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Life Cycle of the Dagger Nematode *Xiphinema israeliae* and the Host Suitability of Olive and Fig for *X. israeliae* and *X. italiae*

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Abstract: *Xiphinema israeliae* has been reported in the rhizosphere of olives in Crete, Greece. Attempts were made to culture this nematode in pots planted with olive and fig seedlings, using *Xiphinema index* as a control. In these conditions, *X. index* showed a high reproduction rate on fig in a few months and none on olive. The experiments with *X. israeliae* indicated that olive and fig are suitable hosts for this dagger nematode, since juveniles of various life stages were found in plants inoculated exclusively with females, although the rate of nematode reproduction was low. *Xiphinema israeliae* was proved to have a parthenogenetic reproduction and a long life cycle, from female to female, taking more than nine months at a 24–26 °C temperature to complete. Therefore, a quite long period, even a few years, may be necessary to obtain a high number of nematodes in pots under experimental conditions. In contrast, *Xiphinema italiae* did not reproduce on olive and fig after a seven-month period. Accordingly, to our knowledge, this study increases the host range and knowledge about the culturing of these species, as only seven species of *Xiphinema* have been successfully cultured in pots till now. The potential of fig and olive for culturing *X. israeliae* gives an opportunity for further studies of its biology and host range.

Keywords: ecology; plant-parasitic nematode; Mediterranean; life cycle

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1. Introduction

The fitness of a plant as a host for plant-parasitic nematode species can be defined as its capacity to support the nematode's feeding and reproduction on it. Host suitability may be stated objectively as the ratio of the number of nematode units recovered at the end of a nematode-infection assay (final nematode population density (Pf)) to the number of nematode units used to inoculate a plant (initial population density (Pi)) [1]. *Xiphinema israeliae* (Luc, Brown and Cohn, 1982) [2] was firstly described in Israel in the rhizosphere of *Citrus* sp. and golf greens with the description based exclusively on morphometric characteristics [2]. The species in Israel had been earlier misidentified as *X. diversicaudatum* from populations found in avocado and grapevines, indicating potential hosts for *X. israeliae*. In greenhouse studies, the nematode multiplied slightly on grapevine and citrus, but the life cycle was not completed during a nine-month period, probably due to unfavorable culturing conditions [2].

Recently, *X. israeliae* was found in the rhizosphere of cultivated and wild olives in Crete, Greece. This was the first report of the nematode outside the area of its original description, and its molecular profile (ribosomal and mitochondrial) was defined [3]. Males were found only in one case of cultivated olive [3]. Further samplings in Crete indicated its presence in 7.5% of cultivated olive (out of 146 samples) and 2.8% of wild olive (out of 36 samples) [4]. Additionally, *Xiphinema italiae* is another dagger nematode species, which is quite common in the Mediterranean area [5]. In Crete, it was found present in cultivated (7.5%) and wild (2.8%) olives out of 146 and 36 samples, respectively, as stated before for *X. israeliae* [4].

The problems and difficulties of rearing *Xiphinema* and *Longidorus* nematodes under artificial culture conditions were addressed 56 years ago [6]. This inability to culture nematodes successfully in pots is a major limiting factor in conducting studies of nematode biology and host suitability. For the majority of longidorid nematodes, the host range is based on their occurrence in the rhizosphere of certain plants only [6]. In the work described herein, several experiments were conducted with *X. israeliae* and one experiment with *X. italiae* to determine the host suitability of these nematodes for reproducing in olive and fig plants grown in pots. *X. israeliae* has a similar size to *X. index* and probably a similar ability to thrive in fine sand. Since the substrate used in pots in the current work had previously been found favorable for the development of *X. index*, *X. index* was used as a control. Fig (*Ficus carica* L.) is a common tree in the Mediterranean area and a good host for *X. index* [7,8]. Therefore, *X. index* was used as a positive control for the experiments on fig and as a negative one for the experiments on olive, which is a poor host [7].

2. Materials and Methods

2.1. Substrates, Planting Material and Growing Conditions

The pots (250 mL and 1.5 L) were filled with fine sand, coarse sand and gravel. Fine sand, commercially available for construction, originated from a quarry located 0.5 km from the seaside. Coarse sand (with sizes >500 μm and <2 mm) and gravel (with sizes >2 mm and <8 mm) were collected from the beach, sieved, thoroughly washed with running tap water and dried. To improve drainage, a thin layer of gravel was placed at the bottom of the 1.5 L pot and three further thin layers alternating with thick layers of fine sand in the form of "sandwich". Similarly, coarse sand was used at the bottom and between the fine sand of the smaller pots. Fine sand was used as the top layer in all the pots to monitor their irrigation needs.

Olive plants cv. Koroneiki, derived from rooted cuttings, were obtained from a nursery. Fig plants were either raised from seeds [7,8] or from cuttings. Seeds were collected from mature fig fruits, dried at room temperature and germinated in fine sand in a growth room (Figure 1a). Fig cuttings were taken from a single fig tree at the end of winter and rooted in the following spring.

