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Nematode Management in the Strawberry Fields of Southern Spain

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Abstract: (1) Background: Spain is the sixth strawberry producer in the world, with about 6500 ha producing more than 350,000 tons, and an annual commercial value about 390 million €. Stunted and dead strawberry plants are frequently associated with plant-parasitic nematodes, but nematode diseases have not been characterized to date in the country. (2) Methods: A poll on the perception of the impact of nematodes on strawberry production was carried out by face-to-face interviews with farm advisors. In addition, nematological field surveys were carried out at the end of the growing season in 2017 and 2018 to determine prevalence and abundance of plant-parasitic nematodes in strawberry crops. The host suitability to *Meloidogyne hapla* of seventeen strawberry cultivars and the tolerance limit to *M. hapla* at progressively higher initial population densities (*Pi*) were assessed in pot experiments in a growth chamber. Comparison of the relative efficacies of several soil disinfestation methods in controlling nematode populations (*M. hapla* and *Pratylenchus penetrans*) was carried out in experimental field trials for twelve consecutive years. (3) Results: *Meloidogyne* spp., *Pratylenchus penetrans*, and *Hemicycliophora* spp. were the main plant-parasitic nematodes in the strawberry fields in South Spain. Root-knot nematodes were found in 90% of the fields, being *M. hapla* the most prevalent species (71% of the fields). A tolerance limit of 0.2 *M. hapla* juveniles per g of soil was estimated for strawberry, and currently cropped strawberry cultivars did not show resistance to *M. hapla*. Nematode population densities were reduced by more than 70% by soil fumigation with 1,3-dichloropropene, dazomet, dimethyl-disulfide, and methyl iodide. The efficacy of metam-sodium in reducing nematode populations was about 50% and that of chloropicrin, furfural, and sodium-azide, less than 40%. Combination of solarization with organic manures (biosolarization) reduced soil nematode populations by 68–73%. (4) Conclusions: Plant-parasitic nematodes (*Meloidogyne*, *Pratylenchus*, and *Hemicycliophora*) are widely distributed in the strawberry fields of Southern Spain. Strawberry is a poor host for *M. hapla* with a tolerance limit of 0.2 J2 per g of soil, and low population increases in cropping cycles of 7–8 months. Strawberry cultivars show a range of susceptibility and tolerance to *M. hapla*, but no resistance is found. Nematodes are effectively controlled by chemical fumigation of soils, but soil biosolarization is equally effective, and therefore, can be proposed as a sustainable alternative for pathogen control in strawberry cultivation.

Keywords: *Meloidogyne*; *Pratylenchus*; soil biosolarization; soil chemical fumigation; strawberry

1. Introduction

The Strawberry (*Fragaria* × *ananassa* Duch.) is an important crop worldwide, which is mainly produced in China, USA, Mexico, Egypt, Turkey, and Spain [1]. Strawberry-growing in Spain is concentrated in the south-western region (Huelva province), which constitutes up to 93% of total Spanish production with 6867 ha yielding 377,596 tons, which had a market value of 392 million € in 2016 [2]. Strawberry production in Huelva started in the late 1970s and increased until 2015 when it leveled off at about 350,000 tons per year. Fruits for fresh consumption are mainly exported to northern European countries. Strawberries are grown under temporary, plastic high-tunnels in annual cropping cycles from October to May, year after year without any crop rotation. Most strawberry transplants cultivated in Huelva are produced in open-field nurseries in central-northern Spain and shipped to fruit-production fields. The harvest period is from January to late May, and in the summer months (July to September), the fields are left fallow, and the soil is disinfested.

Several species of plant-parasitic nematodes have been reported as causing damage to strawberries, and the northern root-knot nematode (RKN) *Meloidogyne hapla* and the northern root lesion nematode (RLN) *Pratylenchus penetrans* are its most important nematode pests worldwide [3–6]. Foliar nematodes, such as *Aphelenchoides fragariae*, *Aphelenchoides ritzemabosi*, *Aphelenchoides besseyi*, and *Ditylenchus dipsaci* have been mentioned as being strawberry pests in USA, Europe, Australia, and the former USSR [3]. Needle and dagger nematodes from the *Longidorus* and *Xiphinema* genera have been linked to the transmission of viruses and decline in strawberries [3]. The sting nematode, *Belonolaimus longicaudatus*, has had a great constraining effect on commercial strawberry production in Florida [7]. Stunted plants and reduced yields are frequently associated with *M. hapla*, *P. penetrans*, *D. dipsaci*, and *Hemicycliophora* spp. in Spain [8–11], but the diseases they cause had yet to be characterized in the country.

In any nematode-plant combination, plant growth and yield losses depend primarily on soil nematode densities at planting (*Pi*), but also on the nematode potential to reproduce on the host plant, the plant tolerance, and how extensive the cropping period is [12–15]. In order to manage nematodes in a sustainable way, it is crucial to draw up accurate information on the nematode population densities that cause yield losses and quantify them in terms of plant damage and nematode reproductive functions. The plant damage function models allow the estimation of (i) the tolerance limit (*T*), defined as the nematode *Pi* up to which no measurable yield loss occurs; (ii) the minimum yield (*m*), when at high values of *Pi*, increasing numbers of nematodes may have no further impact on crop yield. The nematode reproductive function models estimate the maximum multiplication rate (*a*) or a maximum value obtained for the *Pf*/*Pi* rate, with *Pf* being the nematode densities at harvest, and the equilibrium density (*E*), which is the value of *Pi* that makes *Pf* = *Pi* [15]. Estimating these critical values is essential to design integrated management programs since they will determine if it is worth implementing any control measure. Plant damage and reproductive function models for *M. hapla* in strawberry have not been estimated to date.

Conventionally in intensive crops, nematode control has relied upon reducing *Pi* by soil fumigation with chemicals, previously by methyl-bromide, but at present 1,3-dichloropropene is used [16]. However, the use of most soil fumigants is forbidden or strictly restricted within the European Union (Directive 2009/128/CE) and elsewhere for environmental and safety reasons. Extensive research has been done on alternative chemical [17–19] and non-chemical methods [18–20] for controlling nematode diseases. However, the efficacy of these alternative methods in reducing soil nematode densities is lower than soil fumigation, and many have not proven consistency enough when used in intensive crop farming [17–20]. Nevertheless, profitable production can be achieved with lower efficacies, providing that *Pi* is reduced to below the tolerance limit for the crop. Long-term field trials comparing the nematicide efficacies of several soil disinfestation methods would provide valuable information for the management of nematode.

Although genetic resistance is a preferred strategy for nematode management, resistance genes against *Meloidogyne* spp. or *Pratylenchus* spp. have not been identified to date in strawberries. However, variable responses to *M. hapla* [21–23] and *P. penetrans* have been reported [23,24] in strawberry cultivars,

including tolerance. The relative susceptibility to the main plant-parasitic nematodes of specific crop cultivars could be exploited in order to regulate increases in the nematode population in the absence of resistance genes [25].

The main objectives of this research were:

1. To determine the prevalence, abundance, and incidence of plant-parasitic nematodes in the strawberry fields in Southern Spain.
2. To determine the host suitability to *M. hapla* of currently cropped strawberry cultivars.
3. To establish plant damage and reproductive function models for *M. hapla* in strawberry.
4. To compare the efficacies of various soil disinfestation methods against populations of *M. hapla* and *P. penetrans*.

2. Materials and Methods

2.1. Nematological Survey

To determine the impact of plant-parasitic nematodes on strawberry crops in Southern Spain, a two-way approach was taken: (i) a poll to a "group of experts" on their perception of nematode caused diseases and how to manage them and (ii) a nematological field survey.

2.1.1. A Poll on Perception of Nematode Caused Diseases on Strawberries

From February to March 2017, a poll on the perception of the impact nematodes on strawberry production was carried out by face-to-face interviews with 60 farm advisors who had at least five years of field experience. Farm advisors deemed to be an "expert group" since they are experienced agronomists who have accumulated knowledge on strawberry diseases and plant protection obtained by advising in strawberry crop management through years. Before these interviews took place, a questionnaire was distributed to farm advisors, which was designed to evaluate their opinions about prevalence, abundance, and incidence of nematode diseases and how effective nematode control methods used in the area were. The answers to the questionnaire were collected and grouped on a spreadsheet. Descriptive and exploratory statistical data analyses were performed on data using frequency distributions and by calculating central tendency and dispersion measures.

