Journal of Nematology 35(1):29–34. 2003.

Host Suitability of the Olive Cultivars Arbequina and Picual for Plant-Parasitic Nematodes[1]

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Abstract: Host suitability of olive cultivars Arbequina and Picual to several plant-parasitic nematodes was studied under controlled conditions. Arbequina and Picual were not suitable hosts for the root-lesion nematodes *Pratylenchus fallax, P. thornei,* and *Zygotylenchus guevarai.* However, the ring nematode *Mesocriconema xenoplax* and the spiral nematodes *Helicotylenchus digonicus* and *H. pseudorobustus* reproduced on both olive cultivars. The potential of *Meloidogyne arenaria* race 2, *M. incognita* race 1, and *M. javanica,* as well as *P. vulnus* and *P. penetrans* to damage olive cultivars, was also assessed. Picual planting stocks infected by root-knot nematodes showed a distinct yellowing affecting the uppermost leaves, followed by a partial defoliation. Symptoms were more severe on *M. arenaria* and *M. javanica*-infected plants than on *M. incognita*-infected plants. Inoculation of plants with 15,000 eggs + second-stage juveniles/pot of these *Meloidogyne* spp. suppressed the main height of shoot and number of nodes of Arbequina, but not Picual. Infection by each of the two lesion nematodes (5,000 nematodes/pot) or by each of the three *Meloidogyne* spp. suppressed ($P < 0.05$) the main stem diameter of both cultivars. On Arbequina, the reproduction rate of *Meloidogyne* spp. was higher ($P < 0.05$) than that of *Pratylenchus* spp.; on Picual, *Pratylenchus* spp. reproduction was higher ($P < 0.05$) than that of *Meloidogyne* spp.

Key words: Helicotylenchus digonicus, H. pseudorobustus, Meloidogyne arenaria, M. incognita, M. javanica, Mesocriconema xenoplax, Olea europaea, olive nurseries, *Pratylenchus fallax, P. penetrans, P. thornei, P. vulnus,* reproduction, ring nematodes, root-knot nematodes, root-lesion nematodes, Spain, spiral nematodes, *Zygotylenchus guevarai.*

Olive (*Olea europaea* L.) is grown extensively in the Mediterranean Basin, the subtropical regions of Australia, southern Africa, and North and South America. Of 8.0 million ha of olive grown worldwide in 2001, 90% was located in Mediterranean countries (FAO, 2002). In Andalusia, southern Spain, olive is planted on more than 1.3×10^6 ha of land (Barranco, 1999). In Spain, improved cultivars are grown self-rooted, without rootstock. The improved cultivars Arbequina and Picual are widely used by the olive industry in Spain because of their high yield and oil content.

Olive roots can be infected by several plant-parasitic nematodes. Nematode species found associated with adult olive trees include *Mesocriconema xenoplax (=Criconemella xenoplax), Helicotylenchus* spp., *Heterodera mediterranea, Meloidogyne* spp., *Pratylenchus* spp., and *Rotylenchulus* spp. (Castillo et al., 1999; Diab and El-Eraki, 1968; Lamberti and Vovlas, 1993). In addition, some 34 species of plant-parasitic nematodes were recently found associated with olive planting stocks in a nematode survey of olive nurseries in Andalusia (Nico et al., 2002).

In spite of their prevalence in the olive rhizosphere, no data are available concerning the host suitability of olive trees to species such as *M. xenoplax, Helicotylenchus digonicus, H. pseudorobustus, Pratylenchus fallax, P. thornei,* and *Zygotylenchus guevarai* (Nico et al., 2002). Also, there is a need to determine the damage potential of these nematodes to olive plants. Several authors have reported damage to olive by the root-knot nematodes *M. arenaria, M. incognita,* and *M. javanica* and the root-lesion nematode *P. vulnus* (Lamberti and Baines, 1969a, 1969b; Sasanelli et al., 1997). However, no data are available on the response of the improved cultivars Arbequina and Picual to parasitism by these nematode species. Similarly, the effect of the lesion nematode *P. penetrans* on olive growth is not known, though it is prevalent in olive nurseries in southern Spain (Nico et al., 2002).