The plants (olives and fig seedlings) grew in a growth room with artificial light and a 16 h photoperiod (Figure 1c). The temperature in the growth room was regulated to a minimum of 20 °C and a maximum of 28 °C with a heater and a fan from December 2019 until July 2020, and thereafter, until December 2023 when the study was finalized, it was adjusted constantly to 24–26 °C with an air conditioner. The figs originating from rooted cuttings grew outdoors in the shade of a shelter to protect them from direct sunlight.

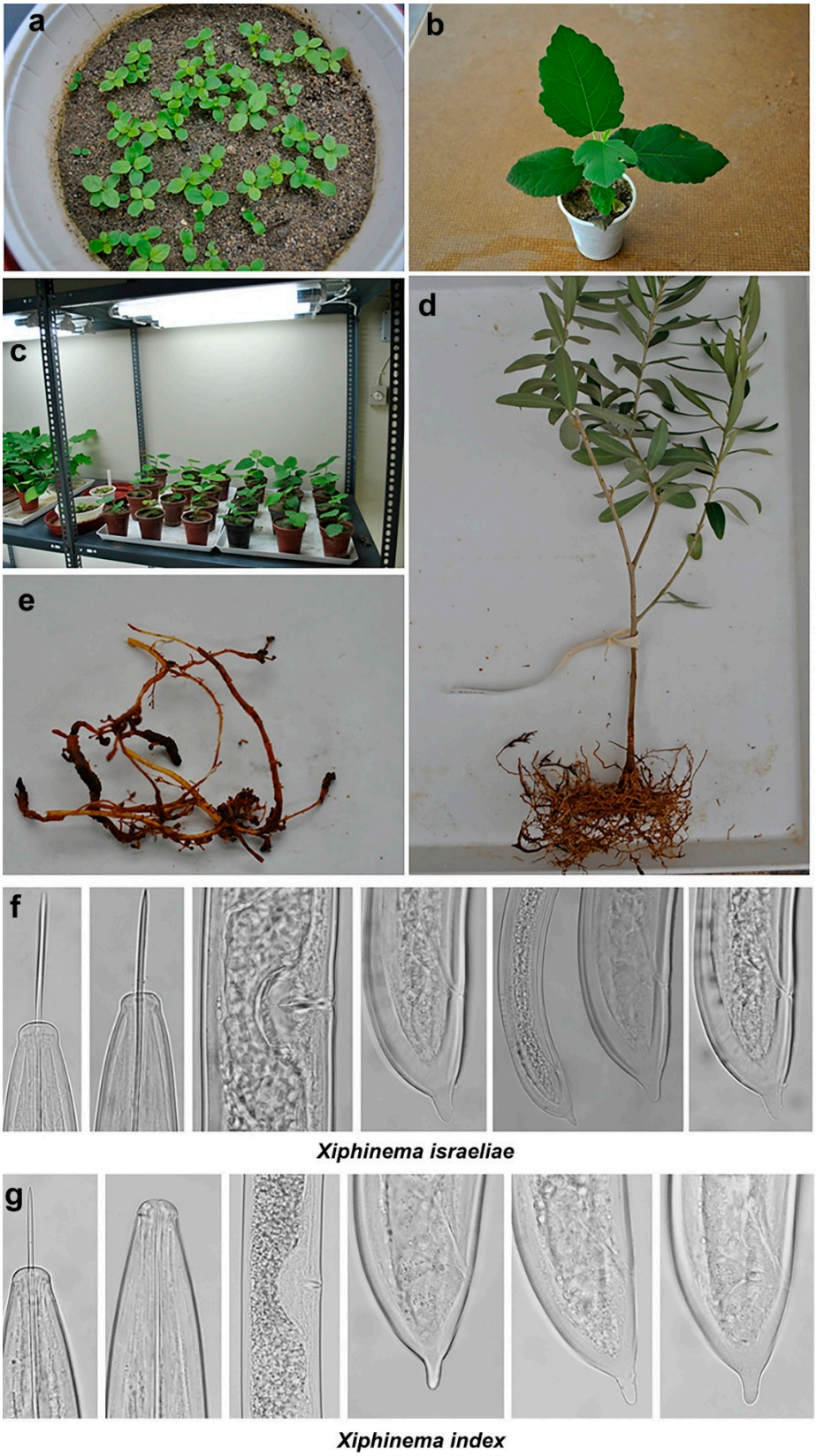

Figure 1. (**a**) Germinated seeds of fig. (**b**) Fig seedling in 100 mL cup. (**c**) Fig plants in growth room. (**d**) Olive plant cv Koroneiki after uprooting. (**e**) Root galls in fig roots caused by the feeding of *X. index*. Photomicrographs of females of (**f**) *Xiphinema israeliae* and (**g**) *Xiphinema index*, details of lip region, vulval and tail regions showing details of the mucro.

2.2. Nematodes

The identity of all the nematode species populations was identified by integrative taxonomy using molecular (ribosomal and mitochondrial) markers and morphological characteristics (Figure 1f,g) as described previously [3].

