Evaluation of the phytopathological reaction of wild and cultivated olives as a mean of finding promising new sources of genetic diversity for resistance to root-knot nematodes

Juan E. Palomares-Rius[†], Institute for Sustainable Agriculture (IAS), Spanish National Research Council (CSIC), Avda. Menéndez Pidal s/n, 14004, Córdoba, Campus de Excelencia Internacional Agroalimentario, ceiA3, Spain; **Angjelina Belaj**, IFAPA Centro Alameda del Obispo, Córdoba, Spain; **Lorenzo León**, IFAPA **Raúl De la Rosa**, IFAPA; **Hava F. Rapoport**, IAS-CSIC; and **Pablo Castillo**, IAS-CSIC

[†]Corresponding author: Juan E. Palomares-Rius

E-mail: palomaresje@ias.csic.es

running title: olive reactions to *Meloidogyne*

Abstract

Olive (*Olea europaea* L.) is one of the most important fruit-crops in the Mediterranean Basin, occupying significant acreage in these countries and often accompanied with important cultural heritage and landscape value. This crop can be infected by several *Meloidogyne* species (*M. javanica*, *M. arenaria* and *M. incognita*, among others), and only a few cultivars have been found with some level of resistance to these nematodes. Recent innovations in intensive olive growing using high planting densities, irrigation and substantial amounts of fertilizers, could increase the nematode population to further damaging levels. In order to further understand the interactions involved between olive and pathogenic nematodes and in the hope of finding solutions to the agricultural risks, this research aimed to determine the reaction of important olive cultivars in Spain and wild olives to *M. javanica* infection, including genotypes of the same and other *O. europaea* subspecies. All the olive cultivars tested were found to be good hosts for *M. javanica*, but substantially different high levels of reproduction were found in three cultivars (cv. Gordal Sevillana, cv. Hojiblanca and cv. Manzanilla de Sevilla). In the wild accessions, the *O. europaea* subsp. *cerasiformis* (genotype W147) and the subsp. *europaea* var. *sylvestris* (genotype W224) were resistant to *M. javanica* at different levels, with strong resistance in W147 (Reproduction factor (Rf) = 0.0003) and moderate resistance in W224 (Rf = 0.79). The defense reaction of W147 to *M. javanica* showed a strong increase of phenolic compounds but no hypersensitive reaction.

Keywords: histopathology, *Meloidogyne*, olive, reproduction, resistance, root-knot nematodes

Introduction

Olive (*Olea europaea* L.) is one of the most important fruit-crops in the Mediterranean Basin, occupying significant acreage in these countries and often accompanied with important cultural heritage and landscape value. Furthermore, this crop is expanding worldwide because of the high demand for olive oil related to health benefits (Rallo et al. 2016). In addition to oil production, some cultivars are specifically cultivated for consumption as table olives, while others are used for both purposes. The cultivated olive tree belongs to the *O. europaea* complex which consists of six different subspecies associated with specific, often isolated, geographical areas (Green 2002; Besnard et al. 2018): (i) subsp. *europaea* with two botanical varieties [var. *sylvestris* (namely oleasters or wild olive) and var. *europaea* (cultivated olive)] distributed in the Mediterranean Basin; (ii) subsp. *laperrinei* in the Saharan mountains; (iii) subsp. *cuspidata* distributed from southern Africa to southern Egypt and from Arabia to China); (iv) subsp. *guanchica* native from the Canary Islands; (v) subsp. *maroccana* in southern Morocco; and, (vi) subsp. *cerasiformis* in Madeira Islands. This broad range of wild relatives, including both genotypes of other subspecies and those pertaining to the var. *sylvestris* of its own subspecies, represents promising new sources of genetic diversity for resistance to plant pathogens, including root-knot nematodes (*Meloidogyne* spp.).

The olive tree rhizosphere has been found to be a good habitat for many species of plant-parasitic nematodes (Castillo et al. 2010; Ali et al. 2014). Only some of them, however, have been shown to be pathogenic or to directly feed on olive roots, among which one of the most widespread and damaging is the genus *Meloidogyne* (Castillo et al. 2010; Ali et al. 2014; Archidona-Yuste et al. 2018). A number of species of this genus have been found to infect olive (Tarjan 1957; Minz 1961; Yang and Zhong 1980; Abrantes et al. 1991; Castillo et al. 2003; Archidona-Yuste et al. 2018): *M. javanica*

(Treub, 1885) Chitwood, 1949; *M. incognita* (Kofoid & White, 1919) Chitwood, 1949, *M. hapla* Chitwood, 1949, *M. arenaria* (Neal, 1889) Chitwood, 1949, *M. lusitanica* Abrantes & Santos, 1991, *M. baetica* Castillo, Vovlas, Subbotin & Troccoli, 2003, and *M. oleae* Archidona-Yuste, Cantalapiedra-Navarrete, Liebanas, Rapoport, Castillo & Palomares-Rius, 2018.

The aforementioned *Meloidogyne* spp. have been reported associated with olive in 19 countries, representing a real threat for olive culture worldwide (Ali et al. 2014). Plant-parasitic nematodes can also be found in sporadic distributions in wild olives, olive nurseries, and established orchards, and have been demonstrated to cause heavy root galling and severe reduction in plant growth in pathogenicity tests (Castillo et al. 2010; Lamberti et al. 1969; Nico et al. 2002; 2003; Sasanelli et al. 2002; Sasanelli et al. 1997). Estimates of olive losses in the United States due to *Meloidogyne* spp. and *Tylenchulus semipenetrans* (Cobb, 1913) ranged from 5 to 10% (Koenning et al. 1994; Singh et al. 2013). In some cases plant-parasitic nematodes are associated with damaging syndromes such as "drying syndrome" in newly established olive orchards in Argentina (Pérez et al. 2001) or with vascular diseases (Lamberti et al. 2001; Saeedizadeh et al. 2003). Damage in nursery plants is more severe due to nematode parasitism and more importantly, plant-parasitic nematode populations are disseminated from nurseries to uninfested areas (Nico et al. 2002). Furthermore, the olive crop is changing from traditional low-input orchards without irrigation to new, highly mechanized orchards, with irrigation, high fertilizer inputs, and high planting densities, often belonging to new cultivars adapted to these crop conditions (Rallo et al. 2016). These new high-density irrigated orchards tend to have soil conditions more conducive for the establishment of the diseases caused by nematodes, mainly *Meloidogyne* spp. (Ali et al. 2016).