Host suitability to plant-parasitic nematodes can be assessed by measuring their reproduction on plants after artificial inoculations (Lewis, 1987). The reproduction factor (Rf) has been widely used in nematological studies to define resistance and susceptibility of plants to a nematode (Marull and Pinochet, 1991; Pinochet et al., 1991). When a compatible host-parasite interaction has been established by a nematode, infection of the plant can be followed by a sequence of disruptions in the physiological processes that lead to pathogenesis (Melakerberhan and Webster, 1993). Reductions of normal-growth plant parameters may be used as indicators of plant physiology impairment related to pathogenesis.

The objectives of this study were to: (i) evaluate the host suitability of the olive cultivars Arbequina and Picual to *M. xenoplax, Helicotylenchus digonicus, H. pseudorobustus, Pratylenchus fallax, P. thornei,* and *Zygotylenchus guevarai,* and (ii) investigate the damage potential of *P. vulnus, P. penetrans, M. arenaria* race 2, *M. incognita* race 1, and *M. javanica* to these olive cultivars.

Materials and Methods

Inoculum preparation: The nematode populations used in this study were obtained from olive planting stocks collected in commercial nurseries at Córdoba, Jaén, and Sevilla provinces of Andalusia, southern

Received for publication 29 August 2002.
[1] Research supported by grant CAO99-010-C3-01 from Instituto Nacional de Tecnologías Agroalimentarias (INIA) and a grant from Agencia Española de Cooperación Internacional (AECI).
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The authors thank N. Greco and J. H. O'Bannon for reviewing the manuscript and J. Martin Barbarroja for technical assistance.
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This paper was edited by P. Timper.

Spain (Nico et al., 2002). Plant-parasitic nematodes used in this study, as well as their origin and location, are described in Table 1.

Meloidogyne spp. were increased on tomato plants (*Lycopersicon esculentum* Mill. cv. Roma) starting from a single egg mass for each species. Root-knot nematode populations were identified to species and race based on features of their perineal pattern and differential host experiments (Hartman and Sasser, 1985). Inoculum for experiments was obtained by extracting eggs and second-stage juveniles (J2) from 2-month-old cultures using 1% sodium hypoclorite (Hussey and Barker, 1973) followed by centrifugal flotation (Coolen, 1979).

Inoculum of *Pratylenchus* spp. was increased on carrot disks starting from a single mature female (Castillo et al., 1995). Inoculum from the infected carrot disks was extracted on a modified Baermann funnel.

Cultures of *M. xenoplax* and *Helicotylenchus* spp. were started from 100 specimens hand-picked from the extracted soil samples and placed in sterile water. Tomato seedlings (cv. Roma) were inoculated by pipeting the nematodes into a single depression near the base of the plant. The inoculated plants were incubated under greenhouse conditions for at least 2 months and renewed several times to increase nematode populations. Nematodes used for inoculum were extracted from soil by centrifugal-flotation (Coolen, 1979).

Host suitability experiment: Reproductive potential of *P. fallax, P. thornei, Z. guevarai, M. xenoplax, H. digonicus,* and *H. pseudorobustus* was evaluated on the olive cultivars Arbequina and Picual. Single, 6-month-old olive planting stocks of uniform size were transplanted into clay pots containing 1 liter of an autoclaved potting mixture (sand: clay loam, 2:1, v/v). Plants were inoculated by adding 1,000 nematodes (Pi) in 10 ml of sterile distilled water around the root ball of each plant at transplanting, except for *M. xenoplax* for which 100 nematodes per plant were used as inoculum. Nematode concentrations were determined in 1-ml aliquots of water suspension. Plants were grown in a growth chamber at 25 ± 1 °C, 60 to 90% relative humidity, and a 14-hour photoperiod of fluorescent light at 360.5 ± 24.7 $\mu Em^{-2}S^{-1}$. Plants were watered with 100 ml of water on alternate days and fertilized weekly with 100 ml of a nutrient solution (Hoagland and Arnon, 1950). There were 10 replicated plants per inoculation treatment in a randomized complete block design. The experiment ended 120 days after inoculation with *Helicotylenchus* spp., and 90 days after inoculation with the other nematode species. At the end of the experiment, each plant was cut at the soil level and its roots washed free of soil. Final soil and root populations of nematodes (Pf) were determined by flotation-centrifugation and maceration-centrifugation, respectively (Coolen, 1979). From the Pf value, the nematode reproduction factor Rf (Pf/Pi) was calculated. The experiment was conducted twice except for *Helicotylenchus* spp.