Three nematode populations of *X. israeliae* were used: (1) *X. israeliae* population 138 collected from a very old olive tree in an abandoned orchard, in the south of the province of Heraklion, Crete (OLI 138, Pyrgiotissa) [4] (the soil was extremely stony and dry, and sampling was carried from December 2019 to April 2021); (2) *X. israeliae* population 94 collected from an old olive tree in a cultivated orchard, in the south of the province of Lasithi, Crete (OLI 94 Kentri) [4], in December 2021 and March and October 2022; and (3) *X. israeliae* population 95 collected from nearby to the previous olive orchard (OLI 95 Kentri) [4] in December 2021 and October 2022. The *Xiphinema index* that was used in the experiments as control was extracted from fig plants (pot culture) and was originally obtained from a grapevine in Crete.

The *X. italiae* originated from a grapevine in Crete, and the extracted specimens were used immediately for inoculation.

The nematodes were extracted from field samples through sieves and separated from debris through a modified extraction dish lined with a 95 μm net [9]. Nematodes collected in the dish after 24–48 h were used for plant inoculation. These were selected under a dissecting microscope, based on their activity and vitality, and immediately inoculated into plants. In the case of females, only non-gravid females were selected. At the end of each experiment, the plants were uprooted (Figure 1d), and the whole substrate of each pot was checked for the presence of nematodes as described before. The extraction dish was checked daily till nematode recovery ceased, and the total number of nematodes per pot was determined. The reproduction factor (Rf) was estimated by dividing the final population (Pf) with the initial one (Pi), excluding weak specimens and those with a translucent body, suspected of being the original, almost dead, inoculated nematodes. According to the nematode life stages recovered, the biology was characterized as following: (a) Complete life cycle, if the number of females in the final population was higher to the initially inoculated one. (b) Egg laying and development, when different juvenile life stages were observed (the juvenile life stages were identified by their total length and the lengths of the functional and replacement odontostyle [2]). (c) Survival, if active females were found in numbers lower than the initial population used for inoculation. (d) Failure, when no nematodes were found at the end of the experiment.

2.3. Preliminary Experiment with Xiphinema israeliae and Xiphinema index in Cups Planted with Fig in Growth Room

Plastic cups of 100 mL (28 in total) filled with alternate layers of coarse and fine sand, containing a single fig seedling (Figure 1b), were inoculated with 1–5 specimens (females or juveniles) of *X. index* (20 cups) and 2–10 specimens (females or juveniles or both in equal numbers) of *X. israeliae* (8 cups). They were slightly watered with a washing bottle when the plants started indicating wilting symptoms and maintained in the growth room for five months (20–28 °C). Specimens of *X. israeliae* from population 138 were used for inoculations. Nematodes were extracted after a five-month period as described above and counted, with the aim of investigating the ability of the nematode to develop and reproduce on fig.

2.4. Experiments with Olive and Fig Seedlings in Pots in Growth Room

2.4.1. *Xiphinema israeliae*

Four 1.5 L plastic pots were filled with alternating layers of the field soil (population 138, collected in December 2019) and thin layers of gravel to ensure sufficient drainage.

The field soil had been previously sieved through an 8 mm sieve to remove stones. Based on the nematode density in field soil, it is estimated that in each pot, 30–40 nematode specimens (*ca.*10–15 females and 15–30 juveniles) were present. An olive plant was planted in each pot, and the plants grew in the growth room for six, eight or 10 months (20–28 °C).

One plastic pot of 1.5 L, filled with alternate layers of fine sand and thin layers of gravel and planted with olive, was inoculated with 40 specimens (10 females and 30 juveniles) and another one with seven specimens (3 females and 4 juveniles) extracted from the field soil (population 138) and left to grow for 10 or 11 months (20–28 °C).

Further inoculations were carried out at different dates on olives grown in 1.5 L pots, in the period 2020–2021, when adult nematodes from samplings were available (Table 1); females only were exclusively used for inoculation, as males were not found in any sampling.

Table 1. Reproduction factor (Rf) values of *Xiphinema israeliae* population 138 originating from the field and developing in pots planted with olive and fig kept in a growth chamber.

***X. israeliae* Population 138**												
	Nematodes from Field (5 Pots for Olive, 12 Pots for Fig)								**Nematodes from Pot Culture (8 Pots for Olive, 3 Pots for Fig)**			
	Complete Life Cycle		**Egg Laying, Development**		**Survival**		**Failure**		**Complete Life Cycle**		**Egg Laying, Development**	
	Olive	Fig	Olive	Fig	Olive	Fig	Olive	Fig	Olive	Fig	Olive	Fig
Pots [a]	1/5	3/12	2/5	6/12	1/5	1/12	1/5	2/12	2/8	2/3	6/8	1/3
Months	12	9–10	12	4–12	12	7	12	7–12	12	12	10–12	12
Pi	10	5–15	10	10	5	10	10	3–10	15	9–18	6–25	4
Rf	23.1	4–8.5	0.5–1	0.7–6	0.4	0.1	-	-	5.3–11.5	0.8–6.4	0.7–4	0.5
Pf females	52	6–20	2–4	1–5	2	1	-	-	21–35	10–41	3–11	0

[a] Pots: number of pots/total number of pots; Pi: initial nematode population per pot by females; Pf: final nematode population (females and juveniles) per pot excluding number of weak and with translucent body females; Rf = Pf/Pi: reproduction factor; Pf females: final population of females per pot indicating completion of life cycle if Pf females > Pi. Two values separated by dash indicate range of minimum and maximum values.