89 Host-plant resistance could be the easiest, safest, and cheapest long-term approach
90 to controlling the damage caused by plant-parasitic nematodes (Castillo et al. 2010),
91 provided, for example by breeding for resistant rootstocks. Previous studies have
92 identified a few olive cultivars associated with some degree of resistance to the most
93 common species of *Meloidogyne*, such as *M. incognita*, *M. javanica*, *M. arenaria*, and *M.*
94 *hapla* (Lamberti and Baines 1968; Al-Sayed and Abdel-Hameed 1991; Sasanelli et al.
95 1997). In general, commercial cultivars are grown self-rooted, but the use of resistant
96 rootstocks, alternative cultivars such as cv. Allegra in California (McKenry 1994), or
97 wild types, could also be of great interest. Apart from the indications above, most
98 preliminary reports indicate that most olive cultivars as good, rather than resistant, hosts
99 for *Meloidogyne* spp., such as cv. Arbequina and cv. Picual as hosts for *M. javanica*, *M.*
100 *incognita* and *M. arenaria* (Nico et al. 2003), and cv. Cima di Bitonto as a host for *M.*
101 *javanica* (Sasanelli et al., 2009). Knowledge of resistance or susceptibility of wild olives
102 is absent. Thus searching for resistance in traditional cultivars and/or newly bred
103 cultivars, as well as in wild olive genotypes, requires further attention. Progress in
104 understanding the reaction of olive cultivars, wild olive genotypes, and other related
105 subspecies to *Meloidogyne* spp. represents a critical step to finding new solutions for
106 sustainable olive agriculture.
107 The specific objectives of this research were to: (i) determine the host suitability
108 of widely used, mainly Spanish, commercial olive cultivars, wild olive genotypes, and
109 related subspecies of the *O. europaea* complex to *M. javanica*, and (ii) to assess the
110 histopathological plant-nematode interaction in susceptible and resistant hosts in order to
111 understand the mechanisms involved. The species *M. javanica*, currently found to be the
112 most prevalent *Meloidogyne* species in both cultivated and wild olives in Spain
113 (Archidona-Yuste et al. 2018), was used for standardized evaluation.

Material and methods

Nematode inocula. An isolate of *M. javanica* from a commercial orchard, cv. Manzanilla de Sevilla, in La Campana, Sevilla province, was identified to species level based on features of the female perineal pattern, isozyme malate and esterase patterns and molecular data (SCAR-based polymerase-chain-reaction assays, *cox*II-16S rRNA and specific PCR) (Esbenshade and Triantaphyllou 1985; Hartman and Sasser 1985; Zijlstra et al. 2000). Inoculum of the *M. javanica* isolate was increased on tomato (*Solanum lycopersicon* L. cv. Tres Cantos) grown in clay pots filled with an autoclaved (120ºC, 2 h) sandy soil mixture, starting from a single egg mass in a growth chamber adjusted to 25 ± 1°C, 60 to 90% relative humidity, and a 16-h photoperiod of fluorescent light at 360 ± 25 μE $m^{-2}s^{-1}$ for 2 months. The inoculum consisted of eggs and second-stage juveniles (J2) extracted from 2-month-old tomato plants using 1% sodium hypochlorite (Hussey and Barker 1973) followed by centrifugal flotation (Coolen 1979).

Plant material. The wild olive genotypes and the related subspecies were obtained from the *ex situ* wild repository established at IFAPA Centre "Alameda del Obispo" (Belaj et al. 2016; León et al. 2018), Córdoba, while the olive cultivars (Table 1) came from the World Olive Germplasm Collection of IFAPA (WOGC) which is also maintained at the same research centre (Belaj et al. 2016). While both the wild and the cultivated genotypes are represented by two-three trees per genotype in their respective collections, the plant material utilized and further vegetatively propagated by semi-hardwood cuttings, was obtained from one and always the same tree. The following procedure was followed to rapidly produce homogeneous plants suitable for the experiments: Cuttings from branches of the preceding year's growth (0.5 cm of diameter,

and with a length of 12-14 cm approximately) were selected from each tree under study for vegetative propagation. The stem cuttings were surface-disinfested with fungicide (a 1% $CuSO_4$ solution) for 5 min and washed four times in sterile distilled water to prevent fungal contamination. Afterwards, the lower end of the cuttings was dipped for 5 seconds in 3000 ppm indole butyric acid powder (Rootone® F, Compo, Barcelona, Spain) to promote rhizogenesis. After that, the treated and dried stem cuttings were planted in propagation trays filled with perlite and kept under suitable conditions for rooting (25 ± 1°C, 60 to 90% relative humidity) for 2 months in the greenhouse chamber. The rooted cuttings were transferred into 1 l plastic pots filled with peat and maintained for approximately 6 months in a shade house for hardening (Del Río and Caballero, 2005).

Plants of uniform root system and shoot size were selected and transplanted into 75 mm x 77 mm x 180 mm plastic pots (one plant per pot) filled with an autoclaved (120°C, 1 h, twice) soil mixture (sand/clay loam, 2:1, vol/vol). Plants were watered on alternate days with 100 ml of sterilized tap water and fertilized with 100 ml of a 0.1% solution of a 20-5-32 (N-P-K) + micronutrients fertilizer (Poly-Feed™, Haifa, Israel) and pruned to maintain a single shoot every week. After a 7-day recovery period the plants were inoculated with *M. javanica* inoculum.

Growth chamber experiments. The experiments were conducted in a growth chamber under the conditions described above, which are considered optimal for the development and reproduction of *M. javanica* (Trudgill and Perry 1994). Plants were inoculated individually by adding 10,000 eggs + J2 of *M. javanica* in 10 ml of sterile distilled water. The nematode suspension, corresponding to a theoretical inoculum density of 10 nematodes/cm^3 soil, was added to four holes in the soil around the base of the plant. The nematode inoculum density of the water suspension was determined by counting nematode specimens in 10 1 ml aliquots.

Plants were watered with 100 ml of water on alternate days and fertilized weekly with 100 ml of the previously mentioned nutrient solution. Each genotype was replicated eight times and the experiment was arranged in a completely randomized design. The experiment was conducted twice, with a duration of 120 days after nematode inoculation.

Assessment of plant growth variables and data analyses. Plant growth, root galling, and nematode reproduction were rated at the end of both trials. Plant growth of the genotypes was assessed by comparing root fresh weight with that of un-inoculated control plants. Before assessment of root weight, the root system of a plant was gently washed free of adhering soil and debris, and root galling rated on a 0 to 6 scale, where 0 = no galls; 1 = 1–10; 2 = 11–20; 3 = 21–40; 4 = 41–70; 5 = 71–90; and 6 ≥ 91 galls (Nico et al. 2003).

