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Solarization of soil in piles for the control of *Meloidogyne incognita* in olive nurseries in southern Spain

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The potential of solarization to control *Meloidogyne incognita* in piles of soil used at olive nurseries in southern Spain was studied in 1999 and 2000. Kaolin and soil infested with free eggs and egg masses of the nematode in nylon bags were buried 20 and 40 cm deep inside conical piles of soil 80 cm high and with a base diameter of 1 m. Soil piles were solarized for 3 weeks in July and August. The effect of various periods of solarization was assessed by egg hatch bioassays in sterile water, and by infectivity to tomato plants. Maximum soil temperature at 20 cm depth in solarized piles was 47·4°C in 1999 and 48·2°C in 2000, compared with 32·9°C and 31·7°C in nonsolarized piles. Solarization reduced egg hatch by > 95% compared with nonsolarized samples, irrespective of type, burial depth and location of inocula in a soil pile. Egg hatch of egg mass-infested samples buried at 20 cm depth was higher than that of free eggs buried at the same depth. The differential effect associated with burial depth and type of inoculum was not found in solarized piles. In nonsolarized piles, hatch of free eggs from samples buried at 40 cm depth was higher than that from samples buried at 20 cm depth. Egg hatch in samples from solarized piles was lower than that from nonsolarized piles. A bioassay of tomato plants in 2000 confirmed the reduction in infectivity of free eggs buried in solarized soil piles. Under the conditions in southern Spain, solarization of 40 cm-high piles of soil for 3 weeks can therefore be used for the control of root-knot nematodes in potting soil for olive nursery production.

Keywords: control, egg hatch, egg masses, nematode reproduction, *Olea europaea*, root-knot nematodes

Introduction

The production of pathogen-free olive (*Olea europaea*) planting stock involves the use of plant material and soil mixtures free of pathogens for plant propagation. In Andalucia, southern Spain, the use of homogeneous planting stocks produced by rooting leafy olive stem cuttings in perlite under mist conditions (Caballero & del Río, 1999) has resulted in a steady increase in new olive orchards during the last decade. Andalucia now has more than $1{\cdot}3 \times 10^6$ ha of olive production (Barranco, 1999), making this region the main olive-producing area worldwide (FAO, 2002).

In Andalucia, commercial production of 8- to 10-month-old olive planting stocks is by perlite rooted stems in plastic bags with 2–3 L of field soil. The soil originates from alluvial sandy or loamy cultivated soils that may be infested with soilborne plant pathogens, including plant-parasitic nematodes. Plant-parasitic nematodes associated with olive planting stocks in olive nurseries in southern Spain include ring (*Mesocriconema xenoplax*), root-knot (*Meloidogyne* spp.) and root-lesion (*Pratylenchus* spp.) nematodes (Nico *et al*., 2002). Root-knot and root-lesion nematodes reduce growth of olive planting stocks (Lamberti & Baines, 1969; Nico *et al*., 2003). Therefore, in the system of olive planting stock production in Andalucia, soil for growth of olive stems rooted in perlite should be analysed and disinfested before use.

Soil solarization has successfully controlled soilborne fungi (Katan, 1980, 1981), bacteria (Raio *et al*., 1997), and plant-parasitic nematodes (Stapleton & Heald, 1991). Soil is solarized by covering level, finely tilled soil at field capacity with transparent plastic sheets. Solarization of small piles of nursery substrates used as potting mixtures for glasshouse-grown plants is a novel approach for the management of soilborne plant pathogens (Stapleton *et al*., 1999). The efficacy of this specific use of soil solarization for the control of soilborne pathogens, particularly plant-parasitic nematodes, requires testing because disinfestation may be incomplete in deep layers of soil, or where the piles of soil are in shadow. Sometimes, *Meloidogyne* spp. are heat-tolerant and difficult to control by soil solarization (Katan, 1987). The gelatine of *Meloidogyne* egg masses protects against desiccation (Orion, 1995) and possibly against high temperatures.

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The objective of this study was to assess the effects of soil solarization for the control of *M. incognita* in a soil mixture used for nursery production of olive planting stocks in southern Spain.

Materials and methods

Two experiments (I and II) were carried out at the experimental farm of the Institute of Sustainable Agriculture (Instituto de Agricultura Sostenible), Córdoba, Spain (latitude 37°N, longitude 4°W, altitude 110 m) in July 1999 and August 2000. A soil mixture (sand:clay loam, 2:1, v/v) was amended (experiment II) or not (experiment I) with an organic growing mixture (Max, Vehnemoor GmbH, Sedelsberg, Germany), and is typical of substrates used in olive nurseries in southern Spain. For each experiment, six conical piles of the soil mixture were prepared according to the natural slope angle (~40°, 80 cm high and 1 m base diameter) and arranged in two rows of three piles each, 1 m apart, on a cement surface. Three of the six soil piles were covered with polyethylene for the solarization treatments and three remained uncovered (controls). There were three replicates per treatment, and solarized and nonsolarized treatments alternated within a row. For the solarization treatment, soil piles were covered with 50 μm transparent, low-density polyethylene film without additives (Macresur Acolchados, Macresur SA, Almería, Spain). Prior to mulching, the piles of soil mixtures were sprinkler-irrigated to field capacity (–20·8 kPa). The soil throughout the piles was checked for uniformity of humidity using a hygrometer (Celinfa MHP, Barcelona, Spain).