2.1.2. Nematological Field Survey of Strawberry Fields

To determine how prevalent and abundant plant-parasitic nematodes were in the strawberry growing area of Southern Spain, field surveys were conducted at the end of the growing season, in May 2017 and May–June 2018. The fields to be sampled were chosen geographically on the Universal Transverse Mercator (UTM) 1 × 1 km grid, but only squares with more than 15 ha of strawberry cultivation, according to the Land Use System of Geographic Information of Andalusia (SIOSE) [26], were included in the survey. Two fields located approximately at the center of each UTM 1 × 1 km square were selected for sampling. Farm advisors from local marketing organizations, phytosanitary companies and cooperatives, helped to identify suitable fields for sampling on the basis of (i) representative cultivation sites, (ii) cultivars, (iii) accessibility, and (iv) how willing growers were to participate in the survey. The selected sites were geo-localized using a GPS 60CSX® device (Garmin Ltd., Olathe, KS, USA), and their geographical coordinates were recorded.

Fifty-two fields were sampled. Nematode soil population densities were estimated from composite soil samples dug with a spade around the roots of 10 to 12 plants distributed randomly at each site. Nematodes were extracted from two 250 g sub-samples of soil using the Whitehead tray method [27] and identified and counted under a compound microscope. To identify the *Meloidogyne* species, females were collected from infected roots under a stereo microscope. A minimum of 10 females per site was used to identify the RKN species in accordance with their perineal [28] and isoesterase electrophoretic patterns [29].

2.2. Host Suitability of Strawberry Cultivars

2.2.1. Production of *M. hapla* Inoculum

A population of *M. hapla* collected from strawberry roots at Cumbres Malvinas, Palos de la Frontera (37°14'N–6°53'W), started with the offspring from one female, was reared on susceptible tomato *Solanum lycopersicum* 'Roma' in polypropylene pots (50 cm long, 17 cm wide, 14.5 cm high) containing 10 L of a sterilized sand-silty soil (84% sand: 10% silt: 6% clay) with 0.5% organic matter, a pH of 7.7, and electrical conductivity of 0.38 mS cm^{-1}, for a period of 90 days at 24 ± 2 °C in a growth chamber. *Meloidogyne hapla* second-stage juveniles (J2) were extracted from the tomato roots using the Hussey & Barker method [30], and the resulting egg suspension was concentrated by passing it through a 25-µm sieve. The retained eggs were washed off on Whitehead trays [27], and the hatched J2 within 72 h were used as inoculum.

2.2.2. Establishment of Pot Experiments

Seventeen commercial strawberry cultivars: 'Calderon', 'Calinda', 'Candonga', 'Charlene', 'Flaminia', 'Flavia', 'Fortuna', 'Marisol', 'Marquis', 'Melissa', 'Palmeritas', 'Petaluma', 'Primoris', 'Rabida', 'Rociera', 'Sabrina', and 'Savana' were tested. Bare root plantlets from nurseries at high-altitudes in central-northern Spain were singly transferred to polypropylene pots (12 cm diameter at the top and 10 cm at the bottom, 11 cm high) containing 0.75 L of the sterile sand-silty soil described previously. Plants were allowed to grow for two weeks and then inoculated with two *M. hapla Pi* levels (0 and 1 J2 per g of soil). Nematodes were inoculated in c.a. 6 mL of water distributed over three holes (3–5 cm deep) made in the soil around the plant. Each cultivar-*Pi* combination was replicated five times, and the pots were arranged in five blocks in a complete randomized block design, within a growth chamber at "IFAPA Camino de Purchil" in Granada, Spain. The experiment was conducted at 24 ± 2 °C with a photoperiod of 16 h light and 50% relative humidity. Plants were fertilized with a slow-release fertilizer Osmocote® (15% N + 10% P_2O_5 + 12% K_2O + 2% MgO_2 + microelements) (Scotts Company, Heerlen, Netherlands) by adding approximately 3 g of it onto the surface of each pot just after transplanting. The experiment was conducted twice, during the first trimester of 2017 and 2018.

Nematode densities per pot (soil + roots) (*Pf*), root gall indices, and fresh top weight were determined 70 days after the inoculation of the nematode. Nematodes were extracted from the strawberry roots using the Hussey & Barker method [30] and from the 250-g soil subsamples by the Whitehead tray method [27]. Root-gall indices were determined on a 0–5 scale, where 0 = no galling; 1 = trace infection with few small galls; 2 = < 25% roots galled; 3 = 26 to 50%; 4 = 51 to 75%; 5 = > 75% roots galled [31]. The multiplication rate (*Pf*/*Pi*) was calculated as a measure of the capacity of the cultivar to reproduce the nematode. The relative yield loss was calculated for each cultivar as being the rate between the average top weight of nematode-inoculated plants (*Y*1) and that of non-inoculated plants (*Y*0) (1).

$$\text{Relative yield loss} = 1 - (Y1/Y0) \quad (1)$$

Multiplication rates and relative yield losses were compared among cultivars by ANOVA and Kruskal-Wallis tests.

2.3. Estimation of Plant Damage and Reproductive Function Models for M. hapla in Strawberry

2.3.1. Production of *M. hapla* Inoculum

Meloidogyne hapla J2 were obtained as described in Section 2.2.1.

2.3.2. Establishment of Pot Experiments

Bare root plantlets of strawberry 'Fortuna' from high-altitude nurseries in central-northern Spain were singly transferred to polypropylene pots (14.4 cm diameter at the top and 14 cm at the bottom, 14 cm high) containing 1.5 L of the sterilized sand-silty soil described previously. Plants were allowed to grow for two weeks and then inoculated with nine *M. hapla Pi* levels (0, 1, 2, 4, 8, 16, 32, 64, and 128 J2 per g of soil). Each treatment (*Pi*) was replicated five times, and the pots were arranged in five blocks in a complete randomized block design, within a growth chamber at "IFAPA Camino de Purchil" in Granada, Spain. The experiment was conducted under the same conditions as described in 2.2.2., and it was repeated twice in time, during the first trimester of 2017 and 2018.

Nematode densities per plant (soil + roots) on harvesting (*Pf*), multiplication rates (*Pf*/*Pi*), root gall indices, and fresh top weight, including fruits, were determined 90 days after the inoculation of the nematode as described in 2.2.2. Relative yields (*y*: the fresh weight of strawberry plants expressed as a fraction of the fresh weight of plants obtained at *Pi* = 0 J2 per g soil) were averaged over replicates per nematode density and fitted to the Seinhorst's Equation (2) for yield losses [15]. The tolerance limit for yield losses (*T*) and the relative minimum yield (*m*) were estimated by non-linear regression with the Marquardt estimation method.

$$y = m + (1 - m) \times 0.95^{(Pi/T-1)} \quad (2)$$

The relationship between *Pi* and *Pf* was used to estimate the maximum nematode multiplication rate (*a*) and the equilibrium density (*E*, when *Pi* = *Pf*) [15]. The maximum multiplication rate (*a*) was estimated by selecting the *Pi* with the highest slope on the regression line *Pf* vs. *Pi*. The maximum population density (*M*) was estimated from the experimental data, and *E* was calculated according to the Equation (3) [15].

$$M = (a \times E)/(a - 1) \quad (3)$$

2.4. Soil Disinfection Efficacy in Field Trials

The relative efficacies of various soil disinfestation techniques for reducing nematode populations in the soil were compared by carrying out field trials at two experimental sites during twelve consecutive cropping cycles, from the 2006–2007 season to 2017–2018.

2.4.1. Experimental Fields and Strawberry Growing Conditions

The experimental sites were located at Palos de la Frontera (37°14′ N, 6°53′ W), which was naturally infested with *M. hapla*, and Moguer (37°17′ N, 6°51′ W) infested with *P. penetrans*. The soil was classified as loamy-sands with an organic matter content of 0.4–0.8%, pH 6.7–6.9, and electrical conductivity of 0.08–0.11 mS cm^{-1}.

At both sites, conventional crop management was followed as recommended for strawberry production in the region [32]. Briefly, strawberry plants were planted in October in raised beds (50 cm wide × 30 cm high), which were protected by black plastic mulch and had a localized fertirrigation system. These beds were covered by high-tunnels with 0.15 mm translucent polyethylene plastic, which allowed 60–75% of the photosynthetic active radiation from November to May to enter. High-tunnels, which were 8.3 m wide and covering six beds, were mounted and removed every season and built using semi-circular steel bars with a 3.3 m high tunnel apex. Bare-root strawberry plantlets from commercial nurseries were transplanted in the field in October and placed in double rows per raised bed 25 cm apart within and between rows. Thirty individual plots (3.3 × 25 m), with three raised beds per plot, were delimited on each field site every year as experimental units. Nematode soil population densities were determined every season in July, before soil disinfestation treatments, and data were collected only from plots with more than 50 nematodes per 100 g of soil.