Final soil and root nematode population densities (*Pf*) were determined. Soil was washed thoroughly with tap water through a 710-µm mesh sieve and the filtered water was collected in a beaker and thoroughly mixed with 4% kaolin (v/v). This mixture was centrifuged at 1100 g for 4 min, the supernatants were discarded, pellets were re-suspended in 250 mL $MgSO_4$ ($\delta = 1{\cdot}16$), and the new suspensions were centrifuged at 1100 g for 3 min. The new supernatants were sieved through 5 µm mesh, and nematodes collected on the sieve were washed with tap water, transferred to Petri dishes and counted under a stereomicroscope (Coolen 1979). To assess nematode population in *Meloidogyne*-infected roots, the complete root system of a plant was washed free of soil and cut into 1-2-cm segments, and *M. javanica* (eggs, sedentary stages and J2s) were extracted by maceration followed by centrifugation. Root tissues were homogenized in 250 mL of a 1% solution of NaOCl using a Waring blender at 1800 g for 1 min, and homogenates were centrifuged and extracted as described above (Coolen 1979; Hussey and Barker 1973). Population densities were used to calculate the reproduction index [Rf

189 = final population density in soil and roots (*Pf*) divided by initial population density (*Pi*)].
190 All data of root symptom severity, nematode reproduction and root fresh weight were
191 transformed into $\log_{10}(X + 1)$, before analyses (Gómez and Gómez 1984). Similarity
192 between the experiment repetitions was tested by preliminary analyses of variance
193 (ANOVA) using experimental runs as factors, which determined that the experiment ×
194 genotype interaction was not significant ($P \geq 0.05$) and thus permitted combining the data
195 of both experimental runs for further analyses (Gómez and Gómez 1984). Analyses of
196 variance were carried out using Statistix 10.0 (NH Analytical Software, Roseville, MN,
197 USA). Significant differences among means of root weight, gall rating, and nematode
198 reproduction were estimated using the least significant difference multiple range test (P
199 = 0.05). Data from uninoculated control treatments were not included in analyses of gall
200 ratings and nematode reproduction, to avoid the use of zero in the ANOVA.

201 **Histopathological study.** Galled roots from the different subspecies of olive and
202 cultivated plants infected by *M. javanica* and controls (similar root zones from
203 uninoculated plants) were selected at the end (120 days) of the experiments (2-3
204 roots/plant from 3-4 plants in every genotype and treatment for each experiment
205 repetition), gently washed free of adhering soil and debris, fixed in FAE solution
206 [formalin, acetic acid, 95% ethanol and distilled water (10:5:50:35 v/v/v/v)] for at least
207 48 h, dehydrated in a tertiary butyl alcohol series (70-85-90-100%) and embedded in
208 paraffin (58°C melting point; Merck, Darmstadt, Germany) for histopathological
209 observations. Embedded tissues were sectioned longitudinally and transversely at 12 µm
210 with a rotary microtome and stained with a combination of tannic acid - ferric chloride,
211 safranin and fast green, by which nuclei, chromosomes, and lignified or suberized cell
212 walls stain red, cytoplasm and cellulosic cell walls stain green, and the tannic acid – iron
213 chloride aids in cell wall definition and is considered to be a general test for phenols

(Reeve, 1951; Jensen, 1962; Ruzin, 1999). The stained sections were examined microscopically (optical microscope Leica DMRBFHC, Leica Microsystems, Heerbrugg, Switzerland), and photographed (digital camera Leica DFC450C).

Two genotypes (cv. Ayvalik and the wild genotype W19) developed typical galls but were damaged during histological processing, so no microphotographs could be obtained. The wild olive genotype from Morocco (W224) was not included in the final histological analysis because of the few and small galls produced. Similarly, genotype W147 from Madeira Island and belonging to subsp. *cerasiformis* was also excluded from this analysis because roots were not galled in the two experimental repetitions.

In an additional experiment, 12 plants from genotype W147 were inoculated with a high inoculum level of 15,000 J2s in order to further explore early interactions with the host plant, such as a possible hypersensitive reaction or repellence of the nematode by the root. These plants were sampled at 4, 11, 25 and 70 days after inoculation (DAI) (3 plants per sampling time). Half of the roots for each plant were processed according to the histopathological procedure described above, and the other half processed for fuchsine acid staining following Byrd et al. (1983). Unfortunately, wild olive plantlets of genotype W224 were not available for the additional study of resistance.

Results

Suitability of wild and cultivated olive genotypes as hosts of *Meloidogyne javanica*. Symptoms on aboveground plant parts did not appear, either on nematode-inoculated nor un-inoculated plants. In some cases differences in root fresh weight were detected between inoculated and un-inoculated plants. For the wild olives, W166 was the only genotype with a significant difference in root fresh weight between inoculated and un-inoculated plants, with greater root fresh weight in un-inoculated plants (Table 2).

Root fresh weights were great for the inoculated cv. Hojiblanca and cv. Manzanilla de Sevilla (Table 3), while cv. Gordal Sevillana and cv. Picual had lower root fresh weight when inoculated with *M. javanica*. In the other five cultivars there was no difference in fresh root weights between the inoculated and un-inoculated plants (Table 3). Variation in fresh root weights were observed, however, among the genotypes of wild olive and commercial cultivars, likely related to the original plant size and growth level at the beginning of the experiment (Table 2 and 3).

While differing among olive genotypes, relative levels of root galling and Rf values followed similar patterns. The wild genotype W158 belonging to subsp. *cuspidata* had the highest levels of root galling and Rf value, followed by the two genotypes (W1048 and W46) belonging to *O. europaea* subsp. *guanchica*, as well as the subsp. *maroccana* genotype W215 (Table 2). Most of the other wild genotypes and related subspecies had statistically lower levels of root galling and Rf values than the corresponding non-inoculated plants. Two genotypes, W147 and W224, belonging to subsp. *cerasiformis* and subsp. *europaea* var. *sylvestris*, respectively, had exceptionally low levels of root galling and Rf values (Rf < 1) when inoculated with *M. javanica*. In regard to the commercial cultivars, all had high to moderate root galling (from 0.95 to 3.00) and high Rf values (Rf from 2.17 to 8.64) (Table 3). However substantially higher Rf values were observed in cv. Gordal Sevillana, cv. Hojiblanca and cv. Manzanilla de Sevilla in comparison to the other cultivars (Table 3).

Root morphological and histopathological reaction of olive genotypes to *Meloidogyne* spp.

The root-system morphology had typical galls produced by *M. javanica* (Fig. 1), characterized by galling at the tips of growing roots. Histopathological sections of the root galls of wild olives (Fig. 2) and olive cultivars (Fig. 3) infected by *M. javanica* had

typical feeding sites with 5-6 giant cells close to each nematode female, with bigger galls having more than one female per gall (Figs. 2 and 3). The giant-cell cytoplasm was dense, granulated and homogenous, and contained numerous hypertrophied nuclei. Disruption of xylem vessels was detected close to the massively enlarging giant cells.

