nematode should be limited to soils free of *Meloidogyne* spp. By displacing native host plants with nonhost or nematicidal species, cover crops could help to reduce numbers of plant-parasitic nematodes in olive orchards, as was demonstrated for other fruit crops (102).

Thermotherapy is a heat-based disinfection measure used since the nineteenth century to eradicate pests and pathogens from seeds and other propagation stocks (100). This measure was successfully used to produce virus-free fruit-tree planting stocks (66) and *Agrobacterium tumefaciens*-free grape cuttings (13). Thermotherapy has also been used to eradicate nematodes from infected citrus (39) and grapevine (63) propagation stocks. In order to be successful, the lethal thermal threshold for the nematode must be reached without exceeding the thermal plant tolerance. Studies in our laboratory indicate that the root-lesion nematode *P. vulnus* can survive heat treatments of 25 to 35°C regardless of duration, but its viability is severely reduced at 45 to 55°C during 1 to 15 min (Fig. 15). However, hot water treatments (35 to 55°C) of olive cultivars Arbequina and Picual planting stocks significantly impaired subsequent plant growth unless the planting stocks were pretreated for 10 min at 30 and 35°C. Eradication of *P. vulnus* from artificially infected olive planting stocks was achieved by hot water treatment of infected roots at 50°C for 2 min, but 40 or 45°C during 4 to 20 min did not significantly affect nematode survival on infected root (M. Teva Fernández, R. M. Jiménez-Díaz, and P. Castillo, *unpublished*).

Conclusions, Olive Certification Scheme, and Future Prospects

The removal of many nematicides from the market due to environmental and hu-

Fig. 14. View of an olive orchard in southern Spain showing different crucifer species used as cover vegetation for preventing soil erosion, improving soil structure and fertility, increasing water infiltration, and reducing surface runoff.

Fig. 15. Effects of in vitro treatment of *Pratylenchus vulnus* specimens with hot water at 25 to 55°C for 1 to 15 min. A, Percent mortality after treatment over time. B, Relationship between nematode mortality (%) and time of exposure at 40 and 45°C.

man health concerns has focused attention on the development of alternative control strategies for managing plant-parasitic nematodes in many crops, including olive. Because few resistant rootstocks have been identified in olive, exclusion should be the first and primary control strategy to consider for management of plant-parasitic nematode in olive orchards. The use of pathogen-free planting material and unfested soils is essential for minimizing the effects of single or concomitant species of plant-parasitic nematodes and other pathogens during the early years of olive cultivation and for preventing impairment of growth and yield loss. For producing certified olive trees, the rootstocks or scion material (for grafted olive trees) should be grafted or budded only onto rootstocks of equivalent (i.e., propagation-stock) or higher certification status. Plants to be certified should be kept in nursery soil that has been proved free from the nematode species that can cause significant damage to olive growth (e.g., *X. diversicaudatum*, *M. arenaria*, and *P. vulnus*), and also from all quarantine nematodes (5). Finally, the lack of sufficient levels of nematode resistance or tolerance can be compensated for, to some extent, by the use of other control measures, preferably nonchemical ones, including soil solarization, biofumigation, and biological treatments as we have described. We believe that fulfillment of this strategy would help in maintaining the critical role that olive production plays in Mediterranean farming systems as a major source of oil and fruits, as well as a net contributor to agricultural sustainability in countries with Mediterranean-type climate.

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