2.4.2. Soil Disinfestation Treatments

Soil disinfestation treatments were applied to individual plots. Nine treatments plus an untreated control were established in a randomized complete block design with three replicates per treatment at each location every season. The product dosage, means of application, and type of plastic mulch are described in Tables 1 and 2. The untreated control was included in the twelve field trials at the two sites, and the remaining treatments were tested at least three times in different cropping cycles (n = 9).

Table 1. Soil disinfestation treatments applied to a field naturally infested with *Meloidogyne hapla* to determine their efficacies in reducing soil nematode densities.

Soil Treatment	Dosage (kg/ha)	Application	Plastic Mulch	*n*
Untreated Control	–	–	PE	36
1,3-dichloropropene:chloropicrin (61:33)	300–400	Shank/Drip	PE/VIF	33
Chloropicrin	300–400	Shank	PE/VIF	12
Dazomet	300–500	Broadcast	PE	18
Dimethyl-disulphide	400–600	Shank/Drip	PE/VIF	21
Furfural	600	Drip	PE/VIF	9
Metam-sodium	153	Drip	PE	9
Methyl iodide:chloropicrin (33:67)	150–300	Shank	VIF	9
Sodium-azide	125–160	Drip	PE/VIF	9
Biosolarization with chicken manure	20,000–25,000	Broadcast	PE	12

PE, polyethylene. VIF, Virtually Impermeable Film. *n*, number of replicated plots per treatment.

Table 2. Soil disinfestation treatments applied to a field naturally infested with *Pratylenchus penetrans* to determine their efficacies in reducing soil nematode densities.

Soil Treatment	Dosage (kg/ha)	Application	Plastic Mulch	*n*
Untreated Control	-	-	PE	36
1,3-dichloropropene:chloropicrin (61:33)	300–400	Shank/Drip	PE/VIF	36
Chloropicrin	300–400	Shank	PE/VIF	21
Dazomet	300–500	Broadcast	PE/VIF	27
Dimethyl-disulphide	400–600	Shank	PE/VIF	18
Furfural	600	Drip	PE/VIF	9
Metam-sodium	153	Shank	PE	9
Methyl iodide:chloropicrin (33:67)	150–300	Shank	VIF	15
Sodium-azide	125–160	Drip	PE/VIF	9
Biosolarization with chicken manure	20,000–25,000	Broadcast	PE	21

PE, polyethylene. VIF, Virtually Impermeable Film. *n*, number of replicated plots per treatment.

Prior to any treatment application, the soil in each individual plot was thoroughly tilled and subsequently irrigated with a sprinkler for two consecutive days to ensure the soil was moist at a minimum depth of 20 cm. Soil fumigants were applied by shank-injection or drip-irrigation. Fumigants were injected into the soil at depths of 20 cm with two chisels, simultaneously pressing the bed. Chemicals applied by drip-irrigation were delivered through a single drip line, and emitters were placed on bed centers at a depth of 1 cm every 20 cm, with a flow rate of 1 $L \cdot m^{-1} \cdot h^{-1}$. Dazomet was broadcast by a Mix Tiller Dry® (Forigo Roteritalia Srl., Ostiglia, Italy). The beds were covered with a black plastic mulch. Average soil temperatures during fumigation were between 25–29 °C at both locations.

Biosolarization is a modified form of solarization that combines organic soil amendments, with passive solar heating under a transparent plastic mulch, which creates multiple pest and pathogen inactivation mechanisms in the soil [20]. Biosolarization was applied in mid-July each season. Dried chicken manure from nearby chicken farms was uniformly distributed onto the surface of the soil and then incorporated into the top 20-cm layer by crosswise plowing using a rotary cultivator (Rotavator; Howard Iberica S.A., Granollers, Spain). Plots were then drip irrigated until the soil reached field capacity. Solarization was carried out under a low-density transparent polyethylene film (0.03 mm thick) during July and August for about 6 weeks. After that, the polyethylene film was removed, and the soil was prepared for planting.

2.4.3. Estimation of Soil Nematode Densities

Composite soil samples were taken from each plot just before applying the soil disinfestation treatments (*P*0) and after the treatments at planting (*Pi*) to determine soil nematode densities. On each sampling time, twelve soil cores were taken per plot using a vertical soil core sampler (2 cm diameter × 20 cm deep), and cores were mixed in a composite soil sample. Nematodes were extracted from subsamples of 250-g of soil by the Whitehead tray method [27].

Treatment efficacies were determined using the Schneider-Orelli's correction formula (4), based on nematode soil densities reductions from *P0* to *Pi* (mortality) and corrected by the natural mortality in the untreated plots from the corresponding field trial [33].

$$\text{Mortality} = [1 - (Pi/P0)]$$

$$\text{Schneider-Orelli's corrected efficacy} = [(mt - mc)/(1 - mc)] \times 100 \quad (4)$$

where '*mt*' is the mortality rate for a treated sample, and '*mc*' is the mortality rate for the untreated control. Efficacies were calculated for each field trial separately due to seasonal variations and treatments were compared with the ANOVA and Kruskal-Wallis tests.

2.5. Statistical Analyses

Data are expressed as the mean ± standard error of the mean and were analyzed with the Statgraphics Centurion XVI® (Statpoint Technologies Inc., Warrenton, VA, USA) statistical software. Data from repeated experiments in time were analyzed by ANOVA to check for differences between experiments; since no significant differences were found, the data were grouped in one single set of data. The Kolmogorov-Smirnov and Brown-Forsythe tests were applied to data to check for normality and homoscedasticity of variances; if significant, data were arcsine-transformed and subjected to the same tests once more. When normality and homoscedasticity of variances could be assumed, data were analyzed by ANOVA. If F values were significant, the means were compared by the Bonferroni test ($p < 0.05$). When the homoscedasticity of variances could not be assumed, Welch's ANOVA was used. When normality was not reached after transformation, the data were analyzed by Kruskal-Wallis non-parametric tests, and if H values were significant, means were compared by Dunn's multiple comparison test ($p < 0.05$).

3. Results

3.1. Nematological Survey

3.1.1. A Poll on Perception of Nematode Caused Diseases in Strawberries

Farm advisors participating in the poll in the strawberry growing area of Huelva had 5 to 30 years of experience dealing in strawberry crop management. Each farm advisor supervises, on average, 163 ha of berry crops distributed over 20 fields. According to their perception, nematodes were the fourth most prevalent cause of disease in strawberries, with 63% of fields infested, and fifth in terms of incidence with an average rate of 5% of infested plants per site. Yield losses caused by nematodes were estimated on average to be 6% of the total harvest, which makes an economic loss of about 23.4 million € per year. They reported that soil disinfestation is carried out annually in 84% of the fields, before planting and growers use mixtures of 1,3-dichloropropene plus chloropicrin (55%), metam-sodium (18%), or dazomet (11%). The perceptions farm advisors had about the efficacy of soil disinfestation against plant-parasitic nematodes ranked soil fumigation with 1,3-dichloropropene:chloropicrin as being the most efficient method, followed by dazomet and metam-sodium. Other methods, such as soil solarization, biofumigation, or using chloropicrin alone, were categorized as being hardly efficient.

3.1.2. Nematological Field Survey

Four genera of plant-parasitic nematodes were found to be associated with strawberry crops in Huelva (Table 3). Root-knot nematodes (*Meloidogyne* spp.) were widely distributed and occurred in 90% of the fields, with *M. hapla* being the most prevalent species (71% of the fields), while *Meloidogyne incognita*, *Meloidogyne javanica*, and an undetermined *Meloidogyne* species were found in only 6–8% of the fields. Other nematode pests, such as *P. penetrans* and *Hemicycliophora* spp., were found in 20% of the fields and *D. dipsaci* only in 6% of the sampled fields.

Table 3. Prevalence and abundance of plant-parasitic nematodes associated with strawberry crops in South Spain.

	Prevalence (%)	**Abundance**	
Meloidogyne hapla	71	452	(20–2560)
Meloidogyne incognita	8	122	(13–480)
Meloidogyne javanica	6	320	(33–890)
Meloidogyne sp.	6	8	(8–11)
Pratylenchus penetrans	20	27	(3–94)
Hemicycliophora spp.	20	143	(5–1200)
Ditylenchus dipsaci	6	9	(5–20)

Abundance is expressed as mean (range of variation) of nematodes found in 100 g of soil in 52 fields.