The genotypes W147 and W224 produced no or minimal nodulation in response to *M. javanica* infection, and small rates of reproduction (Table 2). The genotype W147 (subsp. *cerasiformis*) at 4 DAI had very few juveniles in the few roots tips. During this period, nematodes were moving within the root tip searching for specific suitable cells in order to induce feeding sites. These juveniles were surrounded by high levels of phenolic compounds and some necrotic cells (Fig 4-l, m, and n). At 11 DAI, a few nematodes were sedentary, but the others were still in the process of looking for appropriate cells for inducing the feeding site. The root tissue where nematodes were present was darker than other areas of the root, due to the presence of phenolic compounds. Some root tips were very slightly galled (Fig 4-b and c). At 25 DAI, the nematodes in feeding sites with giant cells visible were crowded in specific galls, and exhibited different stages of development inside the gall ranging from J2 sedentary, J3 (Fig. 4-d, e and f), or J4 (Fig. 4-g). Males were also detected at this time. Viewed in histopathological sections, the nematodes were surrounded by feeding sites, but these were saturated with very dark areas due to the presence of phenolic compounds. Also the cytoplasm of the giant cells was generally denser and darker than the surrounding uninfected tissues. However the nematodes were feeding on those cells and clearly undergoing development (Fig. 4-d, e, p, q, s and t). At 70 DAI, only a few small females had developed which were solitary and occupied tip positions (from 2 to 5 females per plant), and only a small number of eggs were produced inside them and in the egg mass (Fig. 4-h-k). Overall, fewer nematodes were detected in the roots than observed at 25 DAI using fuchsine acid. Because of the low number of

289 females present within the root tissue it was not possible to locate females in the
290 histological sections.

291

292 ## Discussion

293 Olive production in the Mediterranean Basin is threatened by soil pathogens such
294 as *Verticillium dahliae* and *Meloidogyne* spp., and biotic interactions with soil
295 microorganisms. In the case of nematodes of the genus *Meloidogyne*, extensive sampling
296 in Morocco found this group of nematodes in 23% and 52% of in olive orchards and
297 nurseries, respectively (Hamza et al. 2017). Studies in Andalusia (Spain) revealed the
298 frequent presence of *Meloidogyne* spp. in both cultivated and wild olives, with *M.*
299 *javanica* being the most prevalent species in Spain (Archidona-Yuste et al. 2018).
300 Furthermore, the recent transition to intensive olive growing, with high-density planting,
301 irrigation and substantial amounts of fertilizers, could increase the nematode population
302 densities to further damaging levels. Based on confirmed levels of pathogenesis
303 (Sasanelli, 2009) and frequent association with olive (Hamza et al. 2017; Archidona-
304 Yuste et al. 2018), *M. javanica* was used as a standard species for the study. This species
305 proved to be suitable to this role, and, following controlled inoculations, produced levels
306 of infection readily measurable by root galling and nematode reproduction. Overall, both
307 the cultivated and wild olive genotypes were found to be suitable hosts, although among
308 the cultivars studied some appeared to be particularly susceptible, and within the wild
309 genotypes there may be candidates for resistance or for better understanding resistance
310 mechanisms.

311 Consistent with the findings of this study, Mediterranean olive cultivars are
312 generally considered good hosts for *Meloidogyne* spp. (Nico et al. 2003; Castillo et al.
313 2010). However, interestingly, statistically significant higher Rf values were found in

three olive cultivars (cv. Gordal Sevillana, cv. Hojiblanca and cv. Manzanilla de Sevilla) in comparison to other cultivars used in Spain and other tested in this study. These cultivars, in particular cv. Hojiblanca and cv. Manzanilla de Sevilla are planted in extensive areas of Spain (MAGRAMA 2017). Information about the susceptibility of cultivars to nematodes is critical when establishing orchards in areas infested with *Meloidogyne* spp., as the plants could increase populations up to damaging levels in only a few years after planting. Furthermore, the nematode damage risk is particularly high when the plant is still young and more susceptible. Other olive cultivars considered in this study, those associated with high density plantings in hedgerows, cv. Arbequina, cv. Sikitita and cv. Koroneiki, had similarly lower Rf values, however, were still good hosts for *M. javanica*. The high intensity field conditions in which these cultivars are often grown commercially could be particularly risky for nematode infection and long-term experiments are necessary to assess the nematode pressure under these conditions.

Perennial plants such as the olive present a particular challenge for combating infection. Resistance genes are exposed to much longer periods of continual pressure than plants in annual production systems (Saucet et al. 2016), increasing the risk that nematodes will 'break' the plant resistance (Lespinasse et al. 2003). Nematicides are restricted in their use in woody plants and in a low-income crop such as olive are not economically feasible, thus alternative control measures are required. Wild olives offer the possibility of resistance sources for *Meloidogyne* spp., specifically as rootstocks. Recently, the use of resistant rootstocks for other diseases (Verticillium wilt) has proven useful, even in the presence of *Meloidogyne* spp. (Palomares-Rius et al. 2016).

In contrast to the majority of the interactions studied in the experiments, the genotype W147, which belonged to subsp. *cerasiformis*, showed a resistant reaction to *M. javanica*. This genotype had a minimal number of nematodes in the roots and

surrounding soil, and the slight gall-like swellings observed were probably due to the presence of very few females developing at a slow rate, as we found in the fuchsine acid staining experiment (Fig 4. h-k). Other cultivars with resistance specific to *Meloidogyne* spp. (as cv. Coratina and cv. Leccino) (Sasanelli, 2009) had low Rf values (< 1), which differed from the resistance behavior observed in the genotype of the subsp. *cerasiformis*, in which the Rf value was extremely low (Rf = 0.0003). In that genotype, only when inoculated with high numbers of juveniles, did very few of them penetrate the roots at 4 and 11 DAI and they seemed aggregated to only certain roots. This parameter, however, could be difficult to assess in experimental conditions due to root heterogeneity in woody plantlets. However, this is not the normal situation in field soil, in which the majority of the inoculum is in the form of eggs which will hatch sequentially. The genotype W147, even with the high inoculation number of juveniles, did not break resistance and only a few females were detected. Nonetheless the fuchsine staining and histological sections showed a substantial nematode reaction to the plant once inside the root, with strong staining of phenolic compounds in tissues close to the nematodes, and in some cases the necrosis of some cells (Fig. 4-l, m and n).