Fig plants grown in 250 mL or 1.5 L pots were inoculated exclusively with females. Based on the results of the olives grown in pots filled with field soil, the olives were uprooted after a period of 12 months (24–26 °C), while the figs were uprooted after a period of 4–12 months. The figs that grew for a 4–6-month period had been planted in 250 mL pots (20–28 °C), while those that grew for longer periods were in 1.5 L pots (24–26 °C).

The inoculations on olive and fig with the three nematode populations, originating from the field, were as follows: (a) population 138 used to inoculate five olives and twelve figs, (b) population 94 used to inoculate five olives and four figs and (c) population 95 used to inoculate three olives and four figs.

To confirm that the females that were laid and developed in pots were able to reproduce, extracted females from the pot substrate from some of the previous pots were inoculated again on olive and fig plants and monitored as described before. The inoculations on olive and fig seedlings, with females of the three nematode populations originating from pots, were as follows: (a) population 138 used to inoculate eight olives and three fig seedlings, (b) population 94 used to inoculate one olive and (c) population 95 used to inoculate one olive and one fig.

2.4.2. *Xiphinema index*

Three pots of 1.5 L, filled with alternating layers of fine sand and gravel and planted with olive, were inoculated with ten females of *X. index* and grew for eight to ten months (24–26 °C). Four further olives were inoculated with ten females of *X. index* each and grew for one year (24–26 °C). Plastic pots of 250 mL, filled with alternating layers of fine sand and

coarse sand and planted with fig seedlings, were inoculated with *X. index* as follows: Two pots were inoculated with 10 females of *X. index* and grew for four months (24–26 °C), and one pot was inoculated with 15 females of *X. index* and grew for four months (24–26 °C). Two pots of 1.5 L planted with fig seedlings were inoculated with three females of *X. index* each and grew for 12 months (24–26 °C).

2.4.3. *Xiphinema italiae*

Olive and fig seedlings in 1.5 L pots (five per each plant species) were inoculated with 20 females of *X. italiae* and grew for seven months (24–26 °C).

2.5. Experiments with Xiphinema israeliae and Xiphinema index in Pots with Fig Outdoors

Figs originating from rooted cuttings and planted in 1.5 L plastic pots growing outdoors, under a shelter, were inoculated with 20 females (three pots) and 20 juveniles (one pot) of *X. israeliae*, from population 138, which was collected from the field in March 2020. Four pots were each inoculated with 20 females of *X. index*. The plants were uprooted after six- and seven-month periods (March–November 2020) from nematode inoculation.

3. Results

3.1. Preliminary Experiment with Xiphinema israeliae and Xiphinema index in Cups Planted with Fig in a Growth Chamber

Of the 28 plants in the 100 mL plastic cups, 23 survived (18 inoculated with *X. index* and 5 inoculated with *X. israeliae*) and were checked for the presence of nematodes after five months of incubation. In the case of *X. index*, nematodes were found only in one plant inoculated with one female, where 17 juveniles were found (first, second and third life stages), indicating that the female laid eggs and the juveniles were able to molt. In the case of *X. israeliae*, nematodes were found in three plants. In one plant inoculated with five females, there was found only one female, which had a translucent body and moved sluggishly and was probably one of the originally inoculated females. In another plant inoculated with three females and three juveniles, two active females were found after five months, which were either those original inoculated or derived from the juveniles. Finally, in the third plant inoculated with 10 females, 27 juveniles (of three different stages) and 3 active females were found. This indicates that fig is a suitable host for *X. israeliae*, since the females laid eggs and the juveniles were able to develop and molt. However, it is not known whether the females were newly developed inside the pot or correspond with those originally inoculated.

3.2. Experiments with Olive and Fig in Pots in a Growth Chamber

3.2.1. *Xiphinema israeliae*

Olive

Xiphinema israeliae population 138

Two olives that grew in pots filled with field soil (ca. 10–15 females and 15–30 juveniles per pot) were uprooted after six months. The Rf ranged from 0.6 to 1.0 (9–10 females and 17–22 juveniles), indicating that the nematode density did not change significantly. One pot was checked after eight months, and the Rf ranged from 2.1 to 2.8 (28 females and 57 juveniles), but based on the number of females, it cannot be proved that the nematode completed a life cycle. However, the increase in the number of females indicates that juveniles molted. From the last pot, which was checked after ten months, the Rf was estimated as from 11.2 to 14.9 (70 females and 377 juveniles). Since in this case the number of females exceeded the initial population, composed by females and juveniles, it is proved

that the nematode successfully completed a life cycle in a period more than eight and less than ten months in these experimental conditions.

When nematode inoculation was performed with both females and juveniles in two pots, it was considered that a life cycle was completed when the number of females in the final population exceeded the total number of the initial population composed of females and juveniles. From the two pots inoculated with females and juveniles, the results were as follows: pot (1), where the Pi was composed of 10 females and 30 juveniles, the Pf was 16 females and 24 juveniles, which is an indication of nematode juvenile development to females, since the number of females in Pf exceeds the number of females in Pi in a period of ten months; pot (2), where the Pi was composed of 3 females and 4 juveniles, the Pf was found to be 10 females and 21 juveniles, indicating that the nematodes also completed a life cycle in a period of 11 months.

