Use of Poultry Manure Combined with Soil Solarization as a Control Method for *Meloidogyne incognita* in Carnation

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Abstract

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The effectiveness of a combination of soil solarization and poultry manure (raw or pelletized) amendments for the control of root-knot nematode (*Meloidogyne incognita*) was tested in carnation (*Dianthus caryophyllus*) crops grown in in-ground beds under plastic-covered greenhouse conditions in southern Spain. Our trials demonstrated that soil solarization alone did not provide sufficient control of root-knot nematode, because the carnation growing season in this region only partly coincides with the most effective period for solarization, resulting in an insufficient duration of treatment during a key period for effectiveness. Chemical fumigation with 1,3-dichloropropene + chloro-

picrin prior to planting was effective in reducing nematode population densities in soil. Its effects spanned 9 months after planting, resulting in acceptable crop yields. In comparison, the combination of soil solarization and raw or pelletized poultry manure was slightly less effective than chemical fumigation for control of this pathogen but crop yields after 9 months were similar. However, the higher root gall indices observed after 9 months, in comparison with chemically fumigated plots, indicated the need for a reapplication of the organic manure treatment at the start of each successive growing season.

Spain is the second-largest producer of cut flowers within the European Union (EU). A total of 2,236 ha of land is used for production, with an annual yield of 359 million dozens of stems and a commercial value of approximately €600 million (27). The main crop is carnation (*Dianthus caryophyllus* L.), grown mainly in the provinces of Cádiz and Seville, in the south of the country. Production is carried out in ground beds under protected growing conditions in nonheated plastic greenhouses of usually less than 5,000 m². Rooted carnation cuttings are imported from The Netherlands, Israel, Italy, or other Spanish regions and planted from mid-June until the end of July. The first stems are harvested in late November, with subsequent harvests occurring at determined intervals until May to June of the following year, when temperatures become too high to obtain good-quality flowers. At this time, some farmers rotate with a spring-summer vegetable crop (tomato, melon, or watermelon), while others prune the carnation plants and discontinue harvesting until the end of the summer, when cropping is resumed. In the latter case, the crop is maintained in the field for as long as 2 years (32).

Nematodes of the genus *Meloidogyne* are among the most destructive soilborne carnation pathogens, causing economic losses of between 10 to 20% annually (5). Conventionally, the control of this pathogen has been carried out using chemical soil fumigants, such as methyl bromide and 1,3-dichloropropene + chloropicrin. The phasing out of methyl bromide (37) and the European ban on 1,3-dichloropropene, coupled with curtailments in the use of other nematicides (10), has led to a shortage of viable alternatives for soil disinfestation in EU countries. Alternative chemical fumigants such as dimethyl-disulfide, recently approved by the United States

Environmental Protection Agency for agricultural use (7,9), are yet to be registered in Europe.

The sole use of nonchemical control methods for soilborne plant pathogens is often inadequate, given their narrow spectrum of activity or lack of consistency, in comparison with methyl-bromide fumigation (4,11,18). Among these nonchemical alternatives, soil solarization has proven effective in the management and control of many soilborne pathogens for a variety of crops, primarily through physical modes of action (3,12,15,23,30). Nevertheless, in some cases, this method has been less effective in the control of root-knot nematodes (38). A possible explanation could be the mobility of their juveniles through soil temperature gradients that may enable them to seek refuge at deeper soil layers (8,21), where soil temperatures do not reach the lethal threshold. At a later point in time, after planting, the nematodes are able to recolonize the soil and infect host plants (35,38). In addition, when the planting and growing season overlaps with the optimum period for soil solarization, usually during the hottest months of the year, the effectiveness of this method is impaired, due to the duration of the solarization period falling short of the required minimum.

The viability of soilborne plant pathogens has been shown to be compromised by N-rich organic amendments, such as poultry manure, through the liberation of toxic volatiles, including ammonia (NH₃) and nitrous acid (HNO₂) (6,22,24,31,36). This strategy, used in combination with soil solarization, which consists of using a transparent plastic film that limits gas emission to the atmosphere and increases soil temperature, has been shown to further improve the control of soilborne plant pathogens (11,13,14,26,34).