The mechanisms providing resistance to nematodes may be too complex to easily determine, and the genotype W147 does not seem to be an exception. One concern is that the nematodes only penetrated a few roots in this genotype when high numbers of juveniles were used for inoculation in the histopathological and fuchsine acid stain study. This might have occurred because of low attraction to the juveniles for the large proportion of roots which are not rapidly growing, a situation often found in woody plant, especially olive, root systems which are characterized by highly varied rates of growth. This hypothesis, however, is difficult to clearly test.

We suggest that what might occur is that competition for use of the feeding sites and the presence of phenolic compounds could lead to nematode death or lack of completion of the adult stage for the majority of nematodes in the roots, allowing only a few of them to create a proper feeding site and enable minimum reproduction. The few observed female nematodes were found alone in isolated positions of the root, supporting the hypothesis that strong competition among nematodes for scarce resources could induce the death of developing nematodes in egg and/or juvenile inoculated plants. This resistance is not associated with the typical hypersensitive response of giant cell death, such as occurs with the gene *Mi* (Williamson and Hussey, 1996), because the few giant cells detected in W147 contained developed nuclei and vacuolization was not present. A similar reaction to that observed in W147 has been observed in resistant grape rootstocks (RS-3 and 10-23B), which express genetic resistance during *Meloidogyne* spp penetration, development and reproduction (Anwar and McKenry, 2000). The resistance to *M. exigua* Göeldi, 1887 in *Coffea canephora* L. (gene *Mex-1*) is also expressed during nematode penetration and development (Alpizar et al. 2007).

Among the wild olives it was interesting to observe the variability in host suitability among different genotypes of the same subspecies. This was seen with *O. europaea* subsp. *europaea* var. *sylvestris* to which the majority of the tested genotypes belonged. The second most resistant genotype to *M. javanica* was the accession W224 (*O. europaea* subsp. *europaea* var. *sylvestris*). In this case, similar to W147, only a few and small galls were detected. For W224 *M. javanica* Rf values was also low, but not as low as W147 (Table 2), as some females were able to reproduce on the roots. A small Rf value (< 1) have also been reported for olive cultivars (cv. Coratina, cv. Leccino, cv. Ascolana and cv. Moraiolo) resistant to *Meloidogyne* and a selection of the wild olive DA 12I (Sasanelli, 2009).

In conclusion, this research provides considerable new data about the broad susceptibility of olive cultivars used in commercial groves, and new and valuable sources of resistance that require future study. Future studies on these olive genotypes should include other species of *Meloidogyne* spp. (*M. arenaria*, *M. incognita* and *M. lusitanica*) and other kinds of nematodes (*Xiphinema* spp. and *Rotylenchus* spp.) affecting olive. The potential of the two genotypes W147 and W224, belonging to subsp. *cerasiformis* and subsp. *europaea* var. *sylvestris*, respectively, as resistant rootstocks is promising. Further effort is necessary in the characterization of the defensive reaction, grafting capabilities, and adaptability of these genotypes to the olive agricultural system.

Acknowledgments

The authors thank J. Martín-Barbarroja (IAS-CSIC) and G. León Ropero (IAS-CSIC) for their excellent technical assistance. This research was supported by grants P12-AGR 1486 and AGR-136 from the 'Consejeria de Economia, Innovacion y Ciencia' of the Junta de Andalucia, and Union Europea, Fondo Europeo de Desarrollo regional, 'Una manera de hacer Europa', grant 201740E042, "Análisis de diversidad molecular, barcoding, y relaciones filogenéticas de nematodos fitoparásitos en cultivos mediterráneos" from Spanish National Research Council (CSIC), grant 219262 ArimNET_ERANET FP7 2012–2015 Project PESTOLIVE 'Contribution of olive history for the management of soil-borne parasites in the Mediterranean basin' from the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA).

Literature cited

Abrantes, I. M. de O., and Santos, M. S. N. 1991. *Meloidogyne lusitanica* n. sp.

(Nematoda: Meloidogynidae), a root-knot nematode parasitizing olive tree (*Olea europaea* L.). J. Nematol. 23:210-224.

Ali, N., Chapuis, E., Tavoillot, J., and Mateille, T. 2014. Plant-parasitic nematodes associated with olive tree (*Olea europaea* L.) with a focus on the Mediterranean Basin: A review. C. R. Biol. 337:423-442.

Ali, N., Tavoillot, J., Chapuis, E., and Mateille, T. 2016. Trend to explain the distribution of root-knot nematodes *Meloidogyne* spp. associated with olive trees in Morocco. Agric. Ecosyst. Environ. 225:22-32.

Alpizar, E., Etienne, H., and Bertrand, B. 2007. Intermediate resistance to *Meloidogyne exigua* root-knot nematode in *Coffea arabica*. Crop Prot. 26:903-910.

Al-Sayed, A., and Abdel-Hameed, S. H. 1991. Resistance and susceptibility of olives to *Meloidogyne incognita* and *Rotylenchulus reniformis*. Ann. Agric. Sci. Moshtohor 29:1221-1226.

Anwar, S. A., and M. V. McKenry. 2000. Penetration, development and reproduction of *Meloidogyne arenaria* on two new resistant *Vitis* spp. Nematropica 30:9-17.

Archidona-Yuste, A., Cantalapiedra-Navarrete, C., Liébanas, G., Rapoport, H. F., Castillo, P., and Palomares-Rius, J. E. 2018. Diversity of root-knot nematodes of the genus *Meloidogyne* Göeldi, 1892 (Nematoda: Meloidogynidae) associated with olive plants and environmental cues regarding their distribution in southern Spain. PLoS ONE 13:e0198236. doi: 10.1371/journal.pone.0198236.

Belaj, A. Gurbuz, M., Sikaoui, H., Moukhli, A., Khadari, B., Mariotti, R., and Baldoni, L. (2016). *Olive genetic resources*. Pages 27-54 in: The Olive Tree Genome, Compendium of Plant Genomes. E. Rugini, L. Baldoni, R. Muleo and L. Sebastiani, eds. Springer, Dordrecht, Netherlands. doi: 10.1007/978-3-319-48887-5_3

Besnard, G., Dupuy, J., Larter, M., Cuneo, P., Cooke, D., and Chikhi, L. 2014. History of