The results from the nematode inoculation of olives with females originating from the field and from pots are presented in Table 1. In the five pots inoculated with nematodes originating from the field, in a rate of 5–10 female nematodes per pot, the results were as follows: (a) in one case the nematode completed a whole life cycle (Rf = 23.1) in a one year period; (b) in two cases, egg laying and nematode development occurred (Rf = 0.5–1), since at least one juvenile and various juvenile life stages were found; (c) in one case, two active females were found out of the five inoculated, indicating a one year period of nematode survival; and (d) in one case no nematodes were found.

In the case of the eight pots inoculated with females originating from pots, in a rate of 6–25 females per pot, the results were as follows: (a) in two cases, the nematode completed a whole life cycle (Rf = 5.3–11.5) in a one-year period; (b) in six cases, egg laying and nematode development occurred (Rf = 0.7–4), since juveniles of various stages were found.

Xiphinema israeliae population 94

Results are presented in Table 2. For nematodes originating from the field and inoculated at a rate of 10–50 females per pot, out of the five pots the results were as follows: (a) in four cases egg, laying and nematode development occurred (Rf = 0.2–1), since juveniles of various life stages were found; (b) in one, case five active females, out of the ten originally inoculated, were found, indicating a one-year period of nematode survival.

Table 2. Reproduction factor (Rf) values of *Xiphinema israeliae* population 94 originating from the field and developing in pots planted with olive and fig kept in a growth chamber.

	***X. israeliae* Population 94**			
	Nematodes from Field (5 Pots for Olive, 4 Pots for Fig)			**Nematodes from Pot Culture (1 Pot for Olive)**
	Egg Laying, Development		**Survival**	**Complete Life Cycle**
	Olive	Fig	Olive	Olive
Pots [a]	4/5	4/4	1/5	1/1
Months	12	12	12	12
Pi	10–50	14–50	10	2
Rf	0.2–1	0.07–2	0.5	3
Pf females	1–27	6–43	5	5

[a] Pots: number of pots/total number of pots; Pi: initial nematode population per pot by females; Pf: final nematode population (females and juveniles) per pot excluding number of weak and with translucent body females; Rf = Pf/Pi: reproduction factor; Pf females: final population of females per pot indicating completion of life cycle if Pf females > Pi. Two values separated by dash indicate range of minimum and maximum values.

One pot was inoculated with two females originating from pots, and a life cycle was completed (Rf = 3) within a period of one year.

Xiphinema israeliae population 95

Results are presented in Table 3. For nematodes originating from the field and inoculated at a rate of 25–30 females per pot, out of three pots the results were as follows: (a) in one case, the nematode completed a whole life cycle (Rf = 8.4) in a one-year period; (b) in one case, egg laying and nematode development occurred (Rf = 0.5), since juveniles of various life stages were found; (c) in one case, 16 active females, out of the 25 originally inoculated, were found, indicating a one-year period of nematode survival. One pot was inoculated with 18 females originating from pots, and a life cycle was completed (Rf = 4.0) within a period of one year.

Table 3. Reproduction factor (Rf) values of *Xiphinema israeliae* population 95 originating from the field and developing in pots planted with olive and fig kept in a growth chamber.

***X. israeliae* Population 95**						
	Nematodes from Field (3 Pots for Olive, 4 Pots for Fig)				**Nematodes from Pot Culture (1 Pot for Olive, 1 Pot for Fig)**	
	Complete Life Cycle	**Egg Laying, Development**		**Survival**	**Complete Life Cycle**	
	Olive	Olive	Fig	Olive	Olive	Fig
Pots [a]	1/3	1/3	4/4	1/3	1/1	1/1
Months	12	12	12	12	12	12
Pi	30	25	7–30	25	18	8
Rf	8.4	0.5	0.4–2.3	0.6	4	8.8
Pf females	32	5	6–20	16	45	21

[a] Pots: number of pots/total number of pots; Pi: initial nematode population per pot by females; Pf: final nematode population (females and juveniles) per pot excluding number of weak and with translucent body females; Rf = Pf/Pi: reproduction factor; Pf females: final population of females per pot indicating completion of life cycle if Pf females > Pi. Two values separated by dash indicate range of minimum and maximum values.

Fig

Xiphinema israeliae population 138

The results from the inoculation of fig with females originating from the field and from pots are presented in Table 1. In the case of the 12 pots inoculated with females originating from the field, with a rate of 5–15 females per pot, the results were as follows: (a) in three cases, the nematode completed a whole life cycle (Rf = 4.0–8.5) in a 9–10 month period; (b) in six cases, egg laying and nematode development occurred, since juveniles of various life stages were found in a period of 4–6 months and in a period of 12 months (Rf = 0.7–6.0); (c) in one case, one active female, out of the ten originally inoculated, was found, indicating a 7-month period of nematode survival; (d) in two cases, no nematodes were found after periods of 7 and 12 months.

In the case of the three pots inoculated with nematodes originating from pots, in a rate of 4–18 females per pot, the results were as follows: (a) in two cases, the nematode completed a whole life cycle (Rf = 0.8–6.4) in a one-year period; (b) in one case, egg laying and nematode development occurred (Rf = 0.5) since juveniles of various life stages were found.