Nematode inocula

A stock culture derived from a single egg mass of *M. incognita* was used in the study which originated from infected feeder roots of a 'Manzanilla' olive planting stock from a nursery at Alcolea (Córdoba), southern Spain (Nico *et al*., 2002). Stock culture was established by placing an egg mass of the nematode beneath the root system of a 'Roma' tomato seedling. Plants were grown in 12 cm pots filled with sterile loamy soil and incubated in a growth chamber at 25 ± 1°C, 60–90% relative humidity, and a 14-h photoperiod of fluorescent light at 360 ± 25 μE m^{-2} s^{-1} (Nico *et al*., 2002). Two months after inoculation, egg masses from infected tomato roots were well-formed, and the inoculated plants were removed and the roots washed free of soil, and finely chopped. Two types of inocula, egg masses and free eggs, were obtained from infected tomato roots and tested. Free eggs were extracted from 5 g subsamples of finely chopped tomato roots shaken in a 0·5% (v/v; 5% active chlorine) NaOCl solution for 4 min (Hussey & Barker, 1973). Eggs were collected on a 5 μm-pore sieve and gently rinsed with tap water for 5 min to remove residual NaOCl. The average number of eggs in water suspension was determined in 10 1 mL aliquots and the inoculum dose achieved by dilution in sterile water. To obtain mature egg masses, tomato roots were washed free of soil and stained with Phloxine B (Gurr, Sigma-Aldrich, Poole, UK) (15 mg per L of tap water) for 20 min and single egg masses removed from the roots.

Experiment I

Five mature egg masses or approximately 1000 free eggs, each in 2 mL sterile distilled water, were mixed with 10 cm^3 of sterile kaolin powder and the mixture transferred to a 4 × 6 cm bag made with nylon mesh (5 μm mesh diameter). Nylon bags with the nematode-infested kaolin were attached to steel wires (Fig. 1a) and buried in the piled soil mixture. Wires with bags were placed on the

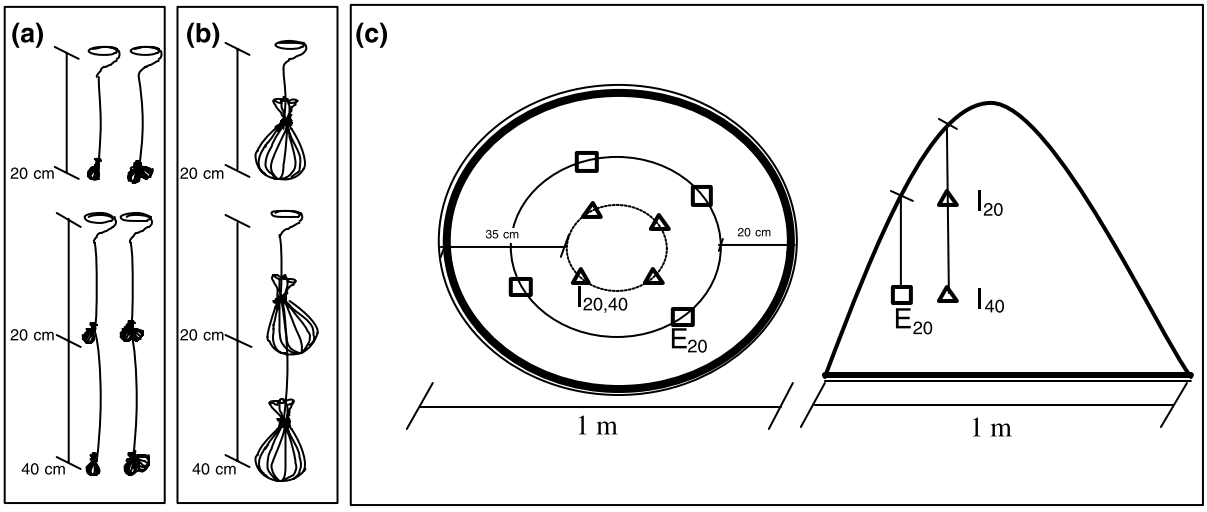

Figure 1 (a) Nylon bags containing 10 cm^3 of nematode-infested kaolin (five mature egg masses or 1000 free eggs) attached to steel wires and buried in piled soil (experiment I). (b) Nylon bags containing 500 cm^3 of nematode-infested soil (10 000 free eggs) attached to steel wires and buried in piled soil (experiment II). (c) A schematic view of a conical soil pile indicating the location of buried bags. E_{20}, bags located at 20 cm depth from the sloped side of a pile; I_{20}, I_{40}, bags located at 20 and 40 cm depth from the top of a pile.

outside of the soil pile (external) or inside the pile at the top (internal) at various depths at each of three locations (Fig. 1c): (i) externally, 20 cm from the pile edge and 20 cm deep (E20); (ii) internally, 35 cm from the edge and 20 cm deep (I20); (iii) internally, 35 cm from the edge and 40 cm deep (I40). Bags with nematode inoculum were retrieved from the piles after 0, 6, 10, 17 and 21 days (free eggs), or 0, 10 and 21 days (egg masses) after the start of solarization. Soil piles were solarized for 3 weeks in July and August 1999.