437 the invasive African olive tree in Australia and Hawaii: evidence of sequential
438 bottlenecks and hybridization with the Mediterranean olive. Evol. Appl. 7:195-211.
439 Besnard, G., Terral, J.-F., and Cornille, A. 2018. On the origins and domestication of the
440 olive: a review and perspectives. Ann. Bot. 121:385-403.
441 Byrd, D. W. Jr., T. Kirkpatrick, and K. R. Barker. 1983. An improved technique for
442 clearing and staining tissues for detection of nematodes. J. Nematol. 15:142–143.
443 Castillo, P., Nico, A., Navas-Cortés, J.A., Landa, B. B., Jiménez-Díaz, R. M., and Vovlas,
444 N. 2010. Plant-parasitic nematodes attacking olive trees and their management. Plant
445 Dis. 94:148-162.
446 Castillo, P., Vovlas, N., Subbotin, S., and Troccoli, A. 2003. A new root-knot nematode,
447 *Meloidogyne baetica* n. sp. (Nematoda: Heteroderidae), parasitizing wild olive in
448 Southern Spain. Phytopathology 93: 1093-1102.
449 Coolen, W. A. 1979. Methods for the extraction of *Meloidogyne* spp. and other nematodes
450 from roots and soil. Pages 317-329 in: Root-knot Nematodes (*Meloidogyne* species)
451 Systematics, Biology and Control. F. Lamberti, and C. E. Taylor, eds. Academic
452 Press, London, UK.
453 Esbenshade, P. R., and Triantaphyllou, A. C. 1985. Use of enzyme phenotypes for
454 identification of *Meloidogyne* species. J. Nematol. 17:6-20.
455 Gómez, K. A., and Gómez, A. A. 1984. Statistical Procedures for Agricultural Research.
456 2nd ed. John Wiley & Sons, Inc., New York, USA.
457 Green, P. 2002. A revision of *Olea* L. (Oleaceae)., Kew Bull. 57:91-140.
458 Hamza, M. A., Ali, N., Tavoillot, J., Fossati-Gaschignard, O., Boubaker, H., El
459 Mousadik, A., and Mateille, T. 2017. Diversity of root-knot nematodes in Moroccan
460 olive nurseries and orchards: does *Meloidogyne javanica* disperse according to
461 invasion processes? BMC Ecol. 17:41. doi: 10.1186/s12898-017-0153-9.

462 Hartman, K. M., and Sasser, J. N. 1985. Identification of *Meloidogyne* species on the
463 basis of differential host test and perineal pattern morphology. Pages 69-77 in: An
464 Advanced Treatise on *Meloidogyne*. Vol. II: Methodology. K. R. Barker, C. C. Carter,
465 and J. N. Sasser, eds. NCSU Graphics, Raleigh, USA.
466 Hussey, R. S., and Barker, K. R. 1973. A comparison of methods of collecting inocula of
467 *Meloidogyne* spp., including a new technique. Plant Dis. Rep. 57:1025-1028.
468 Jensen, W. A. 1962. Botanical Histochemistry. W.H. Freeman and Co., San Francisco
469 and London.
470 Koenning, S. R., Overstreet, C., Noling, J. W., Donald, P. A., Becker, J. O., and Fortnum,
471 B. A. 1999. Survey of crop losses in response to phytoparasitic nematodes in the
472 United States for 1994. J. Nematol. 31:587-618.
473 Lamberti, F., and Baines, R. C. 1969. Pathogenicity of four species of *Meloidogyne* on
474 three varieties of olive trees. J. Nematol. 1:111-115.
475 Lamberti, F., Sasanelli, N., D'Addabbo, T., Ambrico A., Ciccarese, F., and Schiavone,
476 D. 2001. Relationship between plant-parasitic nematodes and *Verticillium dahliae* on
477 olive [*Olea europaea* L.]. Nematol. Mediterr. 29:3-9.
478 León, L., de la Rosa, R., Velasco, L., and Belaj, A. 2018. Using wild olives in breeding
479 programs: implications on oil quality composition. Front. Plant Sci. 9:1-9.
480 Lespinasse, Y., Durel, C. E., Eskes, A., Esmenjaud, D., and Poessel, J. L. 2003.
481 Resistance to biotic stress in fruit trees. Acta Hortic. 622:303-315.
482 McKenry, M. V. 1994. Nematodes of olive. Pages 97-99 in: Olive Production Manual. L.
483 Ferguson, G. S. Sibett, and G. C. Martin, eds. University of California, Oakland,
484 Publ. 3353.
485 Minz, G. 1961. Additional hosts of the root-knot nematode, *Meloidogyne* spp. recorded
486 in Israel during 1958–1959. Ktavim 11:69-70

Nico, A. I., Jiménez-Díaz, R. M., and Castillo, P. 2003. Host suitability of the olive cultivars Arbequina and Picual for plant-parasitic nematodes. J. Nematol. 35:29-34.

Nico, A. I., Rapoport, H. F., Jiménez-Díaz, R. M., and Castillo, P. 2002. Incidence and population density of plant-parasitic nematodes associated with olive planting stocks at nurseries in southern Spain. Plant Dis. 86:1075-1079.

Palomares-Rius, J. E., Castillo, P., Trapero-Casas, J.L., and Jiménez-Díaz, R.M. 2016. Infection by *Meloidogyne javanica* does not breakdown resistance to the defoliating pathotype of *Verticillium dahliae* in selected clones of wild olive. Sci. Hortic. 199:149-157.

Pérez, B. A., Barreto, D., Docampo, D., Otero, L., Costilla, M., Roca, M., and Babbitt, S. 2001. Current status of the drying syndrome (seca) of olive trees in Argentina. (Abstr.) Phytopathology 91:S71.

Powers, T. O, and Harris, T. S. 1993. A polymerase chain reaction method for identification of five major *Meloidogyne* species. J. Nematol. 25:1-6.

Rallo, L., Caruso, T., Díez, C. M., and Campisi G. 2016. Olive Growing in a Time of Change: From Empiricism to Genomics. Pages 55-64 in: The Olive Tree Genome. Compendium of Plant Genomes. E. Rugini, L. Baldoni, R. Muleo, and L. Sebastiani, eds. Springer, Dordrecht, Netherlands.

Reeve, R. M. 1951. Histochemical tests for polyphenols in plant tissues. Stain Tech. 26:91-106.

Ruzin, S. E. 1999. Plant Microtechnique and Microscopy. Oxford University Press, New York.

Saeedizadeh, A., Kheiri, A., Okhovat, M., and Hoseininejad, A. 2003. Study on interaction between root-knot nematode *Meloidogyne javanica* and wilt fungus *Verticillium dahliae* on olive seedlings in greenhouse. Commun. Agric. Appl. Biol.