Xiphinema israeliae population 94

Results are presented in Table 2. For nematodes originating from the field and inoculated at a rate of 14–50 females per pot, in all the four pots egg laying and nematode development occurred (Rf = 0.07–2.0), since at least one juvenile and juveniles of various life stages were found.

Xiphinema israeliae population 95

Results are presented in Table 3. For nematodes originating from the field and inoculated at a rate of 7–30 females per pot, in all the four pots egg laying and nematode

development occurred (Rf = 0.4–2.3), since juveniles of various life stages were found. One pot was inoculated with eight females originating from pots, and a life cycle was completed (Rf = 8.8) within a period of one year.

3.2.2. *Xiphinema index*

Olive

From the seven pots inoculated with ten females of *X. index* each and checked after a 8–12 month period, active nematodes were recovered from four pots as follows: (a) four females in one pot after eight months; (b) one female in one pot after nine months; (c) one female and two juveniles of different life stages (Rf = 0.3) in one pot after ten months; (d) one pot with one female after one year; and (e) three pots without the presence of nematodes after one year.

Fig

The nematodes reproduced successfully and completed a life cycle within a four-month period with an Rf = 3.8 in one pot inoculated with ten females. In the pot inoculated with 15 females, a life cycle was also completed with an Rf = 58 in a four-month period. In the two pots that were inoculated with three females and uprooted after one year, the Rf values were quite high (217 and 411). In all cases, terminal root galls caused by the feeding of *X. index* were observed (Figure 1e).

3.2.3. *Xiphinema italiae*

Olive

Two females per pot were recovered from four out of the five pots inoculated with 20 females each.

Fig

Two females were found in three pots, one female in one pot and six females in one pot out of the five pots inoculated with twenty females each.

3.3. Experiments with Xiphinema israeliae and Xiphinema index in Pots with Fig Outdoors

Two fig plants inoculated with *X. israeliae* were uprooted after six months (March–September 2020). From the one inoculated with 20 juveniles, only 1 active juvenile was found, while from the one inoculated with 20 females no active specimens were found. Two other pots that were inoculated with twenty females were uprooted after seven months; in one pot two active females and juveniles of all life stages were found, while in the other pot three active females and seven active juveniles of all life-stages were found, indicating nematode reproduction and development in both cases. In contrast, *X. index* increased in the four pots after six and seven months, respectively, with an Rf ranging from 11.5 to 22.5, and it completed a life cycle (45–130 females).

4. Discussion

Host suitability to plant-parasitic nematodes can be evaluated by determining their reproduction on plants after experimental inoculations [1]. The reproduction factor (Rf) has been widely used in nematological studies to define the resistance and susceptibility of plants to a nematode [10]. When a compatible host–parasite interaction has been established by a nematode, infection of the plant can be followed by the reproduction of the nematode species [11]. The life cycle and host range of *Xiphinema israeliae* are fairly unknown. Attempts to culture this nematode on grapevine and citrus in a greenhouse environment indicated that the nematode multiplied slightly, but after nine months the

life cycle was not completed [2]. There are no details on the culturing conditions, but the authors characterize both host plants as "unfavorable" [2].

The work described herein was an attempt to investigate whether this nematode can be reproduced on olive and fig seedlings in pots, under controlled conditions. The main difficulty was to obtain a sufficient number of active and vital non-gravid females from the field for inoculating plants in pots. For that, several samplings were conducted. Therefore, plants were inoculated whenever a limited nematode inoculum was available.

The culturing of *X. index* in quite small pots with a good host (fig and grapevine) and inoculated with single females is feasible but with variations in the survival and reproduction rate; some females do not survive, and these which are able to reproduce vary in the number of progenies they lay [8,12,13]. In our work, in an attempt to culture *X. index* and *X. israeliae* in 100 mL plastic cups, planted with fig seedlings, a high percentage of plants survived. However, the ability of *X. index* to reproduce was quite low; only in 1 out of 18 plants were juvenile life stages found, despite each plant being inoculated with 4–5 specimens. For *X. israeliae*, the females laid eggs and juveniles developed only in one out of five pots.

Also, in the *X. israeliae* population 138 experiment on olive grown in field soil, it is not known whether the field soil contained eggs, and therefore, the initial population of 30–40 nematode specimens (10–15 females and 15–30 juveniles) per pot may have been underestimated. However, even in this case, egg hatching would influence the number of juveniles recovered and not of females within the ten months period, when the plant was uprooted. Since the number of females was 4.7–7.0-fold that of the initial nematode population, there is strong evidence that the nematodes completed a whole life cycle.