Experiment II

Free eggs (10 000 eggs) of *M. incognita* in 5 mL sterile distilled water were mixed with 500 cm^3 of amended soil mixture and transferred to 30 × 30 cm mesh bags as before. Nylon bags and infested soil mixture were transferred to the top of the pile and buried 20 and 40 cm deep (I20, I_{40}) (Fig. 1b and c). Bags with free eggs were removed from the soil piles 0, 6, 10, 17 and 22 days after the start of solarization. Soil piles were solarized for 3 weeks from July to August 2000.

Temperature monitoring

Temperatures inside (soil) and above (air) a pile were recorded at 1 min intervals using thermocouple sensors and the values averaged over each 10-min period (CR10X Datalogger, Campbell Scientific, Logan, UT, USA). Thirteen thermocouples were used to monitor temperatures: one for air temperature, three inside one solarized pile at the same locations as the bags with nematode inocula (but outside the bags), three inside nylon bags without nematode inocula and at the same locations as bags with nematode inocula; and six thermocouples were placed in a nonsolarized control pile at locations corresponding to those described for solarized piles. The average temperature ($\bar{T}_h$) for each 10 min period for the whole of the solarization period was calculated. Daily mean temperatures were calculated by the equation: $\bar{T}_{day} = (\Sigma_n \bar{T}_h)/n$, where n = total number of records per day, i.e. 24 × 6 = 144. Temperature fluctuations during the whole period of solarization were evaluated as the temperature at 15·00 h CET (Central European Time) ($\bar{T}_{15}$). This temperature was chosen because solar irradiation is at its maximum at that time (Gaur & Dhingra, 1991).

Survival assay

Nylon bags were removed from the piles of soil mixture at various incubation times and the nematode-infested kaolin or soil assayed for survival of nematode eggs and egg masses. After removal, bags were kept at 4°C until the end of the solarization treatment, according to low-temperature threshold limits of *M. incognita* (Vrain, 1978). In experiment I, free eggs and egg masses for each treatment replicate were collected on a sieve (5 μm mesh diameter) and rinsed with tap water for 2 min to remove kaolin. Egg viability was assessed as the capacity to hatch in sterile deionized distilled water (SDDW) in watch glasses. Free eggs and egg masses were covered with SDDW and incubated at 25 ± 1°C in the dark for 3 weeks. SDDW in each watch glass was renewed at 2- to 3-day intervals throughout the assay. For free eggs, the total numbers of second-stage juveniles (J_2) that emerged by the end of the experiment was recorded and expressed as a percentage of the total initial number of unhatched free eggs. For egg masses, the total numbers of eggs remaining unhatched after incubation was calculated after dissolving the masses in 1% (v/v) NaOCl solution for 5 min. Final relative egg hatch (FRH) was estimated by the equation:

$$FRH = (FH_x/FH_0) \times 100$$

where FH_x is the final percentage egg hatch after solarization treatment, and FH_0 is the final percentage egg hatch for 0 days of solarization. For experiment II, viability of nematode eggs was assessed by an infectivity bioassay using the susceptible tomato cv. Roma. Soil from the bags was transferred to sterilized clay pots and planted with 4- to 6-week-old tomato seedlings cv. Roma, one plant per pot. Plants were incubated in a growth chamber at 25 ± 1°C, 60–90% relative humidity and a 14 h photoperiod of fluorescent light at 360 ± 25 μE m^{-2} s^{-1}. After 60 days' incubation, plants were removed from the pots and the roots washed free of soil. The final nematode population in soil was determined by centrifugal flotation, and in roots by maceration and centrifugation (Nico *et al.*, 2002). Final relative nematode population was determined by the equation:

$$FRP = (FP_x/FP_0) \times 100$$

where FP_x is the final nematode population for soil sampled after x days solarization, and FP_0 is the final nematode population in the same treatment for 0 days of solarization.