TABLE 1. Plant-parasitic nematodes used in the host suitability and pathogenicity experiments.

Nematode species and race	Olive cultivar	Town (Province)
Helicotylenchus digonicus	Manzanilla[a]	Pedrera (Sevilla)
H. pseudorobustus	Hojiblanca[a]	Córdoba
Meloidogyne arenaria race 2	Cornicabra[b]	Villaverde del Río (Sevilla)
M. incognita race 1	Manzanilla[b]	Alcolea (Córdoba)
M. javanica	Picual[b]	Córdoba
Mesocriconema xenoplax	Manzanilla[a]	Alcolea (Córdoba)
Pratylenchus fallax	Arbequina[a]	Pedrera (Sevilla)
P. penetrans	Arbequina[b]	Pedrera (Sevilla)
P. thornei	Arbequina[a]	Pedrera (Sevilla)
P. vulnus	Picual[b]	Villaverde del Río (Sevilla)
Zygotylenchus guevarai	Picual[a]	Huelma (Jaén)

[a] Nematodes were isolated from the rhizosphere of olive planting stocks.
[b] Nematodes were isolated from infected roots of olive planting stocks.

Pathogenicity experiment: The damage potential of the root-lesion nematodes *P. penetrans* and *P. vulnus* and the root-knot nematodes *M. arenaria* race 2, *M. incognita* race 1, and *M. javanica* to the olive cultivars Arbequina and Picual was assessed under controlled conditions. Single, 6-month-old olive planting stocks, uniform in size, were transplanted into clay pots containing 1 liter of the same autoclaved potting mixture used for the host suitability experiment. Plants were inoculated by adding 5,000 individuals (Pi) of *Pratylenchus* spp. or 15,000 eggs + J2 (Pi) of *Meloidogyne* spp. as described in the host suitability experiment. Plants that served as controls were treated similarly to those inoculated but without nematodes. Plants were maintained in a growth chamber under conditions similar to those already mentioned. There were 20 replicated plants per inoculation treatment in a randomized complete block design.

Plants were removed from the soil 120 days after inoculation, their roots washed free of soil, and the shoot and root fresh weights of individual plants were recorded. Growth parameters in inoculated and control plants, including height, main stem and shoot diameters, and number of nodes (expressed as percentage of increase relative to initial measures), were determined. Severity of root galling in plants inoculated with *Meloidogyne* spp. was assessed on a 0-to-6 scale according to the percentage of galled roots, where 0 = no galls, 1 = 1 to 10%, 2 = 11 to 20%, 3 = 21 to 40%, 4 = 41 to 70%, 5 = 71 to 90%, and 6 = 91 to 100%. Symptom severity on leaves of nematode-infected and non-infected planting stocks was rated on a 0-to-5 scale according to the percentage of foliage with yellowing (0 = 0%, 1 = 1 to 25%, 2 = 26 to 50%, 3 = 51 to 75%, 4 = 76 to 100%, and 5 = dead plant). Nematodes were extracted from soil and roots by flotation-centrifugation and maceration-centrifugation, respectively, counted, and Rf values determined.