3.2. Host Suitability of Strawberry Cultivars

There were no differences ($p > 0.05$) in relative yield losses or root galling among the seventeen strawberry cultivars 70 days after *M. hapla* inoculation. Average root galling ranged from 0.3 to 2.0, and relative yield losses compared to non-inoculated plants ranged 8% on 'Calderón' to 19.2% on 'Rabida' (Table 4). Although all cultivars reproduced *M. hapla*, 'Calinda' and 'Charlene' were the best hosts with multiplication rates of 4.2 and 3.3, respectively. The cultivars 'Marisol' and 'Rociera' were poorer hosts with lower ($p < 0.05$) multiplication rates of 1.4 and 1.5, respectively (Table 4).

Table 4. Relative yield losses, root gall indices, and multiplication rates (*Pf*/*Pi*) of *Meloidogyne hapla* on seventeen strawberry cultivars, at 70 days post inoculation of one juvenile per g of soil in pot experiments in a growth chamber.

Cultivar	Yield Loss (%)	Gall Index	*Pf*/*Pi*	
Calderon	8.0 ± 0.1 a	0.7 ± 0.5 a	2.1 ± 0.3	bcd
Calinda	13.9 ± 0.1 a	1.3 ± 0.4 a	4.2 ± 0.3	a
Candonga	14.9 ± 0.1 a	0.3 ± 0.4 a	2.1 ± 0.3	bcd
Charlene	9.8 ± 0.1 a	0.7 ± 0.4 a	3.3 ± 0.2	ab
Flaminia	8.8 ± 0.1 a	1.5 ± 0.6 a	2.1 ± 0.4	bcd
Flavia	8.7 ± 0.1 a	2.0 ± 0.5 a	3.2 ± 0.3	abc
Fortuna	11.0 ± 0.1 a	0.3 ± 0.3 a	2.3 ± 0.2	bcd
Marisol	11.6 ± 0.1 a	0.5 ± 0.3 a	1.4 ± 0.2	d
Marquis	12.7 ± 0.1 a	1.2 ± 0.3 a	1.8 ± 0.2	cd
Melissa	8.0 ± 0.1 a	0.8 ± 0.4 a	1.9 ± 0.3	cd
Palmeritas	15.4 ± 0.1 a	1.0 ± 0.5 a	2.3 ± 0.3	bcd
Petaluma	11.1 ± 0.1 a	0.7 ± 0.5 a	2.1 ± 0.3	bcd
Primoris	12.2 ± 0.1 a	0.3 ± 0.3 a	2.5 ± 0.2	bcd
Rabida	19.2 ± 0.1 a	1.0 ± 0.3 a	2.1 ± 0.2	bcd
Rociera	15.4 ± 0.1 a	1.0 ± 0.2 a	1.5 ± 0.2	d
Sabrina	8.3 ± 0.1 a	0.6 ± 0.4 a	2.5 ± 0.2	bcd
Savana	10.0 ± 0.1 a	0.5 ± 0.6 a	2.2 ± 0.4	bcd

Data are expressed as mean ± standard error of the mean of ten replicates (five replicates per experiment × two experiments). Values in the same column followed by the same letter do not differ significantly according to Bonferroni or Dunn tests ($p < 0.05$).

3.3. Estimation of Plant Damage and Reproductive Function Models for M. hapla in Strawberry

Relative yield data based on fresh top weight 90 days after *M. hapla* inoculation were fitted to the Seinhorst damage function Equation (5) ($p < 0.01$) (Figure 1a). The minimum relative yield (m) was 0.569, and the tolerance limit (T) was 0.202 J2 per g of soil.

$$\text{Rel Yield} = 0.569 + (1 - 0.569) \cdot 0.95^{(Pi/0.202-1)};\ R^2 = 0.683 \quad (5)$$

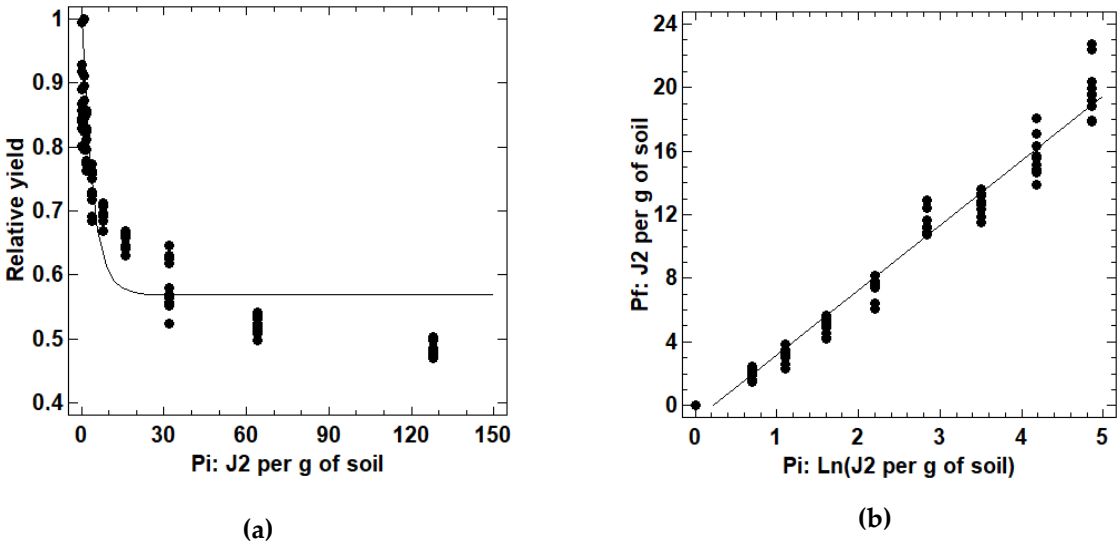


Figure 1. The relative yield of strawberry 'Fortuna' (**a**) and final densities of *Meloidogyne hapla* (**b**) per g of soil 90 days after nematode inoculation in pots in a growth chamber, plotted against progressively higher initial populations (*Pi*). Data from two repeated experiments are included (five replicates per experiment × two experiments).

The maximum final population density (*M*) obtained in the pot experiments was 23 J2 + eggs per g of soil, the maximum multiplication rate (*a*) was 2.4 for the lowest *Pi* = 1 J2 per g of soil, and the equilibrium density (*E*) at which *Pi* = *Pf* was 13 J2 per g of soil (Figure 1b).

3.4. Efficacy of Soil Disinfection in Field Trials

Soil densities of *M. hapla* and *P. penetrans* in non-treated plots, before soil disinfestation, increased slightly through the twelve consecutive cropping cycles for both nematodes (Figure 2). *Meloidogyne hapla* soil densities fluctuated along with the study, and they were higher ($p < 0.05$) at the last season (2017–2018) (265 ± 69 J2 per 100 g of soil) than at the first season (2006–2007) (119 ± 56 J2 per 100 g of soil). However, there was little fluctuation in soil densities of *P. penetrans* with no differences ($p > 0.05$) between the first (54 ± 1 nematode per 100 g of soil) and the last season (82 ± 9 nematodes per 100 g of soil).