Current available control options for root-knot nematodes in carnation production are restricted to a limited number of variably effective options, including soil solarization, biofumigation, newer nematicides, and a few resistant cultivars (5). Given the toxicological and environmental drawbacks associated with purely chemical methods for pathogen control, more intensified research is underway in an attempt to meet the demands for safer and more effective control of pathogens in the context of large-scale agricultural production.

To the best of our knowledge, no field studies comparing the use of soil solarization in combination with poultry manure amend-

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ments with chemical methods for the control of root-knot nematodes in carnation production have yet been carried out. Our objective was to compare the effectiveness of repeated applications of poultry manure amendments with the current industry standard of chemical fumigation of soil using 1,3-dichloropropene + chloropicrin on *Meloidogyne* soil populations and the development of root-knot galls in carnation, and to assess the effect of each method on disease incidence and crop yields.

Materials and Methods

Study site. From 2005 to 2010, a series of three field experiments (experiment 1: 2005–06, experiment 2: 2007–08, experiment 3: 2009–10) was carried out in an experimental unheated plastic greenhouse (1,000 m²) at the IFAPA Centre in Chipiona, Cádiz, Spain (36°44'56"N, 6°24'06"W). In-ground beds containing loamy-sand soil (84% sand, 10% silt, and 6% clay), pH 7.7, electric conductivity 3.8 dS/m, and 0.5% organic matter were disinfested with 1,3-dichloropropene 81.9% + chloropicrin 46.5% (wt/vol) (Telopic c-35) by injection at a dosage of 40 ml/m² into the irrigation system. After covering the soil surface with a transparent polyethylene film for 2 weeks, the soil was uncovered and left to aerate for an additional 4 weeks prior to nematode inoculation.

Infestation of field soil with *Meloidogyne incognita*. Females, eggs, and juveniles of *Meloidogyne incognita* (Kofoid & White) Chitwood were recovered from tomato (*Solanum lycopersicum* L.) roots (20) and soil samples (39) from an experimental field at the IFAPA Centre of Chipiona. Nematodes were identified by perineal pattern of the females (19) and sequence-characterized amplified region polymerase chain reaction (41). For reproduction and population maintenance, eggs and juveniles were used to inoculate susceptible tomato 'Brillante' plants grown in pots.

Field soil was artificially infested by transplanting infected tomato Brillante plants into the field (2.5 plants/m²), where they were allowed to grow for 6 weeks. Aerial parts of the plants were then removed, leaving the buried root systems in the soil for an additional 8 weeks. The soil was then carefully crosswise tilled, drip irrigated until field capacity, and prepared for treatments.

Experimental design. The field was divided into 48 individual plots of 24 m². Prior to treatments, soil nematode population densities were estimated for each plot by removing eight soil cores, which were bulked in one composite sample, and determining nematode counts from two subsamples of 250 cm³ in accordance with the Whitehead tray method (39). Fifteen plots presenting the most homogeneous nematode population densities were chosen for this study. Treatments were applied to 12 (experiments 1 and 3) or 15 (experiment 2) individual plots using a randomized complete block design with three replications. As controls, nontreated and 1,3-dichloropropene + chloropicrin-fumigated plots were included for comparison. Plots receiving organic poultry manure amendments were maintained in the same location throughout the three consecutive experiments, because these amendments are believed to have a cumulative effect on long-term pathogen control, attributable to a gradual shift in microbial populations (25). Non-

treated and chemically fumigated plots were relocated in experiments 2 and 3, following the same procedure described above, with the aim of using a pool of plots with homogeneous initial average nematode population densities in every experiment. One-way analyses of variance (ANOVAs) were performed every year for nematode population densities before treatments to check for homogeneity among plots.

Remaining plots not included in the experimental design were planted every year with either carnation 'Master' (susceptible to *Fusarium oxysporum* f. sp. *dianthi* but resistant to *Meloidogyne* spp.) (28) in order to check for the presence of Fusarium wilt symptoms, or with 'Picaro' (susceptible to *Meloidogyne* spp.) to maintain a rank of nematode population densities in soils in different plots.

Soil treatments, rates and dates of application, carnation planting dates, and harvest periods for each field experiment are listed in Table 1. All treatments were repeated in at least two of the three cropping seasons over the course of this study.