The experiments in 1.5 L pots proved that *X. israeliae* has a parthenogenetic reproduction. In the original description of *X. israeliae* [2], the presence of males was mentioned. However, in the 12 populations which were found in wild and cultivated olives in Crete, Greece, males were found only in one case [3,4]. The three populations used in this study came from areas where males were not found in earlier and in recent samplings. Furthermore, this study indicates that the nematode has a long life cycle, which may take at least ten months in olive and fig, in temperatures of 20–28 °C and 24–26 °C. Summing up the results of the three *X. israeliae* populations tested in the growth room, in 19 out of the 23 pots with olive and in 21 out of the 24 pots with fig, the nematode reproduced and juvenile life stages developed, indicating that both plants are potential suitable hosts for *X. israeliae*. The life cycle was completed in six cases for olive and fig and took more than nine months. However, the nematode reproduction was quite low. The reasons for the slow increase in the nematode population may be attributed either to the culturing conditions (e.g., the fine sand in pots may not provide a favorable environment) or to the nematode biology (e.g., the nematodes may have a low rate of egg laying). Moisture fluctuations and drenching with a nutrient solution have been found to affect the multiplication of longidoridae in pots [14]. A nutrient solution was not applied in any pot with olive and fig in our experiments. The temperature fluctuation in the growth chamber was low in most cases (24–26 °C), and that probably did not have any negative effect on nematode biology. In contrast, in the four figs that grew outdoors the temperature fluctuated, but despite that, signs for nematode development and reproduction were recorded. Another point that is demonstrated in this research is that *X. israeliae* females have a long living period, at least a one year survival. In 3 out of the 23 olives and in 1 out of the 24 figs, active females without juveniles were found, and these were probably the original females used for inoculation that survived and were active for 7–12 months.

In contrast with *X. israeliae*, *X. index* had a population decrease in olive, as has been also demonstrated [7]. Low reproduction rates (Rf = 0.4–1.2) have been recorded for *X. index* in

olive in pot tests, but even these do not demonstrate that olive is a good host [15]. The life cycle of *X. index* on fig seedlings was completed in less than sixteen weeks at 22 °C [7,12,16]. This agrees with our results, where *X. index* completed a life cycle in the growth chamber in less than a period of four months and outdoors under a shed in less than six months.

Xiphinema italiae did not reproduce on olive and fig as after seven months, no juvenile life-stages were found, and the original inoculated population of females decreased to 70–90%. For this nematode, there are only a few studies indicating that this species reproduces on grapevine in pots [6,17] and completes a complete life cycle in less than six months at 28 °C [6]. The presence of *X. italiae* in the rhizosphere of olives in Crete, Greece [3,4] and Spain [18] indicates that either it feeds and reproduces on weeds or occasionally on olive. Probably, the substrates used for the pots and the growing conditions (e.g., moisture) were not appropriate for the nematode's development and reproduction in olive in pots.

The culturing of longidorid nematodes in the lab is an invaluable and crucial resource for studies related to biology, molecular biology and virus transmission, since field sites with nematodes may be at an inconvenient distance or subjected to adverse weather conditions [19]. However, only a few species of *Xiphinema* have been successfully cultured in pots. At the Scottish Crop Research Institute, large containers of 200 l filled with alternate layers of washed coarse river sand and field soil kept at 20 °C in a greenhouse were used for culturing certain *Xiphinema* and *Longidorus* species by planting appropriate host plants [19]. With this method, only three virus vectors *Xiphinema* species, *X. index*, *X. diversicaudatum* and *X. vuittenezi*, have been successfully maintained [19]. Two of the *X. americanum*-group species from Senegal, a population of *X. ifacolum* from Sierra Leone and a population of *X. vulgare* from Florida have been successfully cultured in pots [20–22]. Accordingly, to our knowledge, there is not any report of any other species of *Xiphinema*, except these seven mentioned previously, that can be successfully cultured in pots.

Our results indicate that olive and fig are suitable hosts for culturing *X. israeliae*, although the rate of nematode reproduction was low. Specific root damage was not observed on either olive or fig in all cases of nematode development and reproduction. The nematode was found in the rhizosphere of olives in Crete [3,4], but it is not known whether it feeds preferentially and reproduces on herbaceous plants naturally occurring among olive trees. By ensuring a high population level of the nematode, after a long period (a few years) of growth in pots with olive and fig, sufficient vital specimens can be obtained for further studies on the host range of the nematode and the possibility of its ability for virus transmission.

5. Conclusions

In summary, the results obtained in this research indicated that olive and fig are suitable hosts for *X. israeliae*, since juveniles of various life stages were found in plants inoculated exclusively with females, although the rate of nematode reproduction was low. It was also proved that *X, israeliae* has a parthenogenetic reproduction and a long life cycle, from female to female, taking more than nine months at a 24–26 °C temperature to complete. *Xiphinema index,* which was used as a control, reproduced successfully in fig but not in olive. In contrast, *Xiphinema italiae* did not reproduce on olive and fig after a seven-month period. The potential of fig and olive to culture *X. israeliae* gives the opportunity for further studies of its biology and host range.