Statistical analysis: For the host suitability experiment,

the effect of nematode species, olive cultivar, and experimental trial on the Rf was determined by ANOVA. Effect of nematode species on plant growth, severity of root galling, and reproduction also was determined by ANOVA in the pathogenicity experiment. For the host suitability experiment, treatment means were compared using Fisher's protected least significant difference test (LSD) at $P = 0.05$. In the pathogenicity experiment, orthogonal single-degree-of-freedom contrasts were computed to test the effect of selected experimental treatment including comparisons between uninfected treatments and root-lesion or root-knot-infected treatments (Gomez and Gomez, 1984). All data on nematode population density (X) and relative plant growth were transformed into $\log_{10}(X + 1)$ and to arcsine-square root, respectively, before analyses (Gomez and Gomez, 1984). All analyses were done using Statistix (NH Analytical Software, Roseville, MN).

Results

Host suitability experiment: Similarity between experimental trials allowed the data to be combined for analysis of variance. Rf values for the root-lesion nematodes *P. fallax, P. thornei,* and *Z. guevarai* were less than 1.0 on both Arbequina and Picual (Fig. 1). The Rf values for *M. xenoplax* and the *Helycotylenchus* spp. were more than 1.0 and higher ($P < 0.01$) than the other species (Fig. 1). There was no difference in reproduction rate between the olive cultivars except for the two *Helycotylenchus* spp., where reproduction was greater ($P < 0.05$) on Picual than on Arbequina (Fig. 1).

Pathogenicity experiment: Both Arbequina and Picual olive planting stocks were infected by *M. arenaria* race 2, *M. incognita* race 1, *M. javanica, P. penetrans,* and *P. vulnus* (Tables 2, 3). No disease symptoms on aboveground plant parts were observed in either infected Arbequina or uninoculated control plants (Table 2). However, Picual planting stocks infected by *Meloidogyne* spp. showed disease symptoms on aboveground plant parts. *Meloidogyne*-infected Picual showed yellowing of the uppermost leaves followed by partial defoliation (Table 3). These symptoms were more severe ($P < 0.05$) in *M. arenaria* and *M. javanica*-infected plants than in *M. incognita*-infected ones (Table 3). Plants from both