During the period of soil treatment in experiment 2, soil temperatures were recorded at a 15- and 30-cm depth every 5 min, in soil solarization, poultry manure, and nontreated control plots, using a Campbell datalogger (Campbell Scientific Spain S.L., Barcelona, Spain); then, the average temperatures for every hour were calculated as hourly temperatures. Daily maximum hourly temperatures were compared for each treatment.

Application of soil treatments. Prior to all applications, the soil in each individual plot was thoroughly tilled, using a rotary cultivator (Rotavator; Howard Iberica S.A., Granollers, Spain), and subsequently sprinkler irrigated during two consecutive days (water at 58 liters/m²) to ensure soil moisture at a minimum depth of 20 cm.

Applications of raw-poultry or pelletized-poultry manure. Raw poultry manure (RPM) was obtained from nearby chicken farms and had a 38.72% C and 3.42% N content. Orgevit, a commercial, pelletized product (Memon B. V., Arnhem, The Netherlands), had relatively higher contents of these two elements (44.99% C and 5.33% N) and was used as the source of pelletized poultry manure (PPM). Both products were uniformly distributed onto the surface of the soil (initially at 5 and 3 kg m⁻² for RPM and PPM, respectively), and subsequently incorporated into the upper 15-cm layer, using crosswise plowing. Plots were then drip irrigated until the soil reached field capacity. Dosages of the poultry manure amendments were reduced by 50% from the second year of application. Each plot was covered with transparent polyethylene film after application.

Application of 1,3-dichloropropene 81.9% + chloropicrin 46.5% (wt/vol) (Telopic c-35 EC). A network of 14 polyethylene drip-irrigation tubes (spaced 25 cm apart), interconnected at the border of each plot, was placed over the soil of each plot. Dripper tubes (4 liters/h) were spaced at 33-cm intervals (12 drippers/m²). Virtually impermeable films were used to cover the treated plots prior to chemical applications; then, the plots were irrigated (11 liter/m²) in order to slightly moisten the soil. Telopic c-35 EC was then applied to the soil using this irrigation system (1) with a Venturi device that injected the dosage of the compound in a water flux that was

Table 1. Soil treatments applied to a field infested by *Meloidogyne incognita* prior to planting with carnation 'Picaro', with dates and rates of treatments and planting and harvest dates^z

Experiment 1 (2005–06)	Experiment 2 (2007–08)	Experiment 3 (2009–10)
Nontreated control	Nontreated control	Nontreated control
SS (24 June–22 July 2005)	SS (5 June–20 July 2007)	
RPM (5 kg m ⁻²) + SS (24 June–22 July 2005)	RPM (2.5 kg m ⁻²) + SS (5 June–20 July 2007)	RPM (2.5 kg m ⁻²) + SS (14 July–17 August 2009)
	PPM (3 kg m ⁻²) + SS (5 June–20 July 2007)	PPM (1.5 kg m ⁻²) + SS (14 July–17 August 2009)
1,3-D 81.9% + Pic 46.5% (40 ml m ⁻²) (7–22 July 2005)	1,3-D 81.9% + Pic 46.5% (40 ml m ⁻²) (22 June–14 July 2007)	1,3-D 81.9% + Pic 46.5% (40 ml m ⁻²) (5–17 August 2009)
Planting date: 28 July 2005	Planting date: 20 July 2007	Planting date: 27 August 2009
Harvest period: December 2005–May 2006	Harvest period: December 2007–May 2008	Harvest period: December 2009–April 2010

^z SS = soil solarization, RPM = raw poultry manure, PPM = pelletized poultry manure, 1,3-D = 1,3-dichloropropene, and Pic = chloropicrin.

simultaneously distributed (22 liter/m²) in all three replications of each treatment. A final irrigation (11 liter/m²) followed to wash the tubes and seal the treatments. After 12 to 33 days, all plastic films were removed and the soil was left to aerate for 5 days.

Crop management. One raised bed (0.8 by 6 m) was created in the center of each plot and about 120 rooted carnation cuttings of *D. caryophyllus* Picaro (susceptible to root-knot nematode) were planted in four rows of plants (20 cm between rows and 20 cm between plants within each row).