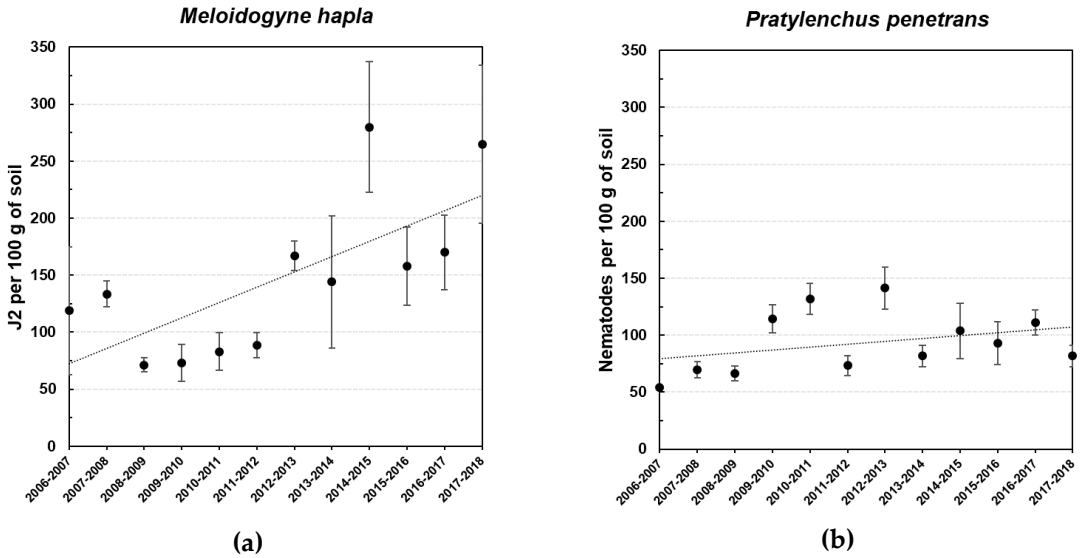
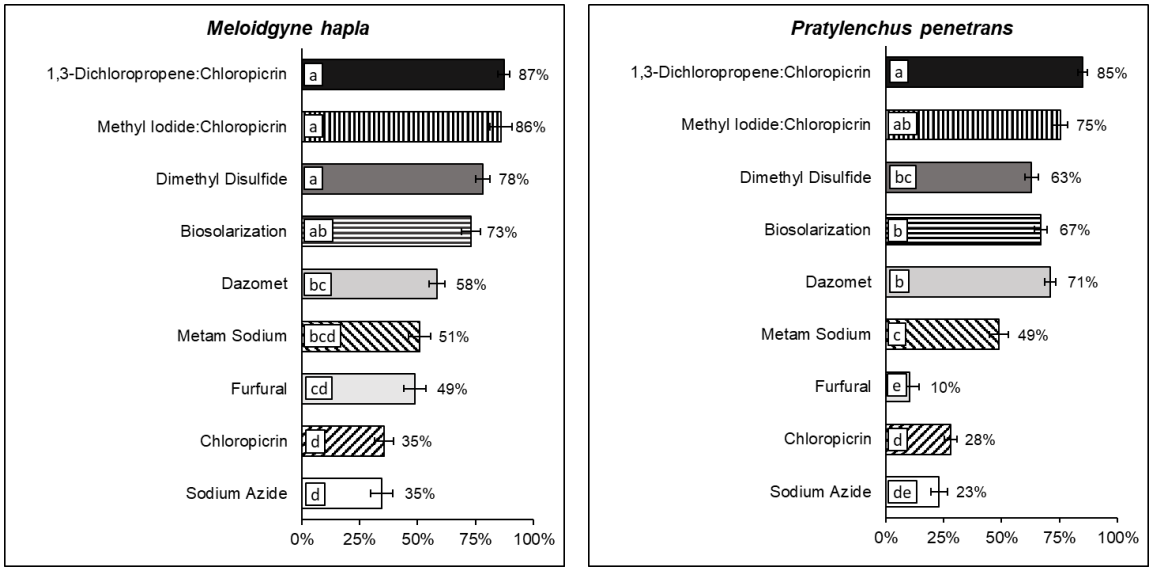


Figure 2. Nematode soil densities per 100 g of soil in non-treated plots before soil disinfestation treatments at two field sites naturally infested with *Meloidogyne hapla* (**a**) or *Pratylenchus penetrans* (**b**) through twelve consecutive cropping cycles. Dots are the average of three replicates per season, and bars represent the standard error of the mean.

The most efficient treatment for reducing soil nematode densities was chemical fumigation with 1,3-dichloropropene:chloropicrin, whose average efficacies were 87% for *M. hapla* and 85% for *P. penetrans*. Methyl iodide:chloropicrin was similarly effective in reducing the soil densities of *M. hapla* and *P. penetrans* (86% and 75%, respectively). Dimethyl-disulfide had similar efficacy (78%) in reducing *M. hapla* soil densities but was less effective than 1,3-dichloropropene:chloropicrin when used against *P. penetrans* (63%) ($p < 0.05$). Other chemicals used were less effective ($p < 0.05$), dazomet reduced *M. hapla* soil densities by 58% and those of *P. penetrans* by 71%. The efficacy of metam-sodium, furfural, chloropicrin, or sodium-azide was lower than 50%. Biosolarization with chicken manure was effective in reducing *M. hapla* soil densities (78%) than 1,3-dichloropropene:chloropicrin ($p > 0.05$) (Figure 3a), but showed lower efficacy ($p < 0.05$) against *P. penetrans* (67%) (Figure 3b).



(a) **(b)**

Figure 3. Relative efficacies of several soil disinfestations treatments in reducing *Meloidogyne hapla* (**a**) and *Pratylenchus penetrans* (**b**) soil densities in strawberry fields infested by the respective nematodes. Data are the mean of nine to thirty-six replicates depending on the treatment. Error bars represent the standard error of the mean. Bars with the same letter do not differ significantly according to the Bonferroni test ($p < 0.05$).

Differences in product dosages, means of application, or in the type of plastic mulch used had no influence on how effective chloropicrin, dazomet, furfural, metam-sodium, methyl-iodide:chloropicrin, or sodium-azide were in reducing soil nematode densities (*M. hapla* or *P. penetrans*) ($p > 0.05$) (data not shown). Shank application increased the efficacy of 1,3-dichloropropene:chloropicrin and dimethyl-disulfide in reducing *M. hapla* soil densities (94 and 83%, respectively) when compared to applying them by drip irrigation (82% and 67%) ($p < 0.05$) but did not in case of *P. penetrans* soil densities. Dimethyl-disulfide was more effective ($p < 0.05$) when sealed under VIF (86%) than under PE (72%) plastic mulch ($p < 0.01$). Biosolarization with chicken manure was more effective in reducing *M. hapla* soil densities when applied at a rate of 25,000 (86%) than at 20,000 kg·ha^{-1} (67%) ($p < 0.05$), but the dosage of chicken manure did not affect the efficacy of biosolarization in reducing *P. penetrans* soil densities.

4. Discussion

Plant-parasitic nematodes infested 90% of the surveyed fields in the strawberry growing area of Huelva (Southern Spain). They were more prevalent than farm advisors had expected (63% of the fields). In general, perceptions of nematode problems are based on observing signs of plant damage rather than on nematode surveys [34]. As a result, diseases caused by nematodes can be frequently overlooked in fields with low to moderate infestation levels where root galls (caused by *Meloidogyne*) and root lesions (caused by *Pratylenchus*) are not so easy to be seen.

Meloidogyne hapla was the most prevalent and abundant species, occurring at 71% of the sites, similar to other strawberry growing areas in Canada and Bulgaria [3–5]. Two other *Meloidogyne* species were present, *M. incognita* (8%) and *M. javanica* (6%), but were not so prevalent. A previous nematological survey on vegetable crops in the area in 1986 [35] reported *M. incognita* in 59% of the sampled fields, but there were no reports of *M. hapla* on strawberry crops until 2002 [8], which indicates that *M. hapla* entered the area at a later stage. The higher prevalence and abundance of *M. hapla* at present indicates that strawberry could be a better host for it than for other RKN species, since *Meloidogyne incognita* and *M. javanica* are highly prevalent in other areas of Spain where there are similar agroclimatic conditions

but on solanaceous or cucurbitaceous host crops, where the prevalence of RKN is crop-dependent [34]. Furthermore, some strawberry cultivars have been reported as being resistant or even immune to *M. incognita* and *M. javanica*, although the host status of strawberry to these two RKN species is still unclear [21,36].

Pratylenchus penetrans has been reported as a pathogen for strawberries and was present in 20% of the strawberry fields sampled in Huelva, but with a lower abundance than *M. hapla*. Root lesion nematodes can interact with other soil pathogens, causing complex diseases in strawberries [37], such as *Macrophomina phaseolina* or *Fusarium*, which appear in the area [38,39].

Hemicycliophora spp. were found in 20% of the fields. These nematodes have been reported to cause an early stunting of strawberry plants and yield reductions since 2010 in Huelva [10].

The high prevalence of plant-parasitic nematodes in strawberry crops in Huelva and the lack of previous reports concerning nematodes, such as *M. hapla* or *Hemicycliophora*, suggest a recent introduction of these nematodes, possibly through the nursery stocks. The sandy soils, which are dominant in the area, are conducive to the spread of nematodes, which, apart from monoculture and strawberry crop intensification, are factors contributing to their spreading.

The wide distribution of plant-parasitic nematodes in the main strawberry growing area of Spain poses a risk for the crop. Despite annual soil disinfestations in 84% of the fields, farm advisors estimated that 6% of their yield losses were due to nematodes. Furthermore, if soils were not disinfested or less effective disinfestation techniques were used, yield losses would probably rise.