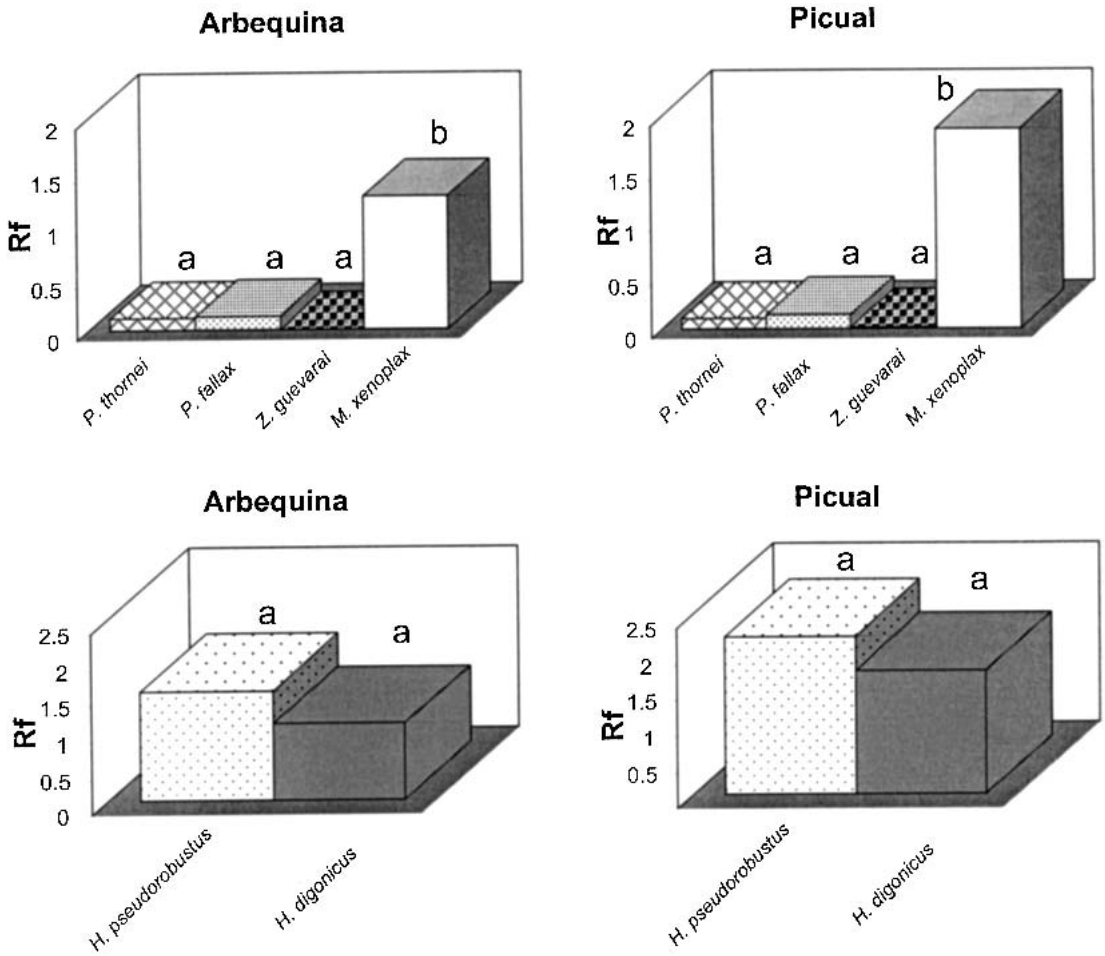

Fig. 1. Host suitability of olive cultivars Arbequina and Picual. Data are the average of two experiments with 10 replicated plants per treatment, except for *Helicotylenchus* spp. which was not repeated. For each olive cultivar, bars with the same letter do not differ ($P > 0.05$) according to Fisher's protected LSD test. Reproduction of the two *Helicotylenchus* spp. was greater ($P < 0.05$) on Picual than on Arbequina.

TABLE 2. Pathogenicity and reproduction of root-knot and root-lesion nematodes on Arbequina olive planting stocks under controlled conditions[a].

Inoculation treatment	Nematode species	Total fresh weight (g)	Root fresh weight (g)	Growth increase during the experiment (%)[b] Shoot height	Main stem diameter	Shoot diameter	Number of nodes	Severity of symptoms[c]	Rf[d]
0	Control	9.62	3.65	10.28	0.46	2.23	3.8	0.00	—[e]
1	*M. arenaria*	7.94	2.95	7.98	0.38	1.57	3.2	0.00	2.20
2	*M. incognita*	9.98	3.49	7.65	0.43	1.59	3.2	0.00	1.59
3	*M. javanica*	7.83	2.87	5.56	0.32	1.50	2.7	0.00	1.85
4	*P. penetrans*	9.99	3.28	9.90	0.36	1.78	3.8	0.00	1.34
5	*P. vulnus*	9.69	3.58	9.74	0.29	1.63	4.0	0.00	1.52
					Contrasts (P)[f]				
0 vs. 1 to 5		ns	ns	ns	ns	<0.001	ns	—	—
0 vs. 1 to 3		ns	ns	0.008	ns	<0.001	0.013	—	—
0 vs. 4 to 5		ns	ns	ns	ns	0.010	ns	—	—
1 to 3 vs. 4 to 5		0.008	ns	0.004	ns	ns	<0.001	—	<0.001
1 vs. 2 to 3		ns	ns	ns	ns	ns	ns	—	0.001
2 vs. 1 and 3		0.001	ns	ns	ns	ns	ns	—	0.002
3 vs. 1 to 2		ns	ns	ns	ns	ns	ns	—	ns
4 vs. 5		ns	ns	ns	ns	ns	ns	—	ns