Statistical analysis

Data were subjected to ANOVA using Statistix 7·0 (NH Analytical Software, Roseville, MN, USA). For each period of solarization, means were compared using Fisher's protected least significant difference test (LSD) at P = 0·05. Orthogonal single degree of freedom contrasts were computed to test the effect of selected treatment combinations (Gomez & Gomez, 1984). Treatment comparisons were made in a sequence of a hierarchical classification of the different sources of variation in the experiment (i.e. in order of position, burial depth, solarization treatment and type of inoculum). When no significant effect could be assigned to a source of variation, the corresponding data were pooled and used for further analysis. The final values for relative egg hatch and nematode population were subjected to linear regression analysis over the period of the solarization treatment. Regression analyses were performed with three replicates for each treatment combination. The percentage final relative egg hatch and final nematode population were transformed to arcsine-square root before analysis of variance

(Gomez & Gomez, 1984). The coefficient of determination, R^2 adjusted for one degree of freedom (R^2_a), and the patterns of residuals plotted against expected values were used to assess the appropriateness of the model to describe the data (Campbell & Madden, 1990). Following regression analysis, the slopes of linear regressions for inoculum location, inoculum source and burial depth were compared by a *t*-test at $P = 0{\cdot}05$ (Gomez & Gomez, 1984).

Results

Air and soil temperatures

Soil temperatures inside and outside nylon bags at a given burial depth and location differed by less than 0·5°C, irrespective of the time, day and treatment. Therefore, only temperatures recorded inside bags were analysed. Temperatures at 15·00 h indicated cycles of warming and cooling over the whole period of the experiment (data not shown), and the temperature range between the warmest and coolest days was 7°C in 1999 and 12°C in 2000. Mean daily air temperatures during the experiments were 28·4°C in 1999 and 28·6°C in 2000 (Fig. 2). Soil temperature also showed cyclic fluctuations, though to a lesser extent than air temperature. All mean daily temperatures inside the pile were higher than in the air, irrespective of solarization treatment, and position and depth of burial (Fig. 2). In both years, the biggest temperature increase following solarization treatment was at 20 cm depth inside (I20) the soil pile, and was 10·6 and 12·8°C higher than air temperature in 1999 and 2000, respectively (Fig. 2). Maximum mean daily temperatures in solarized piles at location I20 were 47·4°C in 1999 and 48·2°C in 2000. The smallest increases in mean daily soil temperature occurred in the nonsolarized piles at location I20 and were 3·4 and 3·1°C in 1999 and 2000, respectively (Fig. 2).

Experiment I

Viability of free eggs and egg masses of *M. incognita*, determined by the final relative percentage of egg hatch, decreased with time regardless of inoculum type, inoculum location and depth inside the soil pile, and solarization treatment (Table 1). Solarization reduced ($P < 0{\cdot}001$) final relative egg hatch in comparison with nonsolarized piles, irrespective of duration of treatment, type of inoculum or location, and depth of burial of inocula within a pile (Table 1). Final relative egg hatch was not influenced ($P \geq 0{\cdot}05$) by type of inoculum in solarized piles at either burial depth or in nonsolarized piles at 40 cm depth (Table 1, Fig. 3). In nonsolarized soil piles final relative egg hatch for inocula buried at 20 cm depth was lower ($P < 0{\cdot}001$) for free eggs compared with egg masses (Table 1).

In nonsolarized soil piles, the final relative egg hatch decreased linearly over time (Table 1, Fig. 4). In contrast, viability of free eggs and egg masses inside solarized piles declined markedly from the first sampling date after 6 days of solarization, and percentage egg hatch was less than 10% from then on.

In solarized and nonsolarized soil piles, the final relative percentage egg hatch of *M. incognita* was not affected ($P \geq 0{\cdot}05$) by location within a pile (i.e. from the top or from the side of the pile), except for free eggs at 20 cm depth in nonsolarized piles and sampled 6 days after the start of treatment (treatments NSol-E_{20}, NSol-I_{20}, Table 1). In the latter, there was a small but significant ($P = 0{\cdot}014$) reduction in viability for eggs inside a pile at the top of a pile compared with eggs outside the sloped side of the pile. Location of inocula in a pile had no overall effect on *M. incognita* egg hatch as indicated by comparisons between slopes and intercepts after linear regression analysis (Fig. 4a and b). In contrast, bags with nematode inocula at 40 cm depth from the top of nonsolarized soil piles had