512 Sci. 68:139-143.
513 Sasanelli, N. 2009. Olive Nematodes and their control. Pages 275-315 in: Integrated
514 Management of Fruit Crops Nematodes. Integrated Management of Plant Pests and
515 Diseases, vol 4. A. Ciancio and K. Mukerji, eds. Springer, Dordrecht, Netherlands.
516 Sasanelli, N., D'Addabbo, T., and Moura Lemos, R. 2002. Influence of *Meloidogyne*
517 *javanica* on growth of olive cuttings in pots. Nematropica 32:59-63.
518 Sasanelli, N., Fontanazza, G., Lamberti, F., D'Addabbo, T., Patumi, M., and Vergari, G.
519 1997. Reaction of olive cultivars to *Meloidogyne* species. Nematol. Mediterr. 25:183-
520 190.
521 Saucet, S. B., Van Ghelder, C., Abad, P., Duval, H., and Esmenjaud, D. 2016. Resistance
522 to root-knot nematodes *Meloidogyne* spp. in woody plants. New Phytol. 211:41-56.
523 Singh, S. K., Hodda, M., and Ash, G. J., 2013. Plant-parasitic nematodes of potential
524 phytosanitary importance, their main hosts and reported yield losses. EPPO Bull.
525 43:334-374.
526 Tarjan, A. C. 1953. Geographic distribution of some *Meloidogyne* spp. in Israel. Plant
527 Dis. Rep. 37:315-316.
528 Trudgill, D. L., and Perry, J. N. 1994. Thermal time and ecological strategies - a unifying
529 hypothesis. Ann. Appl. Biol. 125:521-532.
530
531 Williamson, V.M., and Hussey, R.S. 1996. Nematodes pathogenesis and resistance in
532 plants. Plant Cell. 8: 1735–1745.
533 Yang, B., and Zhong, X. 1980. The identification of root-knot nematodes in *Olea*
534 *europaea* L. Sci. Silvae Sin. 16: 264-265.
535 Zijlstra, C., Donkers-Venne. D.T.H.M., and Fargette, M. 2000. Identification of
536 *Meloidogyne incognita, M. javanica* and *M. arenaria* using sequence characterised

537 amplified region (SCAR) based PCR assays. Nematology 2:847-53.

538

Table 1. Olive (*Olea europaea*) wild genotypes, related subspecies and cultivars included in the study with their respective passport code in the ex situ wild repository and World Olive Germplasm Collection of IFAPA, and geographic origin.

Plant material type	Passport code	Geographic Origin
Wild olive genotypes/related subspecies		
Olea europaea subsp. *cerasiformis*	W147	Portugal, Madeira
O. europaea subsp. *cuspidata*	W158	Ethiopia
O. europaea subsp. *europaea* var. *sylvestris*	W224	Morocco
O. europaea subsp. *europaea* var. *sylvestris*	W223	Morocco
O. europaea subsp. *europaea* var. *sylvestris*	W166	Spain, Extremadura
O. europaea subsp. *europaea* var. *sylvestris*	W19	Spain, Jaén
O. europaea subsp. *guanchica*	W33	Spain, Canary Islands
O. europaea subsp. *guanchica*	W46	Spain, Canary Islands
O. europaea subsp. *guanchica*	W1048	Spain, Canary Islands,
O. europaea subsp. *maroccana*	W215	Morocco
O. europaea subsp. *maroccana*	W228	Morocco
Cultivars (*O. europaea* subsp. *europaea* var. *europaea***)**		
Arbequina	231	Spain
Ayvalik	97	Turkey
Gordal Sevillana	234	Spain
Hojiblanca	v 2	Spain
Koroneiki	218	Greece
Lechín de Sevilla	5	Spain
Manzanilla de Sevilla	21	Spain
Picual	9	Spain
Sikitita	1920	Spain

543 **Table 2.** Host-suitability of wild olive (*Olea europaea*) genotypes (including subspecies other than *europaea*, and var. *sylvestris* of subsp.
544 *europaea* with their respective passport code in the ex situ wild repository and World Olive Germplasm Collection of IFAPA) to *Meloidogyne*
545 *javanica* and effects of root growth[a].

			Variables[b]		
Host genotype	**Passport code**	**Inoculation treatment**	**Root fresh weight (g)**	**Root symptoms[c]**	**Rf[d]**
Olea europaea subsp. *cerasiformis*	W147	Uninoculated control	23.43±5.08 CD	-	-
		M. javanica	18.31±7.02 F	0.5±0.0000 G	0.0003±0.0005F
O. europaea subsp. *cuspidata*	W158	Uninoculated control	69.80±1.98 A	-	
		M. javanica	92.68±15.17 A	4.13±0.44 A	20.64±9.12 A
O. europaea subsp. *europaea.* var. *sylvestris*	W224	Uninoculated control	26.10±3.88 BCD	-	-
		M. javanica	32.77±7.10 CD	0.60±0.21 G	0.79±0.43 E
O. europaea subsp. *europaea.* var. *sylvestris*	W223	Uninoculated control	30.48±2.18 B	-	-
		M. javanica	40.01±11.30 BC	1.08±0.34 F	3.98±2.27 D
O. europaea subsp. *europaea.* var. *sylvestris*	W166	Uninoculated control	81.50±21.38 a A	-	-
		M. javanica	49.40±19.01 b B	2.34±0.60 CD	7.45±2.24 BC
O. europaea subsp. *europaea.* var. *sylvestris*	W19	Uninoculated control	25.93±6.46 BCD	-	-
		M. javanica	25.09±8.04 E	1.73±0.47 E	3.33±1.14 CD
O. europaea subsp. *guanchica*	W33	Uninoculated control	19.55±3.89 D	-	-
		M. javanica	24.58±12.63 E	1.28±0.55 F	3.78±2.29 D
O. europaea subsp. *guanchica*	W46	Uninoculated control	11.90±0.36 E		
		M. javanica	12.36±3.86 G	3.15±1.11 B	3.43±0.86 CD
O. europaea subsp. *guanchica*	W1048	Uninoculated control	11.67±1.24 E		
		M. javanica	12.36±3.86 G	3.15±1.11 B	3.43±0.86 BCD
O. europaea subsp. *maroccana*	W215	Uninoculated control	26.78±3.99 BCD		
		M. javanica	26.86±9.69 DE	2.68±0.59 BC	12.60±8.57 AB
O. europaea subsp. *maroccana*	W228	Uninoculated control	28.90±5.66 BC		

	M. javanica	24.21±14.28 EF	2.28±1.35 DE	10.80±10.17 ABC

[a] Data are the mean of 16 replicated plants per treatment combination from two replicated experiments. Inoculated plants received 10,000 eggs + J2 (Pi) of *M. javanica* while uninoculated plants did not receive nematodes. For each *O. europaea* genotype lowercase letters refers to differences between uninoculated and inoculated treatments within each genotype and are only shown when differences are significant ($P \geq 0.05$) according to LSD test. Upper case letters refer to comparisons of all means of the same treatment (either *M. javanica* inoculated or un-inoculated control) among the different genotypes. Means followed by the same upper case letter do not differ significantly ($P \geq 0.05$) according to LSD test.