[a] Data are means of 20 replicated plants per treatment combination. Plants were inoculated with 5,000 individuals (Pi) of *Pratylenchus* spp. or 15,000 eggs + J2 (Pi) of *Meloidogyne* spp.
[b] Average percentage growth of each parameter during the experiment.
[c] Assessed on a scale of 0 to 5 according to the percentage of foliage with yellowing (0 = 0%, 1 = 1 to 25%, 2 = 26 to 50%, 3 = 51 to 75%, 4 = 76 to 100%, and 5 = dead plant).
[d] Rf (nematode reproduction factor) = Pf (final nematode population per plant)/ Pi (initial nematode inoculum per plant).
[e] — = not tested.
[f] Orthogonal contrast of inoculation treatments. Probability for the t statistic of linear single-degree-of-freedom contrasts; ns = not significant ($P > 0.05$).

cultivars infected by root-knot nematodes showed distorted feeder roots and root galls of large (6 to 8 mm) to moderate (2 to 3 mm) size. Galls occurred singly or in clusters. Severity of root galling in Arbequina and Picual plants infected by *M. arenaria* averaged 1.6 and 1.3, respectively, while galling of *M. incognita*-infected plants was 1.3 and 1.1, and galling of *M. javanica*-infected plants was 1.6 and 1.5. However, neither the olive genotype nor root-knot nematode species influenced the severity of root galling.

Plant growth of Arbequina and Picual olive plants, assessed by the percentage increases in shoot diameter,

TABLE 3. Pathogenicity and reproduction of root-knot and root-lesion nematodes on Picual olive planting stocks under controlled conditions.[a]

Inoculation treatment	Nematode species	Total fresh weight (g)	Root fresh weight (g)	Growth increase during the experiment (%)[b] Shoot height	Main stem diameter	Shoot diameter	Number of nodes	Severity of symptoms[c]	Rf[d]
0	Control	9.96	4.48	11.66	0.85	2.02	4.2	0.10	—[e]
1	*M. arenaria*	10.44	5.29	11.58	0.64	1.59	3.6	1.05	1.47
2	*M. incognita*	10.64	4.97	11.26	0.59	1.44	4.1	0.35	1.33
3	*M. javanica*	9.71	5.04	9.38	0.64	1.48	3.5	1.05	1.57
4	*P. penetrans*	11.72	5.57	12.55	0.55	1.60	3.9	0.15	1.49
5	*P. vulnus*	9.86	4.73	13.07	0.49	1.43	4.0	0.05	2.30
					Contrasts (P)[f]				
0 vs. 1 to 5		ns	ns	ns	0.001	0.001	ns	0.002	—
0 vs. 1 to 3		ns	ns	ns	0.010	0.002	ns	<0.001	—
0 vs. 4 to 5		ns	ns	ns	<0.001	0.004	ns	ns	—
1 to 3 vs. 4 to 5		ns	ns	ns	ns	ns	ns	<0.001	<0.001
1 vs. 2 to 3		ns	ns	ns	ns	ns	ns	0.023	ns
2 vs. 1 and 3		ns	ns	ns	ns	ns	ns	<0.001	ns
3 vs. 1 to 2		ns	ns	ns	ns	ns	ns	0.023	ns
4 vs. 5		ns	ns	ns	ns	ns	ns	ns	<0.001

[a] Data are means of 20 replicated plants per treatment combination. Plants were inoculated with 5,000 individuals (Pi) of *Pratylenchus* spp. or 15,000 eggs + J2 (Pi) of *Meloidogyne* spp.
[b] Average percentage growth of each parameter during the experiment.
[c] Assessed on a scale of 0 to 5 according to the percentage of foliage with yellowing (0 = 0%, 1 = 1 to 25%, 2 = 26 to 50%, 3 = 51 to 75%, 4 = 76 to 100%, and 5 = dead plant).
[d] Rf (nematode reproduction factor) = Pf (final nematode population per plant)/ Pi (initial nematode inoculum per plant).
[e] — = not tested.
[f] Orthogonal contrast of inoculation treatments. Probability for the t statistic of linear single-degree-of-freedom contrasts; ns = not significant ($P > 0.05$).