Regarding the potential damage of *M. hapla* to strawberry, the tolerance limit (*T*) of 0.2 J2 per g soil shows that strawberry is susceptible to damage by *M. hapla*, with similar tolerance limit as other crops, such as alfalfa, carrot, eggplant, and potato [40–43]. However, strawberry is less tolerant to *M. hapla* than spinach (I = 5–20 J2 per g of soil) [44], lettuce (*T* = 7–8) [45], or tomato (*T* = 1–3) [46,47]. Maximum yield losses in strawberry biomass were estimated at 43%, and these results agree with those (30–50%) calculated for other crops, such as tomato [46,47]. Our results are based on the interaction of *M. hapla* with strawberry over a 90 day cycle, but usually the crop cycle in the field lasts up to 180–210 days, and therefore, potential damage could be higher, owing to additional generations of nematodes and increasing numbers of juveniles reinvading the roots and causing added root damage. Nonetheless, strawberries are grown from autumn to late spring (October–May) in Southern Spain, and there may be only a slight increase in population densities during the winter months (December–early March) due to soil temperatures being below the nematode root invasion threshold of 8–10 °C [48].

Meloidogyne hapla on 'Fortuna' strawberries could reproduce and increase its population densities by a multiple of 2.4, 90 days after inoculation, when *Pi* = 1 J2 per g of soil. Values for maximum final population density (*M*), maximum multiplication rate (*a*), and the equilibrium density (*E*) were low compared to other RKN species in vegetable hosts [14,49–51], and low values of *a*, *M*, and *E* have been related to not only poor host status but also to good hosts in unfavorable conditions [15]. Several studies have observed that *M. hapla* parasitizes and reproduces on most strawberry cultivars, but multiplication rates varied depending on the cultivar, ranging from 0.2 to 25, though most cultivars showed multiplication rates below 4 [21–23]. We have tested the most common cultivars cropped in Spain, and all of them were able to maintain or slightly increase the *Pi* successfully, with multiplication rates from 1.4 to 4.2. Cultivars Marisol and Rociera were the best choice for nematode management since the increase in *M. hapla* populations after a cropping cycle would be minimum. In general, strawberries respond as poor hosts for *M. hapla*, and only after long periods of time, nematode densities may reach high levels in the soil. Yield losses in the cultivars tested varied between 8 and 19%, which suggests a range of tolerance to *M. hapla*.

Conventionally, broad-spectrum fumigants have been used to effectively reduce nematode populations and to increase strawberry yields, but the use of these has fallen drastically in the European Union [16,17]. At present, 1,3-dichloropropene:chloropicrin is not included among substances permitted in the European Union (European Directive 2009/128CE), and it can only be used with

temporary permits. Therefore, the only soil fumigants currently authorized in the EU are dazomet, metham-sodium, and metham-potassium.

In the field trials, the most effective treatments for reducing *M. hapla* soil densities were 1,3-dichloropropene:chloropicrin, methyl iodide:chloropicrin, and dimethyl disulfure, whose efficacies ranged from 78% to 87%, which is in keeping with the perception farm advisors have about how efficient soil fumigants are, since they ranked 1,3-dichloropropene:chloropicrin as being the most efficient method, followed by dazomet and metam-sodium. The fumigant 1,3-dichloropropene:chloropicrin has proved to be effective in controlling root-knot nematode globally [16–19], and these field trials confirmed these results. When there is high nematode pressure, 1,3-dichloropropene:chloropicrin may be the best option for controlling nematodes adequately and reaching profitable yields, since even at *Pi* >100 J2 per 100 g of soil, soil fumigation with 1,3-dichloropropene:chloropicrin reduces soil nematode densities below the tolerance limit. Soil fumigation with 1,3-dichloropropene:chloropicrin was more effective and consistent in suppressing *B. longicaudatus* populations in strawberry crops in Florida compared to other chemical nematicides, such as metam-sodium, dazomet, fluensulfone, fluazaindolizine, or fluopyram [19]. Furthermore, mixtures of 1,3-dichloropropene and chloropicrin work synergistically to control a wide range of plant pathogens and pests, which include fungi, nematodes, insects, mites, rodents, weeds, and some bacteria and have been recommended as being the best option for controlling soil borne pathogens in many intensive crops.

Despite not being as effective as 1,3-dichloropropene:chloropicrin, other chemicals can provide a sufficient level of nematode control when nematode densities at planting are lower than 100 J2 per 100 g of soil, that is, dimethyl-disulfide reduces nematode densities below the tolerance limit, at *Pi* lower than 90 J2 per 100 g of soil. In addition, dazomet or metam-sodium would be effective enough to reduce soil infestations under the tolerance limit if nematode densities at planting were lower than 47 and 40 J2 per 100 g of soil, respectively.

Previous research has demonstrated that the effectiveness of fumigants could be enhanced by using highly retentive mulches, such as virtually or totally impermeable films (VIF, TIF) [52–54]. Fumigants used under these mulches result in higher overall exposure to lethal concentrations of them and improves the lateral spread of the fumigant across the soil. We found dimethyl-disulfide to be more effective using VIF than PE plastic mulch, which runs counter to the results shown by Gomez-Tenorio et al. [54], who did not find any differences in the efficacy of dimethyl-disulfide in reducing *Meloidogyne* populations under VIF or PE plastic mulch, which was 100% effective when vermiculite was used as a substrate. Other authors found dimethyl-disulfide to be a less effective nematicide in natural soil than in artificial substrates [55,56]. Therefore, the type of soil/substrate seems to influence the results, and in soils where dimethyl-disulfide is not highly effective, using highly retentive plastic mulches can increase its efficacy in reducing RKN densities.

All chemical treatments applied by shank or drip irrigation disinfected the beds but not the alleys/furrows. When using shanks, 1,3-dichloropropene:chloropicrin and dimethyl-disulfide were more effective in reducing *M. hapla* soil densities (94% and 83%, respectively) when compared to drip irrigation (82% and 67%, respectively) ($p < 0.05$), as previously reported for other crops [57,58], and this is probably due to better distribution of the product in the soil as suggested by Schneider et al. [58]. This was also true for dazomet, a dry granular product applied as a broadcast and tilled into the whole field, dazomet was more effective against *P. penetrans* than other bed-applied fumigants by fumigating the soil used for constructing beds but also alleys/furrows. Improved control of other pathogens, such as *M. phaseolina* with broadcast, applied fumigation as opposed to bed fumigation was previously observed in strawberry crops in Huelva [58].

Non-fumigant soil disinfestation in strawberry production, such as steam, biofumigation, solarization, or soil-less cultivation, are not highly effective methods of reducing nematode soil populations or are uneconomical if used on their own [16–20]. However, combining biofumigation with organic manures plus soil solarization has proved to be an efficient way of controlling weeds and soil-borne diseases in strawberries, such as *Rhizoctonia* spp., *Phytophthora cactorum*, and *Macrophomina*

phaseolina, in Turkey and Spain [20,59,60], and in our field trials, soil biosolarization reached efficacies of 73% reduction for *M. hapla* and 67% for *P. penetrans*, which seems to provide sufficient nematode control in most situations. Nonetheless, this technique could be considered a viable alternative to chemical soil fumigation only if it maintains comparable strawberry yields over time.

5. Conclusions

Plant-parasitic nematodes in strawberry crops of Southern Spain are prevalent over 90% of the fields. *Meloidogyne*, *Pratylenchus*, and *Hemicycliophora* are the genera more frequently found. Strawberry is a poor host for *M. hapla* with a tolerance limit of 0.2 J2 per g of soil, and low population increases in cropping cycles of 7–8 months. Strawberry cultivars currently cultivated in Spain show a range of susceptibility and tolerance to *M. hapla*. Nematodes are effectively controlled by chemical fumigation of soils, but soil biosolarization is proposed as an effective alternative to chemical soil fumigation for strawberry cultivation in Southern Spain.

Author Contributions: M.T., S.V.-L., and L.M. conceived and designed the experiments; J.A.G.-M. and L.M. performed personal interviews and carried out the field experiments; M.T., S.V.-L., M.D.V., and J.A.G.-M. carried out the field sampling for nematodes; M.T., S.V.-L., and M.D.V. carried out the pot experiments in growth chambers; M.T. and S.V.-L. analyzed the data; M.T. and S.V.-L. wrote the paper.