Standard regional growing practices were employed throughout the crop period, including the use of a drip-irrigation system to provide complete nutrient fertigation. Weeds were removed manually during and between crops. The crop was finished by 9 months after planting. Glyphosate 36% (Roundup; 6 liter/ha) was used to kill the carnation plants at the end of each crop cycle, upon which they were removed from the greenhouse.

Assessment of root-knot nematode population densities in soil. Composite soil samples, consisting of eight soil cores extracted from the center of the rows to a depth of 20 cm with a sampling tube (2.5 cm in diameter), were collected from each plot in order to estimate soil nematode population densities before treatments (P_i), at planting (P₀), and 6 (P₆) and 9 (P₉) months after planting. The samples were mixed thoroughly and the nematodes extracted from 250-cm³ soil subsamples, using the Whitehead tray method (38). Second-stage juveniles (J2) that migrated into the water were collected 1 week later, concentrated by sieving through a 25-μm-pore sieve, counted, and expressed as J2s per 100 cm³ of soil (mean ± standard error).

Assessment of disease incidence, severity of infections, and carnation yields. To assess disease severity at P₆ and P₉, four root systems per plot were dug from the soil and gall index was rated on a proportional scale of 0 to 10, with 0 representing a well-developed and healthy root system and 10 representing plants with 100% of the root system galled and completely dead (40). With the exception of the initial June-to-October period, the accumulated number of dead plants was recorded at 3- to 6-week intervals, until the end of the experiment, with a total of eight assessments made per experiment. On this basis, epidemic curves for disease development were plotted and the standardized area under the diseased progress curve (stAUDPC) was calculated throughout the whole experimental period.

Throughout the cropping cycle, flowers from 80 plants in both experiment 1 and 2 and 88 plants in experiment 3 were harvested beginning at the paint brush stage. Flowers were harvested only from plants in the center of each plot, in order to disregard any

border effects. Yield was expressed as the accumulated total flower stems (stems per square meter) harvested throughout each experiment. The quality of yields was assessed through classification into three standard marketable categories, based on flower stem length (extra: 60 to 70 cm, prime: 50 to 60 cm, and second: <50 cm). For all experiments, poor-quality stems were not harvested. Although the percentage of unharvested stems is not shown, it can be easily calculated from the difference between 100% and the addition of the percentages of the three commercial categories.

Statistical analyses. Statistical analyses were performed using Statistix 9.0 software (Analytical Software, Tallahassee, FL). ANOVA and Pearson correlation analyses between variables were performed for (i) soil nematode population densities at different sampling times, (ii) root galling at P₆ and P₉, (iii) percentage of dead plants, (iv) stAUDPC, (v) accumulated production of flower stems, and (vi) percent yield of flower quality categories. Shapiro-Wilk and Levene's tests were performed to check for normality and homoscedasticity of variables. When variables did not fulfill both requirements, numerical data were log (x + 1) transformed and percentages were arcsine square root transformed before being analyzed by ANOVA. When *F* values were significant (*P* < 0.05), means were compared by the Fisher's protected least significant difference test at 95% confidence interval. Kruskal-Wallis tests were used for variables that did not fit parametric requirements after transformation (i.e., gall index).

Results

Maximum hourly soil temperatures in experiment 2 ranged from 29.3 to 36.2 and 28.4 to 32.1°C in the nontreated control plots at the 15- and 30-cm depths, respectively, compared with the soil-solarized (SS) plots with ranges of 32.3 to 47.6 and 31.5 to 39.1°C, respectively. For RPM+SS treatment, the maximum hourly temperature ranges were 33.5 to 41.0 and 31.0 to 38.5°C, respectively. Therefore, as compared with the nontreated plots, soil solarization increased these temperatures by 3.1 to 11.5 and 2.8 to 7°C at the 15- and 30-cm depths, respectively, whereas increases in RPM+SS treatment were 4.2 to 4.8 and 2.6 to 6.4°C, respectively.