[b] Average percentage and standard deviation of each variable during the experiment.

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

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

559 **Table 3.** Host-suitability of several olive (*Olea europaea*) commercial cultivars to *Meloidogyne javanica*) and effects of root growth[a]

			Variables[b]		
Host genotype	**Cultivar**	**Inoculation treatment**	**Root fresh weight (g)**	**Root symptoms[c]**	**Rf[d]**
O. europaea subsp. *europaea.* var. *europaea*	Arbequina	Uninoculated control	3.08±0.71CD	-	-
		M. javanica	3.91±1.36 E	1.75±0.72 B	2.40±2.89 B
O. europaea subsp. *europaea.* var. *europaea*	Ayvalik	Uninoculated control	38.23±3.68 A	-	-
		M. javanica	38.33±12.29 A	0.95±0.16 C	2.61±1.56 B
O. europaea subsp. *europaea.* var. *europaea*	Gordal Sevillana	Uninoculated control	5.08±1.28 a BC	-	-
		M. javanica	3.05±1.30 b DE	2.83±0.38 A	7.11±8.99 A
O. europaea subsp. *europaea.* var. *europaea*	Hojiblanca	Uninoculated control	3.05±0.66 a CD	-	-
		M. javanica	7.51±2.57 b B	3.00±0.84 A	7.26±5.22 A
O. europaea subsp. *europaea.* var. *europaea*	Koroneiki	Uninoculated control	3.20±1.37 CD	-	-
		M. javanica	2.85±1.00 E	2.08±0.63 B	2.17±1.93 B
O. europaea subsp. *europaea.* var. *europaea*	Lechín de Sevilla	Uninoculated control	2.15±1.08 D	-	-
		M. javanica	3.00±1.88 E	1.88±1.11 B	3.27±4.49B
O. europaea subsp. *europaea.* var. *europaea*	Manzanilla de Sevilla	Uninoculated control	2.35±0.73 a D	-	-
		M. javanica	5.63±1.38 b C	2.78±0.47 A	8.64±6.03 A
O. europaea subsp. *europaea.* var. *europaea*	Picual	Uninoculated control	7.40±6.10 a B	-	-
		M. javanica	3.91±1.36 b D	1.75±0.72 B	2.40±2.90 B
O. europaea subsp. *europaea. var*. *europaea*	Sikitita	Uninoculated control	6.93±2.52 B	-	
		M. javanica	8.33±5.84 B	1.75±0.80 B	3.33±3.74 B

560

561 [a] Data are the mean of 16 replicated plants per treatment combination from two replicated experiments. Inoculated plants received 10,000
562 eggs + J2 (Pi) of *M. javanica* while uninoculated plants did not receive nematodes. For each *O. europaea* genotype lowercase letters refers
563 to differences between uninoculated and inoculated treatments of that genotype and are only shown when differences are significant ($P <$
564 0.05) according to LSD test. Upper case letters refers to comparisons of all means of the same treatment (either *M. javanica* inoculated or un-

565 inoculated control) among the different genotypes. Means followed by the same upper-case letter do not differ significantly ($P \geq 0.05$)
566 according to LSD test.
567 [b] Average percentage and standard deviation of each variable during the experiment.
568 [c] Assessed on a 0 to 6 rating scale according to the number of root galls, where 0 = no galls; 1 = 1–10; 2 = 11–20; 3 = 21–40; 4 = 41–70; 5 =
569 71–90; and 6 ≥ 91 galls.
570 [d] Rf (nematode reproduction factor) = *Pf* (final nematode numbers per plant)/ *Pi* (initial nematode inoculum per plant).
571

572

573

574

575 **Figure legends**

576 **Fig. 1.** Root systems of olive (*Olea europaea* subsp. *europaea*. var. *europaea*) cv. 'Picual'
577 (**a**) and cv. 'Gordal Sevillana' **(b)** infected by *Meloidogyne javanica* showing typical
578 galls. (**c-f**) Details of severely nodulated roots in olive cv. Gordal Sevillana.

579 **Fig. 2.** Light micrographs of root cross-sections of wild olives (subspecies other than
580 *europaea*, and var. *sylvestris* of subsp. *europaea*) including the feeding site induced by
581 *Meloidogyne javanica*. **a-d**, *Olea europaea* subsp. *cuspidata* W158; **e-h**, *O. europaea*
582 subsp. *europaea* var. *sylvestris* W223; **i-l**, *O. europaea* subsp. *europaea* var. *sylvestris*
583 W166; **m-p**, *O. europaea* subsp. *guanchica* W33; **q-t**, *O. europaea* subsp. *guanchica*
584 W46; **u-x**, *O. europaea* subsp. *guanchica* W1048; **y-b'**, *O. europaea* subsp. *maroccana*
585 W215; and **c'-f'**, *O. europaea* subsp. *maroccana* W228. Abbreviations: **gc** = Giant cells
586 in a feeding site, (* = individual giant cell); **N** = nematode. Scale bars: 200 µm (a, b, e, f,
587 i, j, m, n, q, u, v, y, z, c' and d'), 100 µm (all the rest).

588 **Fig. 3.** Light micrographs of root cross sections including the feeding site induced by
589 *Meloidogyne javanica* in commercial olive cultivars (*Olea europaea* subsp. *europaea*.
590 var. *europaea*). **a-d**, cv. Arbequina; **e-h**, cv. Gordal Sevillana'; **i-l**, cv. Hojiblanca; **m-p**,
591 cv. Koroneiki; **q-t**, cv. Lechín de Sevilla, **u-x**, cv. Manzanilla de Sevilla; **y-b'**, cv. Picual,
592 **c'-f'**, cv. Sikitita. Abbreviations: **gc** = Giant cells in a feeding site, (* = individual giant
593 cell); **N** = nematode. Scale bars: 200 µm (a, b, e, i, j, m, q, r, u, y, z, c' and d'), 100 µm
594 (all the rest).

595 **Fig. 4.** Light micrographs of fuchsine acid stained roots (**a-k**) and root cross-sections (**l-**
596 **t**) of the wild olive genotype W147. **a**, Gall at 4 days after *Meloidogyne javanica*
597 inoculation (DAI); **b** and **c**, galls at 11 DAI; **d** and **e**, galls at 25 DAI; **f** and **g**, nematodes
598 at 25 DAI; **h** and **i**, galls at 70 DAI; **j**, female with eggs at 70 DAI, **h**, eggs at 70 DAI; **l-**

n, gall and root sections at 4 DAI; **o-t**, gall and root sections at 25 DAI. Abbreviations: **gc** = Giant cells in a feeding site, (* = individual giant cell); **N** = nematode. Scale bars: 100 µm (j), 200 µm (all the rest).