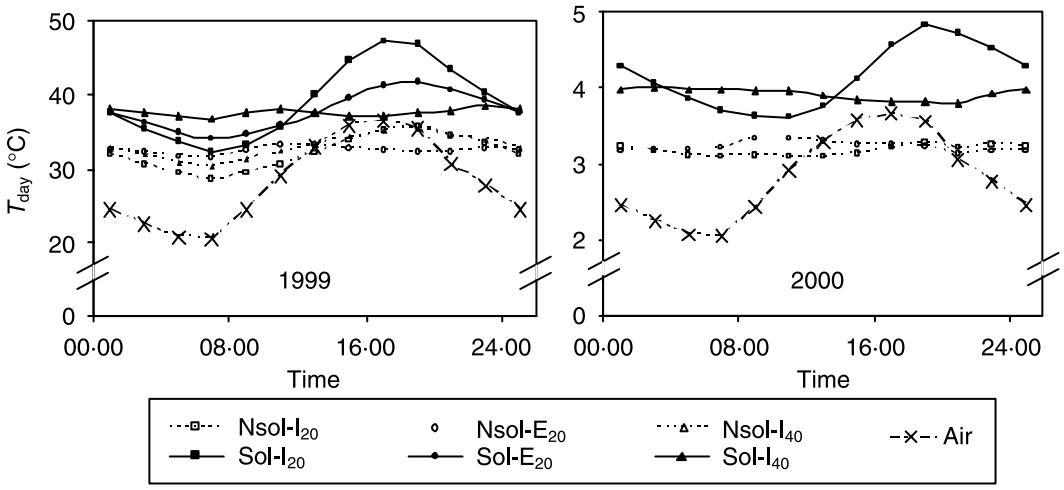

Figure 2 Mean hourly air and soil temperatures at each one of six and four locations of thermocouples used for solarization experiments in 1999 and 2000, respectively. Nsol-I_{20}, nonsolarized 20 cm depth from the top of a pile; Nsol-I_{40}, nonsolarized 40 cm depth from the top of a pile; Nsol-E_{20}, nonsolarized 20 cm depth from the sloped side of a pile; Sol-I_{20}, solarized 20 cm depth from the top of a pile; Sol-I_{40}, solarized 40 cm depth from the top of a pile; Sol-E_{20}, solarized 20 cm depth from the sloped side of a pile.

Table 1 Final relative hatch (%) of *Meloidogyne incognita* eggs after different periods of solarization of piled soil (experiment I)

	Treatment[a]		Solarization time (days)				
Type of inoculum	Code	No.	0	6	10	17	21
Free eggs	Nsol-E_{20}	1	100 a[b]	75·8 b	60·8 c	53·6 c	32·5 d
	Nsol-I_{20}	2	100 a	73·1 b	58·1 c	51·8 c	36·5 d
	Nsol-I_{40}	3	100 a	88·8 ab	85·5 b	78·9 bc	67·0 c
	Sol-E_{20}	4	100 a	1·7 b	2·1 b	0·8 b	1·4 b
	Sol-I_{20}	5	100 a	2·5 b	0·8 b	0·7 b	1·2 b
	Sol-I_{40}	6	100 a	8·0 b	5·0 b	2·1 b	1·2 b
Egg masses	Nsol-E_{20}	7	100 a	–[c]	68·3 b	–	45·8 c
	Nsol-I_{20}	8	100 a	–	66·5 b	–	49·5 c
	Nsol-I_{40}	9	100 a	–	81·4 b	–	64·6 c
	Sol-E_{20}	10	100 a	–	4·4 b	–	2·5 b
	Sol-I_{20}	11	100 a	–	3·8 b	–	2·2 b
	Sol-I_{40}	12	100 a	–	5·4 b	–	2·5 b
Contrasts (P)[d]							
Location of burial	1 vs. 2			0·014	NS	NS	NS
	4 vs. 5			NS	NS	NS	NS
	7 vs. 8			–	NS	–	NS
	10 vs. 11			–	NS	–	NS
Depth of burial	1 vs. 3			< 0·001	–	–	–
	2 vs. 3			< 0·001	–	–	–
	(1–2) vs. 3			< 0·001	< 0·001	< 0·001	< 0·001
	(4–5) vs. 6			< 0·001	NS	NS	NS
	(7–8) vs. 9			–	< 0·001	–	< 0·001
	(10–11) vs. 12			–	NS	–	NS
Solarization treatment	1 vs. (4–5)			< 0·001	–	–	–
	2 vs. (4–5)			< 0·001	–	–	–
	(1–2) vs. (4–5–6)			–	< 0·001	< 0·001	< 0·001
	3 vs. 6			< 0·001	–	–	–
	3 vs. (4–5–6)			–	< 0·001	< 0·001	< 0·001
	(7–8) vs. (10–11–12)			–	< 0·001	–	< 0·001
	9 vs. (10–11–12)			–	< 0·001	–	< 0·001
Type of inoculum	(1–2) vs. (7–8)			–	< 0·001	–	< 0·001
	3 vs. 9			–	NS	–	NS
	(4–5–6) vs. (10–11–12)			–	NS	–	NS

[a]Nylon bags (5 μm mesh) containing nematode inocula (1000 free eggs or five mature egg masses) in 10 cm^3 of sterile kaolin powder buried in conical soil piles and introduced from the side at 20 cm depth (E_{20}) or from the top of the pile at 20 cm (I_{20}) and 40 cm (I_{40}); piled soil was solarized (Sol) for different periods, or not solarized (control, Nsol).

[b]The effects of treatments were assessed by the final relative egg hatch (FRH) in water at 25°C. FRH = FH_x/FH_0, where FH_x = final percentage egg hatch for x days of solarization, and FH_0 = final percentage egg hatch in the sample for 0 days of solarization. Data are means of three replicates; means in rows followed by lower-case letters do not differ significantly ($P \geq 0{\cdot}05$) according to Fisher's protected LSD test.

[c]Not tested.