No symptoms of Fusarium wilt were observed on susceptible Master carnation, nor was this fungus isolated from plants with nematode-induced disease symptoms. Other phytoparasitic nematodes such as *Tylenchus*, *Merlinius*, or *Paratylenchus* spp. were found at very low densities of less than five individuals per 100 cm³ of soil. However, these other species have not been reported as being detrimental to carnation. Consequently, any disease symp-

Table 2. Soil population densities of *Meloidogyne incognita* at different times during the crop cycles of carnation 'Picaro' in three experiments

Treatments ^z	Nematode (J2) soil population densities ^y			
	P _i	P ₀	P ₆	P ₉
2005–06				
Nontreated	73.7 ± 3.6 a	46.8 ± 6.8 a	472.8 ± 61.7 a	666.3 ± 93.6 a
SS	70.2 ± 6.2 a	47.3 ± 8.0 a	592.2 ± 91.9 a	742.7 ± 102.5 a
RPM+SS	72.8 ± 5.2 a	16.8 ± 2.3 b	151.8 ± 23.7 c	208.3 ± 32.4 c
1,3D+Pic	80.8 ± 5.5 a	8.8 ± 0.7 c	224.8 ± 20.2 b	301.3 ± 28.2 b
2007–08				
Nontreated	59.5 ± 3.2 a	55.8 ± 6.5 a	125.8 ± 33.7 a	227.3 ± 55.0 a
SS	59.0 ± 4.5 a	21.7 ± 3.4 b	63.8 ± 16.2 ab	130.3 ± 36.3 ab
RPM+SS	61.3 ± 2.6 a	16.8 ± 3.4 bc	118.0 ± 25.0 a	256.7 ± 52.4 a
PPM+SS	52.0 ± 2.2 a	11.5 ± 1.7 cd	81.5 ± 27.6 ab	127.3 ± 37.3 ab
1,3D+Pic	56.0 ± 4.1 a	10.5 ± 3.6 d	4.3 ± 0.7 b	9.7 ± 1.6 b
2009–10				
Nontreated	25.3 ± 2.2 a	18.2 ± 0.8 a	233.7 ± 19.5 a	926.7 ± 102.0 a
RPM+SS	26.0 ± 2.1 a	8.5 ± 0.6 b	54.3 ± 9.2 b	109.7 ± 20.0 bc
PPM+SS	26.7 ± 1.4 a	9.5 ± 0.8 b	65.0 ± 14.7 b	230.0 ± 48.0 b
1,3D+Pic	24.0 ± 1.1 a	2.7 ± 0.3 c	33.0 ± 4.5 b	50.1 ± 6.4 c

^y Population densities before treatments (P_i), at planting (P₀), and 6 (P₆) and 9 (P₉) months after planting. J2 = second-stage juvenile. Values are expressed as mean ± standard error. Within an experiment, mean values in each column followed by the same letter do not differ significantly at *P* < 0.05 according to Fisher's protected least significant difference.

^z Three independent experiments were conducted, experiment 1: 2005–06, experiment 2: 2007–08, and experiment 3: 2009–10. Abbreviations: 1,3D = 1,3-dichloropropene, Pic = chloropicrin, PPM = pelletized poultry manure, RPM = raw poultry manure, and SS = soil solarization.

toms observed in the plants such as stunting, yellowing, root galling, or death were attributed to *M. incognita*.

Effect of soil treatments on root-knot nematode population densities in soil. Nematode population densities throughout the three experiments are shown in Table 2. For all three experiments, there were not statistically significant differences in the average initial populations (P_1) of J2s of *M. incognita* in the different treatment plots.

Experiment 1 (2005–06). Soil nematode population densities at P_0 were significantly reduced in the RPM plots and in plots fumigated with 1,3-dichloropropene + chloropicrin ($P = 0.0001$). However, no significant differences between solarized and nontreated plots were found. At P_6 and P_9 , nematode soil population densities for the RPM and chemically fumigated plots remained approximately two to three times lower than the solarized and nontreated control plots. Interestingly, nematode population densities in the soil of RPM plots were even lower than those in the chemically fumigated plots ($P = 0.0060$).

Experiment 2 (2007–08). Nematode population densities at P_0 were reduced by all soil treatments, including plots that were solarized only, but the reductions were significantly greater in the chemically fumigated and PPM-treated plots ($P = 0.0001$). However, only plots treated with 1,3-dichloropropene + chloropicrin maintained nematode population densities lower than those in the nontreated plots at P_6 ($P = 0.0029$) and P_9 ($P = 0.0026$).