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References

1. FAOSTAT. Available online: http://www.fao.org/faostat (accessed on 16 January 2019).
2. Ministerio de Agricultura, Pesca y Alimentación. *Anuario de Estadística 2017*. Available online: https://www.mapa.gob.es/es/estadistica/temas/publicaciones/anuario-de-estadistica/2017/ (accessed on 16 January 2019).
3. Brown, D.J.F.; Dalmasso, A.; Trudgill, D.L. Nematode pests of soft fruits and vines. In *Plant Parasitic Nematodes in Temperate Agriculture*; Evans, K., Trudgill, D.L., Webster, J.M., Eds.; CAB International: Wallingford, UK, 1993; pp. 427–462. ISBN 978-0851988085.
4. Bélair, G.; Khanizadeh, S. Distribution of plant-parasitic nematodes in strawberry and raspberry fields in Quebec. *Phytoprotection* **1994**, *75*, 101–107. [CrossRef]
5. Samaliev, H.Y.; Mohamedova, M. Plant-parasitic nematodes associated with strawberry (*Fragaria ananassa* Duch.) in Bulgaria. *Bulg. J. Agric. Sci.* **2011**, *17*, 730–735.
6. Nyoike, T.W.; Mekete, T.; McSorley, R.; Weibelzahl-Karigi, E.; Liburd, O.E. Confirmation of *Meloidogyne hapla* on strawberry in Florida using molecular and morphological techniques. *Nematropica* **2012**, *42*, 253–259.
7. Noling, J.W. Estimating strawberry yield and sting nematode impacts using counts of plant sizes and fruit stems. *Proc. Fla. State Hort. Soc.* **2011**, *124*, 197–201.
8. Vega, J.M.; Páez, J.; López-Aranda, J.M.; Medina, J.J.; Miranda, L.; Montes, F. Nematodes on strawberries in Southern Spain. Effects on yield and control of *Meloidogyne hapla*. *Nematology* **2002**, *4*, 309.
9. RAIF. *Balance Fitosanitario. Provincia de Huelva. Fresa. Campaña 2016–2017*; Red de Alerta e Información Fitosanitaria, Consejería de Agricultura, Pesca y Desarrollo Rural, Junta de Andalucía: Sevilla, Spain, 2017; pp. 1–7. ISBN 978-8484382422.

10. Bascón, J.C.; Arcos, S.; Páez, J.I.; Avilés, M.; Robertson, L.; Vega, J.M.; Navas, A. *Hemicycliophora* spp. ¿Un nuevo problema en el cultivo de la fresa en Huelva? In Proceedings of the Resúmenes del XVI Congreso de la Sociedad Española de Fitopatología, Málaga, Spain, 17–21 September 2012; p. 336.
11. Peña-Santiago, R.; Castillo, P.; Escuer, M.; Guerrero, P.; Talavera, M.; Vieira, P. *Tylenchid Species (Nematoda, Tylenchida) Recorded in the Iberian Peninsula and the Balearic Islands: A Compendium*; Universidad de Jaén: Jaén, Spain, 2004; pp. 1–127. ISBN 978-8484392422.
12. Seinhorst, J.W. The relation between nematode density and damage to plants. *Nematologica* **1965**, *11*, 137–154. [CrossRef]
13. Ehwaeti, M.E.; Phillips, M.S.; Trudgill, D.L. Dynamics of damage to tomato by *Meloidogyne incognita*. *Fund. Appl. Nematol.* **1998**, *21*, 627–635.
14. Greco, N.; Di Vito, M. Population dynamics and damage levels. In *Root Knot Nematodes*; Perry, R.N., Moens, M., Starr, J.L., Eds.; CAB International: Wallingford, UK, 2009; pp. 246–274. ISBN 978-1845934927.
15. Schomaker, C.H.; Been, T.H. Plant growth and population dynamics. In *Plant Nematology*; Perry, R.N., Moens, M., Eds.; CAB International: Wallingford, UK, 2006; pp. 275–301. ISBN 978-1845930561.
16. López-Aranda, J.M.; Miranda, L.; Medina, J.J.; Soria, C.; de los Santos, B.; Romero, F.; Pérez-Jiménez, R.; Talavera, M.; Fennimore, S.A.; Santos, B.M. Methyl bromide alternatives for high tunnel strawberry production in southern Spain. *HortTechnology* **2009**, *19*, 187–192. [CrossRef]
17. López-Aranda, J.M.; Domínguez, P.; Miranda, L.; de Los Santos, B.; Talavera, M.; Daugovish, O.; Soria, C.; Chamorro, M.; Medina, J.J. Fumigant use for strawberry production in Europe: The Current Landscape and Solutions. *Int. J. Fruit Sci.* **2016**, *16*, 1–15. [CrossRef]
18. Medina, J.J.; Miranda, L.; Domínguez, P.; Soria, C.; Pérez-Jiménez, R.M.; Zea, T.; Talavera, M.; Velasco, L.; Romero, F.; De los Santos, B.; et al. Comparison of different chemical and non-chemical alternatives to Methyl Bromide for strawberry in Huelva (Spain). *J. Berry Res.* **2012**, *2*, 113–121. [CrossRef]
19. Watson, T.T.; Desaeger, J.A. Evaluation of non-fumigant chemical and biological nematicides for strawberry production in Florida. *Crop Prot.* **2019**, *117*, 100–107. [CrossRef]
20. Domínguez, P.; Miranda, L.; Medina, J.J.; de los Santos, B.; Talavera, M.; Daugovish, O.; Soria, C.; Chamorro, M.; López-Aranda, J.M. Evaluation of non-fumigant alternative soil treatments for strawberry production in Huelva (Spain). *Int. J. Fruit Sci.* **2016**, *16*, 28–36. [CrossRef]
21. Edwards, W.H.; Jones, R.K.; Schmitt, D.P. Host suitability and parasitism of selected strawberry cultivars by *Meloidogyne hapla* and *M. incognita*. *Plant Dis.* **1985**, *69*, 40–42. [CrossRef]
22. Khanizadeh, S.; Belair, G.; Lareau, M.J. Relative susceptibility of five strawberry cultivars to *Meloidogyne hapla* under three soil water deficit levels. *Phytoprotection* **2012**, *75*, 133–137. [CrossRef]
23. Pinkerton, J.; Finn, C.E. Responses of strawberry species and cultivars to the root-lesion and northern root-knot nematodes. *HortScience* **2005**, *40*, 33–38.
24. Dale, A.; Potter, J. Strawberry cultivars vary in their resistance to northern lesion nematode. *J. Nematol.* **1998**, *30*, 577–580. [PubMed]
25. Verdejo-Lucas, S.; Talavera, M. Pathogenic potential. parasitic success and host efficiency of *Meloidogyne incognita* and *M. javanica* on cucurbitaceous plant genotypes. *Eur. J. Plant Pathol.* **2018**. [CrossRef]
26. SIOSE 2013. Base Cartográfica de Ocupación del Suelo en Andalucía. Available online: http://www.juntadeandalucia.es/medioambiente/mapwms/REDIAM_siose_2013 (accessed on 30 March 2017).
27. Whitehead, A.G.; Hemming, J.R. A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Ann. Appl. Biol.* **1965**, *55*, 25–38. [CrossRef]
28. Hartman, K.M.; Sasser, J.N. Identification of *Meloidogyne* species on the basis of differential host test and perineal-pattern morphology. In *An Advanced Treatise on Meloidogyne. Biology and Control*; Barker, K.R., Carter, C.C., Sasser, J.N., Eds.; North Carolina State University: Raleigh, NC, USA, 1985; Volume I, pp. 69–77. ISBN 978-0931901014.
29. Esbenshade, P.R.; Triantaphyllou, A.C. Electrophoretic methods for the study of root-knot nematode enzymes. In *An Advanced Treatise on Meloidogyne. Methodology*; Barker, K.R., Carter, C.C., Sasser, J.N., Eds.; North Carolina State University: Raleigh, NC, USA, 1985; Volume II, pp. 115–123. ISBN 978-0931901022.
30. Hussey, R.S.; Barker, K.R. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. *Plant Dis. Rep.* **1973**, *57*, 1025–1028.
31. Hussey, R.S.; Janssen, G.J.W. Root-knot nematode species. In *Plant Resistance to Parasitic Nematodes*; Starr, J.L., Cook, R., Bridge, J., Eds.; CAB International: Wallingford, UK, 2002; pp. 43–70. ISBN 978-0851994660.