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References

1. Lewis, S.A. Nematode-plant compatibility. In *Vistas on Nematology*; Veech, J.A., Dickson, D.W., Eds.; The Society of Nematologists: Hyattsville, MD, USA, 1987; pp. 246–252.
2. Luc, M.; Brown, D.J.F.; Cohn, E. *Xiphinema israeliae* n. sp. (Nematoda: Dorylaimoidea). *Rev. Nematologie* **1982**, *5*, 233–239.
3. Tzortzakakis, E.A.; Archidona-Yuste, A.; Cantalapiedra-Navarrete, C.; Nasiou, E.; Lazanaki, M.S.; Kabourakis, E.M.; Palomares-Rius, J.E.; Castillo, P. Integrative diagnosis and molecular phylogeny of dagger and needle nematodes of olives and grapevines in the island of Crete, Greece, with description of *Xiphinema cretense* n. sp. (Nematoda, Longidoridae). *Eur. J. Plant Pathol.* **2014**, *140*, 563–590. [CrossRef]
4. Tzortzakakis, E.A.; Archidona-Yuste, A.; Cantalapiedra-Navarrete, C.; Birmpilis, I.G.; Nasiou, E.; Palomares-Rius, J.E.; Castillo, P. Description of the first-stage juveniles of *Xiphinema cretense* and *X. herakliense*—Distribution of *Xiphinema* and *Longidorus* species in olive orchards and grapevines in Crete, Greece. *Hell. Plant Prot. J.* **2016**, *9*, 73–77. [CrossRef]
5. Lamberti, F. Plant nematode problems in the Mediterranean region. *Helminthol. Abstarcts Ser. B Plant Nematol.* **1981**, *50*, 145–166.
6. Cohn, E.; Mordechai, M. Investigations on the life cycles and host preference of some species of *Xiphinema* and *Longidorus* under controlled conditions. *Nematologica* **1969**, *15*, 295–302.
7. Brown, D.J.F.; Coiro, M.I. The reproductive capacity and longevity of *Xiphinema index* (Nematoda: Dorylaimida) from three populations on selected host plants. *Rev. Nematologie* **1985**, *8*, 171–173.
8. Coiro, M.I.; Brown, D.J.F. The status of some plants as hosts of four populations of *Xiphinema index* (Nematoda: Dorylaimida). *Rev. Nematologie* **1984**, *7*, 283–286.
9. Brown, D.J.F.; Boag, B. An examination of methods used to extract virus-vector nematodes (Nematoda: Longidoridae and Trichodoridae) from soil samples. *Nematol. Mediterr.* **1988**, *16*, 93–99.
10. Pinochet, J.; Verdejo, S.; Marull, J. Host suitability of eight *Prunus* spp. and one *Pyrus communis* rootstocks to *Pratylenchus vulnus*, *P. neglectus*, and *P. thornei*. *J. Nematol.* **1991**, *23*, 570–575. [PubMed]
11. Nico, A.I.; Jiménez-Díaz, R.M.; Castillo, P. Host suitability of the olive cultivars Arbequina and Picual for plant-parasitic nematodes. *J. Nematol.* **2003**, *35*, 29–34. [PubMed]
12. Coiro, M.I.; Brown, D.J.F.; Lamberti, F. Reproduction of *Xiphinema index* (Nematoda: Dorylaimida) on five plant species. *Nematologica* **1990**, *36*, 474–483.
13. Coiro, N.I.; Taylor, C.E.; Lamberti, F. Reproduction of two populations of *Xiphinema index* in relation to host and temperature. *Nematol. Mediterr.* **1990**, *18*, 117–118.
14. Cohn, E.; Mordechai, M. The influence of some environmental and cultural conditions on rearing populations of *Xiphinema* and *Longidorus*. *Nematologica* **1970**, *16*, 85–93. [CrossRef]
15. Sasanelli, N.; Coiro, M.I.; D'Addabbo, T.; Lemos, R.J.; Ridolfi, M.; Lamberti, F. Reaction of an olive cultivar and an olive rootstock to *Xiphinema index*. *Nematol. Mediterr.* **1999**, *27*, 253–256.
16. Coiro, M.I.; Serino, M.; Agostinelli, A. Feeding and reproduction of *Xiphinema index* (Nematoda: Dorylaimida) on two hosts at three temeperatures. *Nematol. Mediterr.* **1991**, *19*, 101–102.
17. Cohn, E.; Tanne, E.; Nitzany, F.E. *Xiphinema italiae*, a New Vector of Grapevine Fanleaf Virus. *Phytopathology* **1970**, *60*, 181–182. [CrossRef]

18. Archidona-Yuste, A.; Navas-Cortés, J.A.; Cantalapiedra-Navarrete, C.; Palomares-Rius, J.E.; Castillo, P. Remarkable diversity and prevalence of dagger nematodes of the genus *Xiphinema* Cobb, 1913 (Nematoda: Longidoridae) in olives revealed by integrative approaches. *PLoS ONE* **2016**, *11*, e0165412. [CrossRef] [PubMed]
19. Brown, D.J.F.; Taylor, C.E. *Nematode Vectors of Plant Viruses*; CAB International, University Press: Cambridge, UK, 1997; p. 286.
20. Coiro, M.I.; Sassanelli, N.; Serino, M. Fecundity and longevity of individual *Xiphinema ifacolum* (Nematoda: Dorylaimidae) on tomato. *Nematologica* **1995**, *41*, 191–196. [CrossRef]
21. Coiro, M.; Lamberti, F.; Sassanelli, N.; Duncan, L.W.; Agostinelli, A. Reproduction and longevity of *Xiphinema vulgare* (Nematoda). *Nematol. Mediterr.* **2002**, *30*, 91–95.
22. Diop, M.T.; Dieme, J.-H.; Mounport, D.; Baujard, P. Laboratory culture of two *Xiphinema americanum*- group species (Nematoda: Longidoridae) from Senegal. *Nematology* **2001**, *3*, 411–415.