Experiment 3 (2009–10). All treatments were effective in reducing the nematode soil population densities at P_0 ($P = 0.0001$). Both RPM and PPM and 1,3-dichloropropene + chloropicrin treatments were effective at maintaining nematode population densities lower than nontreated controls at P_6 ($P = 0.0016$) and P_9 ($P = 0.0003$).

Effect of soil treatments on root-knot disease incidence, severity of infections, and carnation flower yields. The data for root-knot nematode on carnation plants and flower yields are shown in Table 3.

Experiment 1 (2005–06). At P_6 , the percentage of dead plants in the nontreated plots averaged 21.8%, compared with the chemical fumigant and RPM treatments, in which fewer than 1% of the plants had died. However, differences between treatments were not statistically significant. At P_9 , the incidence of dead plants increased to 64 and 72.5% in the solarized and nontreated control plots, respectively. These percentages were significantly higher in comparison with chemically fumigated plots, with a disease inci-

dence under 9%, and RPM-treated plots, with an incidence of 22.8% ($P = 0.0004$). Root gall indices and the stAUDPC values calculated from the disease curves for the different treatments showed a similar pattern, with the highest values observed in nontreated and solarized plots contrasting with the lowest values in the chemically fumigated plots ($P = 0.0007$). Plots treated with RPM produced approximately threefold higher flower yields in comparison with the nontreated controls. Total carnation yield was higher when 1,3-dichloropropene + chloropicrin soil fumigation was applied before crop planting. No differences were observed in stem quality among treatments, except for a higher proportion of second category stems produced in the RPM-treated and chemically fumigated plots. This is mainly attributable to the fact that, in nontreated and solarized plots, the percentage of unharvested stems was higher (*data not shown*).

Experiment 2 (2007–08). During this experiment, the complete death of plants was observed in nontreated controls at only P_9 . This contrasts sharply with the chemically fumigated treatments, in which no plant death occurred, and the low plant death values for plots treated with poultry manure amendments prior to planting ($P = 0.0032$; Table 3). Gall indices were higher in nontreated plots compared with chemically fumigated plots at P_6 ($P = 0.0021$) and P_9 ($P = 0.0010$). The stAUDPC pattern was analogous to that of dead plant incidences, and an inverse relationship was observed for total yield. Yields in chemically fumigated and poultry manure plots were higher than in nontreated control plots ($P = 0.0008$). Yield quality in both chemical and poultry manure soil treatments was better than nontreated control plots ($P = 0.016$ and $P = 0.0001$ for extra and prime category stems, respectively). Intermediate values for all variables analyzed were observed for the soil solarization treatment, which maintained the incidence of plant death to below half that of the nontreated control.

Experiment 3 (2009–10). All treated plots had no incidence of dead plants at P_6 , in contrast with an average incidence of 95.8% death in nontreated control plots. Three months later, the incidence of dead plants in the nontreated control plots was 100%, in contrast to 1,3-dichloropropene + chloropicrin-treated plots with zero incidence and poultry manure-amended plots with disease incidence below 8% ($P = 0.0002$). High values for gall index were observed in samples of carnation roots from nontreated control plots at both P_6 and P_9 , in contrast with 0.5 and 1.6 gall index values for 1,3-dichloropropene + chloropicrin and 1.3–1.9 and 2.2–3.2 gall index

Table 3. Disease incidence, severity, and flower yield of carnation ‘Picaro’ cultivated in a field infested with *Meloidogyne incognita*^u