was suppressed ($P < 0.05$) after infection by *Pratylenchus* spp. and *Meloidogyne* spp. (Tables 2, 3). There were no differences in the total and root fresh weights between nematode-infected and non-infected plants of either olive cultivar (Tables 2, 3). Shoot height, assessed as the percentage growth during the experiment, was suppressed ($P < 0.05$) only on Arbequina infected by *Meloidogyne* spp. (Tables 2, 3). Percentage growth of the main stem diameter was suppressed ($P < 0.05$) on Picual but not Arbequina by all species of *Meloidogyne* and *Pratylenchus* (Tables 2, 3). Finally, the number of nodes was suppressed ($P < 0.05$) by *Meloidogyne* spp. on Arbequina but not on Picual (Tables 2, 3).

Although both Arbequina and Picual supported reproduction of the three *Meloidogyne* spp. and two *Pratylenchus* spp., the Rf value was higher ($P < 0.05$) for *Meloidogyne* spp. than for *Pratylenchus* spp. on Arbequina, and higher ($P < 0.05$) for *Pratylenchus* spp. than for *Meloidogyne* spp. on Picual (Tables 2, 3).

Discussion

Olive cultivars Arbequina and Picual are the most extensively grown across approximately 1.3 million ha of olive groves in Spain. Our results indicate that *Mesocriconema xenoplax, Helycotylenchus digonicus, H. pseudorobustus, Meloidogyne arenaria* race 2, *M. incognita* race 1, *M. javanica, Pratylenchus penetrans,* and *P. vulnus* develop and reproduce on these olive planting stocks. However, the reproduction rates of *M. xenoplax* on Arbequina and Picual were much lower than on some other fruit trees, such as peach, which are regarded as very suitable hosts (Westcott and Burrows, 1991). The Rf values of the two *Helicotylenchus* spp. differed between the two olive cultivars, indicating that Picual is a better host for these nematodes. Reproduction rates of the root lesion nematodes *P. fallax, P. thornei,* and *Z. guevarai* were lower than 1, suggesting that Arbequina and Picual were not suitable hosts for these species. These results agree with field observations, indicating that these nematodes reproduce predominantly on herbaceous hosts and that their presence in fruit tree nurseries (including olive) should not pose a problem on nursery production (Castillo et al., 1998; Pinochet et al., 1991, Verdejo and Pinochet, 1991).

The three root-knot nematodes, *M. arenaria* race 2, *M. incognita* race 1, and *M. javanica,* and the two lesion nematodes, *P. penetrans* and *P. vulnus,* showed a reproduction rate in Arbequina and Picual planting stocks higher than 1, indicating that these olives are suitable hosts for these nematodes.

In spite of the ability of *P. penetrans* and *P. vulnus* to infect and reproduce in olive planting stocks, they did not induce any macroscopic symptoms on either the roots or foliage. In contrast, infections of Picual by *Meloidogyne* spp. induced root galls and leaf chlorosis in the plant. Leaf chlorosis has been reported for woody plants infected by *Meloidogyne* spp. (Pinochet et al., 1992; Talavera et al., 1999) and suggests that root infections by the nematode impair plant physiology (Melakerberhan and Webster, 1993). Root infections by the three root-knot nematodes resulted in moderate root galling in the two olive cultivars, as was previously observed in naturally infested rootstocks from olive nurseries in Andalusia (Nico et al., 2002).

Results of our experiments failed to reveal a significant correlation between infection by either the three *Meloidogyne* spp. or the two *Pratylenchus* spp. and reduction in the plant weight gain of the two olive cultivars. However, shoot diameter appeared to be a highly sensitive parameter for assessing nematode damage on both cultivars. The ability of *P. penetrans* to infect and damage olive plants shown in this study confirms our previous results (Nico et al., 2002). This may have been overlooked in other studies because this nematode was not common in olive orchards surveyed (Inserra and Vovlas, 1981). Although parasitism by these root-knot and root-lesion nematodes can impair olive plant growth under controlled conditions, long-term experiments under field or microplot conditions are necessary to determine the damage that these nematodes may cause to growth and yield of the two cultivars in olive orchards.

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