[d]Orthogonal single degree of freedom contrast of treatments. Probability for the t-statistic of linear single degree of freedom contrasts; NS, not significant ($P \geq 0{\cdot}05$).

a greater final relative egg hatch 6 days after treatment ($P < 0{\cdot}001$) than bags at 20 cm, irrespective of inoculum type (Table 1, Fig. 4c and d). The effect of depth was observed for the whole of the experiment, as indicated by differences when comparing the regression lines for final relative percentage egg hatch for inocula buried at 40 cm ($P < 0{\cdot}001$) and 20 cm ($P = 0{\cdot}006$) depth in nonsolarized soil (Fig. 4c and d). For solarized soil piles, the final relative egg hatch of *M. incognita* was not influenced ($P \geq 0{\cdot}05$) by burial depth after 6 days of treatment (Table 1).

Experiment II

The effects of solarization in 2000 were similar to those in 1999 (Table 2). In nonsolarized soil piles, viability of free eggs of *M. incognita*, as measured by the infectivity of inoculum to tomato seedlings, decreased gradually ($P < 0{\cdot}001$) with time. With solarization the maximum reduction in viability had already occurred after 6 days (Table 2). The infectivity of free eggs was not affected ($P = 0{\cdot}183$) by depth of burial, irrespective of solarization

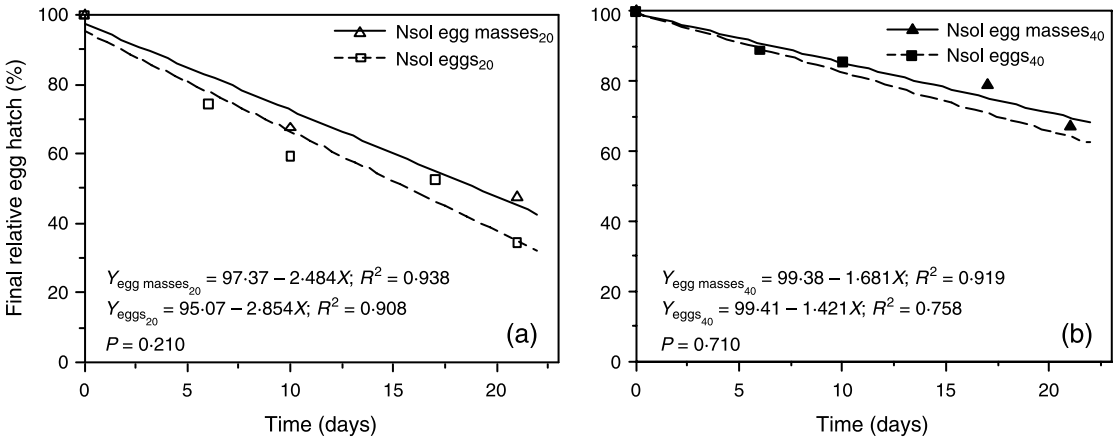

Figure 3 Linear regressions of final relative percentage egg hatch of *Meloidogyne incognita* (*FRH*) in nonsolarized (Nsol) samples over the time period of treatment for experiment in 1999. Effect of type of inoculum (free eggs vs. egg masses) buried 20 cm deep (a) and 40 cm deep (b). *P* indicates '*F*' probability for comparison of gradients of regression lines included in a frame. For Nsol $eggs_{20}$ and Nsol egg $masses_{20}$, each point represents the average of final relative egg hatch (%) between external (E_{20}) and internal (I_{20}) samples at 20 cm depth for free eggs and egg masses, respectively.