32. López-Aranda, J.M.; Medina, J.J.; Marsal, J.I.; Bartual, R. Strawberry production in Spain. In *The Strawberry: A Book for Growers. Others*; Childers, N.F., Ed.; IFAS Publ. University Florida: Gainesville, FL, USA, 2002; pp. 230–237. ISBN 978-0938378112.
33. Schneider-Orelli, O. *Entomologisches Praktikum*; Verlag Sauerländer: Aarau, Germany, 1947; pp. 1–149.
34. Talavera, M.; Sayadi, S.; Chirosa-Ríos, M.; Salmerón, T.; Flor-Peregrín, E.; Verdejo-Lucas, S. Perception of the impact of root-knot nematode-induced diseases in horticultural protected crops of south-eastern Spain. *Nematology* **2012**, *14*, 517–527. [CrossRef]
35. Andreu, M.; Salmerón, T.; Martínez, V.; Tobar, A. Nematodos fitoparásitos de los cultivos hortícolas de Trigueros (Huelva). *Bol San. Veg. Plagas* **1986**, *12*, 319–322.
36. Horst, R.K. Nematodes. In *Westcott's Plant Disease Handbook*; Horst, R.K., Ed.; Springer: Dordrecht, The Netherlands, 2013; pp. 251–268. ISBN 978-9400721401.
37. Lamondia, J.A.; Martin, S.B. The influence of *Pratylenchus penetrans* and temperature on black root rot of strawberry by binucleate *Rhizoctonia* spp. *Plant Dis.* **1989**, *73*, 107–110. [CrossRef]
38. Avilés, M.; Castillo, S.; Bascón, J.; Zea-Bonilla, T.; Martín-Sánchez, P.M.; Pérez-Jiménez, R.M. First report of *Macrophomina phaseolina* causing crown and root rot of strawberry in Spain. *Plant Pathol.* **2008**, *57*, 382. [CrossRef]
39. Avilés, M.; Bascón, J.; Gallardo, M.A.; Orta, M.S.; Borrero, C. New foci of strawberry Fusarium wilt in Huelva (Spain) and susceptibility of the most commonly used cultivars. *Sci. Hortic.* **2017**, *226*, 85–90. [CrossRef]
40. Inserra, R.N.; O'bannon, J.H.; Di Vito, M.; Ferris, H. Response of two alfalfa cultivars to *Meloidogyne hapla*. *J. Nematol.* **1983**, *15*, 644–645.
41. Gugino, B.K.; Abawi, G.S.; Ludwig, J.W. Damage and management of *Meloidogyne hapla* using oxamyl on carrot in New York. *J. Nematol.* **2006**, *38*, 483–490.
42. Watanabe, T.; Masumura, H.; Kioka, Y.; Noguchi, K.; Min, Y.Y.; Murakami, R.; Toyota, K. Development of a direct quantitative detection method for *Meloidogyne incognita* and *M. hapla* in andosol and analysis of relationship between the initial population of *Meloidogyne* spp. and yield of eggplant in an andosol. *Nematol. Res.* **2013**, *43*, 21–29. [CrossRef]
43. Brodie, B.B.; Evans, K.; Franco, J. Nematode parasites of potatoes. In *Plant Parasitic Nematodes in Temperate Agriculture*; Evans, K., Trudgill, D.L., Webster, J.M., Eds.; CAB International: Wallingford, UK, 1993; pp. 87–132. ISBN 978-0851988085.
44. Potter, J.W.; Olthof, T.H.A. Yield losses in fall-maturing vegetables relative to population densities of *Pratylenchus penetrans* and *Meloidogyne hapla*. *Phytopathology* **1974**, *64*, 1072–1075. [CrossRef]
45. Viaene, N.; Abawi, G.S. Damage Threshold of *Meloidogyne hapla* to Lettuce in Organic Soil. *J. Nematol.* **1996**, *28*, 537–545.
46. Potter, J.W.; Olthof, T.H.A. Effects of population densities *Meloidogyne hapla* on growth and yield of tomato. *J. Nematol.* **1977**, *9*, 296–300.
47. Aalders, L.T.; Minchin, R.; Hill, R.A.; Braithwaite, M.; Bell, N.L.; Stewart, A. Development of a tomato/root knot nematode bioassay to screen beneficial microbes. *N. Z. Plant Prot.* **2009**, *62*, 28–33.
48. Lahtinen, A.E.; Trudgill, D.L.; Thlikkala, K. Threshold temperature and minimum time requirements for the complete life cycle of *Meloidogyne hapla* from northern Europe. *Nematologica* **1988**, *34*, 443–451.
49. Giné, A.; López-Gómez, M.; Vela, M.D.; Ornat, C.; Talavera, M.; Verdejo-Lucas, S.; Sorribas, F.J. Thermal requirements and population dynamics of root-knot nematodes on cucumber and yield losses under protected cultivation. *Plant Pathol.* **2014**, *63*, 1446–1453. [CrossRef]
50. Vela, M.D.; Giné, A.; López-Gómez, M.; Sorribas, F.J.; Ornat, C.; Verdejo-Lucas, S.; Talavera, M. Thermal time requirements of root-knot nematodes on zucchini-squash and population dynamics with associated yield losses on spring and autumn cropping cycles. *Eur. J. Plant Pathol.* **2014**, *140*, 481–490. [CrossRef]
51. López-Gómez, M.; Giné, A.; Vela, M.D.; Ornat, C.; Sorribas, F.J.; Talavera, M.; Verdejo-Lucas, S. Damage functions and thermal requirements of *Meloidogyne javanica* and *Meloidogyne incognita* on watermelon. *Ann. Appl. Biol.* **2014**, *165*, 466–473. [CrossRef]
52. Santos, B.M.; Gilreath, J.P.; Motis, T. Managing nutsedge and stunt nematode in pepper with reduced Methyl Bromide plus chloropicrin rates under Virtually Impermeable Films. *HortTechnology* **2005**, *15*, 596–599. [CrossRef]

53. Cabrera, J.A.; Hanson, B.D.; Gerik, J.S.; Gao, S.; Qin, R.; Wang, D. Pre-plant soil fumigation with reduced rates under low permeability films for nursery production, orchard and vineyard replanting. *Crop. Prot.* **2015**, *75*, 34–39. [CrossRef]
54. Gómez-Tenorio, M.A.; Zanón, M.J.; de Cara, M.; Lupión, B.; Tello, J.C. Efficacy of dimethyl disulfide (DMDS) against *Meloidogyne* sp. and three formae speciales of *Fusarium oxysporum* under controlled conditions. *Crop Prot.* **2015**, *78*, 263–269. [CrossRef]
55. Cabrera, J.A.; Wang, D.; Gerik, J.S.; Gan, J. Spot drip application of dimethyl disulfide as a post-plant treatment for the control of plant parasitic nematodes and soilborne pathogens in grape production. *Manag. Sci.* **2014**, *70*, 115–1157. [CrossRef]
56. Mao, L.; Yan, D.; Wang, Q.; Li, Y.; Ouyang, C.; Liu, P.; Shen, J.; Guo, M.; Cao, A. Evaluation of the Combination of Dimethyl Disulfide and Dazomet as an Efficient Methyl Bromide Alternative for Cucumber Production in China. *J. Agric. Food Chem.* **2014**, *62*, 4864–4869. [CrossRef]
57. Desaeger, J.A.; Seebold, K.W.; Csinos, A.S. Effect of application timing and method on efficacy and phytotoxicity of 1,3-D, chloropicrin and metam-sodium combinations in squash plasticulture. *Pest Manag. Sci.* **2008**, *64*, 230–238. [CrossRef]
58. Schneider, S.M.; Ajwa, H.A.; Trout, T.J.; Gao, S. Nematode control from shank- and drip-applied fumigant alternatives to methyl bromide. *HortScience* **2008**, *43*, 1826–1832. [CrossRef]
59. Chamorro, M.; Domínguez, P.; Medina, J.J.; Miranda, L.; Soria, C.; Romero, F.; López-Aranda, J.M.; Daugovish, O.; Mertely, J.; De los Santos, B. Assessment of chemical and biosolarization treatments for the control of *Macrophomina phaseolina* in strawberries. *Sci. Hortic.* **2015**, *192*, 361–368. [CrossRef]
60. Benlioğlu, S.; Boz, Ö.; Yildiz, A.; Kaşkavalci, G.; Benlioğlu, K. Alternative soil solarization treatments for the control of soil-borne diseases and weeds of strawberry in the western Anatolia of Turkey. *J. Phytopathol.* **2005**, *153*, 423–430. [CrossRef]