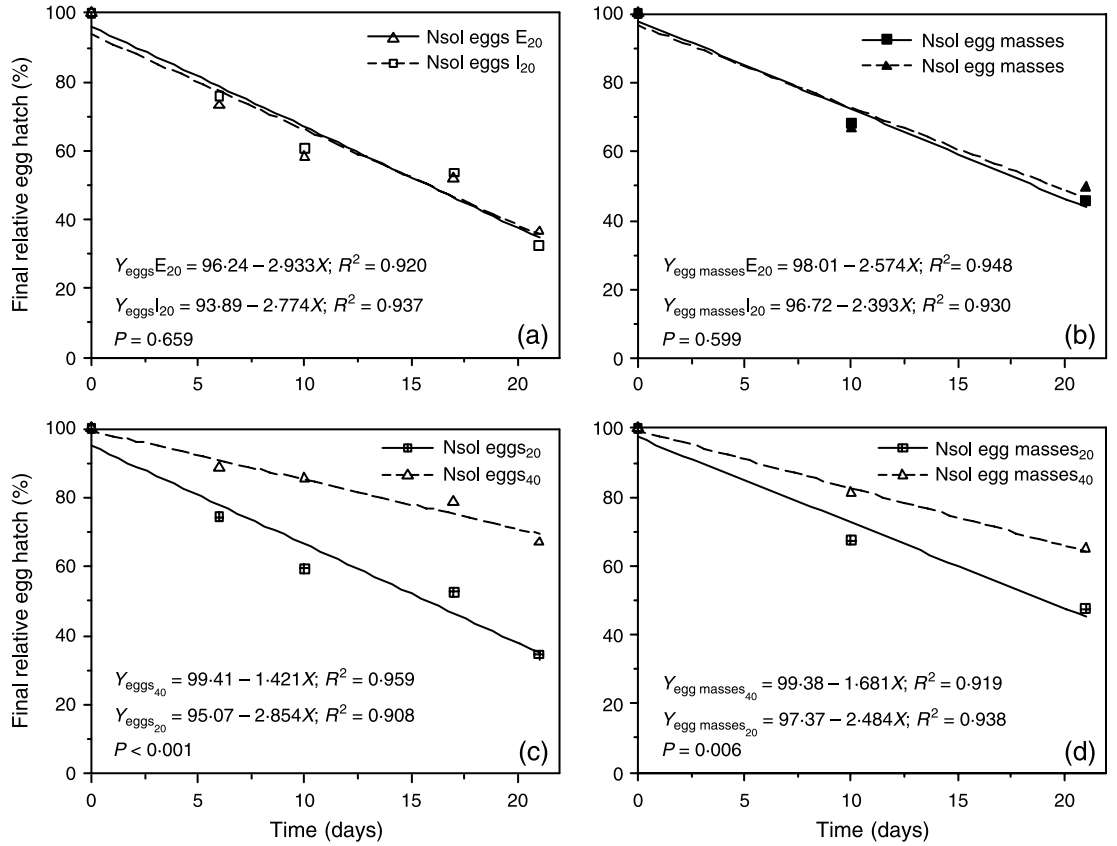

Figure 4 Linear regressions of the final relative percentage egg hatch of *Meloidogyne incognita* (FRH) in nonsolarized (Nsol) samples over the time period of treatment for experiment in 1999. Effect of location of buried bags with free eggs (a) or egg masses (b) within a pile (E_{20}, samples located at 20 cm depth from the sloped side of a pile; I_{20}, samples located at 20 cm depth from the top of a pile); and effect of burial depth (20 cm, 40 cm) of bags with nematode free eggs (c) or egg masses (d). *P* indicates '*F*' probability for comparison of gradients of regression lines included in a frame. (Nsol, nonsolarized; I_{20}, samples located at 20 cm depth from the top of a pile; E_{20}, samples located at 20 cm depth from the sloped side of a pile).

Table 2 Infectivity of *Meloidogyne incognita* eggs to 'Roma' tomatoes after solarization of piled soil containing buried eggs for different periods of time (experiment II)

Treatment[a]		Solarization time (days)				
Code	No.	0	6	10	17	22
Nsol-I_{20}	1	100 a[b]	85·1 b	44·5 c	27·1d	17·9 d
Nsol-I_{40}	2	100 a	75·2 b	54·1 c	37·9 cd	31·0 d
Sol-I_{20}	3	100 a	0·2 b	0·4 b	0·1 b	0·2 b
Sol-I_{40}	4	100 a	0·2 b	0·1 b	2·2 b	0·1 b
Contrasts (P)[d]						
	1 vs. 2		NS	NS	NS	0·002
Depth of burial	3 vs. 4		NS	NS	NS	NS
Solarization						
	(1–2) vs. (3–4)		< 0·001	< 0·001	< 0·001	–[c]
	1 vs. (3–4)		–	< 0·001	0·001	< 0·001
treatment	2 vs. (3–4)		–	< 0·001	< 0·001	< 0·001

[a]Nylon bags (5 μm mesh) containing nematode inoculum (10 000 free eggs) in 500 cm^3 of soil mixture amended with an organic mixture (1:1, v/v) buried in conical soil piles and introduced from the top of the pile at 20 cm (I_{20}) and 40 cm (I_{40}); the piled soil was solarized (Sol) for different periods or not solarized (control, Nsol).

[b]The effect of treatments was assessed by the final relative population (FRP) on tomato plants. FRP = (FP_x/FP_0) × 100, where FP_x = final nematode population for soil sampled at x days of solarization, and FP_0 = final nematode population for soil sampled at 0 days of solarization. Data are means of three replicates; means in rows followed by lower-case letters do not differ significantly ($P \geq 0{\cdot}05$) according to Fisher's protected LSD test.

[c]Orthogonal single degree of freedom contrast of treatments. Probability for the t-statistic of linear single degree of freedom contrasts; NS, not significant ($P \geq 0{\cdot}05$).

[d]Not tested.

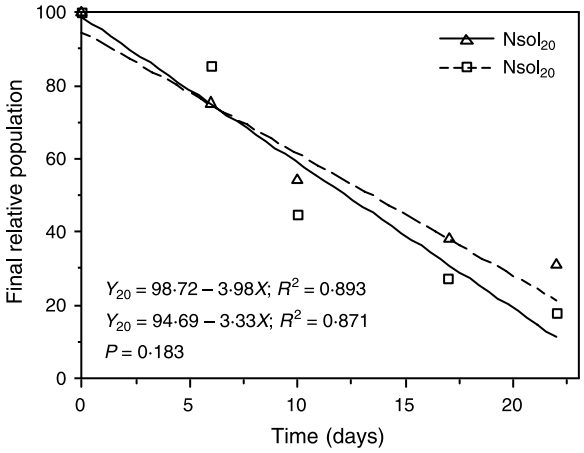

Figure 5 Linear regressions of the *Meloidogyne incognita* final relative population in tomato (cv. Roma) plants inoculated with nematode-infested soil buried in nonsolarized (Nsoil) soil piles and sampled at different time intervals during treatment in 2000. Effect of burial depth (20 vs. 40 cm). *P* indicates '*F*' probability for comparison of gradients of regression lines included in a frame.

treatment (Table 2; Fig. 5), except for eggs buried 40 cm deep, compared with eggs buried 20 cm deep in nonsolarized soil piles after 22 days' treatment (Table 2).

Discussion

The effects of soil solarization on the control of plant pathogens depend mainly on solar radiation, the frequency of cloud cover during treatment, and air and soil temperature. This research evaluated the effects of soil solarization of piles of soil used for production of olive planting stock in Andalucia and control of *M. incognita*. Maximum average daily temperatures in deep zones within the piled soil were lower than those in shallower and central zones of the pile. This may be a constraint for the efficient solarization of piled soil, thus leading to the possibility that some soil would not be completely disinfested. However, the results of the present study demonstrate that solarization of piled soil provides adequate control of *M. incognita* eggs, free or in masses, at a depth of 40 cm after 6 days of treatment, even in the less heated areas. These results are in agreement with those reported for the use of solarization of confined volumes of substrate for the control of plant parasitic nematodes (Giblin-Davis & Verkade, 1988; Stapleton *et al*., 1999).

Free eggs and egg masses both serve as inocula of *Meloidogyne* spp. in nursery substrates. Therefore, the efficacy of solarization for disinfestation must be assessed on both types of inoculum. The gelatinous matrix aggregating *Meloidogyne* egg masses protects against desiccation (Orion, 1995) and other stress factors, such as freezing (Vrain, 1978), high temperatures (Daulton & Nusbaum, 1961), microbial parasitism (Orion & Kritzman, 1991), enzymatic degradation (Punja & Zhang, 1993) and synthetic nematicides (Mojtahedi *et al*., 1991). Data from the study reported here showed that the gelatinous matrix protected egg masses at 20 cm depth in nonsolarized treatments only. Egg masses did not determine a differential survival for eggs located at 40 cm depth in nonsolarized piles or for those located at any soil depth in solarized piles, thus suggesting that solarization may be used as an appropriate control method for both types of inocula. Also, these results are in agreement with those reported by

Walker (1962), where 48°C for a time period longer than 6 min was shown to be the thermal death threshold of eggs and egg masses of *Meloidogyne* spp., as well as those reported by Gokte & Mathur (1995), where these nematodes were eradicated from grapevine rootstocks by treatment at 48–53°C for 10–20 min.

Prevailing weather conditions in summer in Andalucia make soil solarization particularly suitable for the control of soilborne pathogens, e.g. *Verticillium dahliae* (Melero-Vara *et al*., 1995; López-Escudero & Blanco-López, 2001). In the present study, mean daily air temperature was rather similar in 1999 and 2000, suggesting comparable environmental conditions in both years. The maximum average daily temperatures in piled soil (up to a maximum of 40 cm depth) solarized in Córdoba during July and August were similar to those reported for solarization of natural soils at three sites in Andalucia (López-Escudero & Blanco-López, 2001), and higher than those obtained at similar soil depths in Texas (Heald & Robinson, 1987), northern India (Gaur & Dhingra, 1991) and central northern Chile (Herrera *et al*., 1999). This suggests that the occurrence of long periods of intense solar radiation and lack of rainy or cloudy days, typical in the Mediterranean, provide conditions favourable for solarization, and overcome difficulties caused by piling of the soil.

In conclusion, solarization in summer of piled soil in nurseries in Andalucia is appropriate for optimizing production of *Meloidogyne*-free olive plants under the conditions at Córdoba. Solarization of piled soil (at a maximum depth of 40 cm) provided > 95% control of *M. incognita* eggs and egg masses in soil after 10 days' treatment, which should satisfy the standard required for the adequate control of *M. incognita* (Whitehead, 1998). Furthermore, the thermal threshold for inactivating free eggs and egg masses of *M. incognita* reached by solarization may also control other plant-parasitic nematodes potentially hazardous to olive plants, e.g. *Pratylenchus vulnus* and *Pratylenchus penetrans* (Nico *et al*., 2003). If nurserymen were to adopt solarization of piled soil, further adjustments to the method would be required. The normal schedule in nurseries is that rooted olive stems are grown in potted soil in the spring (Sutter, 1994). Solarization of piled soil in July or August would then leave a long period of time between disinfestation and use. Therefore, additional experiments are needed to determine the efficiency of solarization of piled soil for control of *M. incognita* under suboptimal conditions likely to occur in Córdoba during September and October. Solarization during those months would shorten the storage time of large volumes of solarized, piled soil until use.

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