Treatments ^x	Percentage of dead plants ^v		Gall index ^v		stAUDPC ^y	Yield ^z	Categories (% stems) ^w		
	P ₆	P ₉	P ₆	P ₉			Extra	Prime	Second
2005–06									
Nontreated	21.8 a	72.5 a	4.1 a	5.0 a	77.8 a	75 a	47.3 a	18.3 a	4.7 a
SS	8.4 a	64.0 a	4.4 a	4.9 a	70.8 a	145 ab	56.0 a	22.3 a	5.7 a
RPM	1.0 a	22.8 b	2.9 ab	3.8 a	48.5 b	223 b	54.0 a	22.7 a	13.7 b
1,3D+Pic	0.9 a	8.7 b	0.5 b	0.8 b	20.8 c	362 b	42.7 a	24.3 a	23.7 c
2007–08									
Nontreated	21.7 a	100.0 a	3.1 a	4.1 a	43.0 a	3 a	0.0 a	0.0 a	33.3 a
SS	5.8 a	49.2 b	2.9 a	3.6 a	16.0 ab	76 ab	5.7 ab	40.3 b	38.3 a
RPM	2.5 a	10.0 bc	2.7 a	3.3 a	3.1 b	154 c	12.7 b	49.3 b	30.7 a
PPM	0.0 a	25.4 bc	1.3 ab	2.2 ab	3.2 b	134 bc	14.3 bc	46.7 b	30.7 a
1,3D+Pic	0.0 a	0.0 c	0.8 b	1.1 b	0.0 b	183 c	14.3 b	58.7 b	22.0 a
2009–10									
Nontreated	95.8 a	100.0 a	8.0 a	8.0 a	79.1 a	0 a	0.0 a	0.0 a	0.0 a
RPM	0.0 b	7.6 b	1.3 bc	2.2 b	2.1 b	117 b	0.0 a	41.7 b	51.0 b
PPM	0.0 b	4.2 bc	1.9 b	3.2 b	0.6 b	118 b	0.0 a	38.3 b	53.7 b
1,3D+Pic	0.0 b	0.0 c	0.5 c	1.6 b	0.0 b	141 b	0.0 a	41.0 b	52.3 b

^u Within an experiment, mean values in each column followed by the same letter do not differ significantly at $P < 0.05$ according to Fisher’s protected least significant difference or Kruskal-Wallis tests.

^v P_6 and P_9 = 6 and 9 months after planting, respectively.

^w Categories are based on the stem lengths: extra (60 to 70 cm), prime (50 to 60 cm), and second (<50 cm).

^x Three independent experiments were conducted: experiment 1, 2005–06; experiment 2, 2007–08; and experiment 3, 2009–10. Abbreviations: SS = soil solarization, RPM = raw poultry manure, 1,3D+Pic = 1,3-dichloropropene + chloropicrin, and PPM = pelletized poultry manure.

^y Standardized area under disease progress curve.

^z Yield expressed as stems per square meter.

values for the poultry manure treatments at P₆ and P₉ ($P = 0.0001$), respectively. Similarly, a significant reduction in stAUDPC was found in all treated plots in comparison with the nontreated control ($P = 0.0001$). Although carnation yield was zero harvestable stems in the control plots, yields in the 1,3-dichloropropene + chloropicrin-treated and poultry manure-amended plots averaged 125 stems m⁻². Stem quality was also higher from these soil treatments, based on the higher proportions of prime category stems produced ($P = 0.0001$) even though no extra quality stems were produced for any treatment.

Correlation of root-knot nematode soil population densities, disease incidence, and carnation yields. The correlations between nematode J2 soil population densities and disease variables in the three experiments are shown in Table 4. *M. incognita* J2s populations in soil at P₆ or P₉ were positively correlated, in most cases, with gall indices (with the exception of experiment 1), the incidence of dead plants mainly at P₉ and also at P₆ (except for experiment 2), and stAUDPC (except for experiment 2). Nematode population densities at P₆ or P₉ were significantly negatively correlated with carnation yields (except for 9-month accumulated yields in experiment 1). In experiment 3, populations of J2 post treatment and at P₆ or P₉ were significantly correlated with all disease variables.

Discussion

The root-knot nematode is a particularly difficult pathogen to manage, particularly under protected cultivation conditions, because temperatures inside plastic greenhouses are optimal for *M. incognita* to thrive throughout most of the year (8,17). Furthermore, host plants with long crop cycles, such as carnation, provide sufficient time for nematode populations to increase within the host through several generations (33).

Major advances in the control of plant diseases caused by nematodes have been achieved through the application of organic soil amendments, generally in conjunction with plastic tarping over a period of a few weeks (2,22,26,31,34). Similarly, we have pioneered a method for the control of *Meloidogyne* spp. in carnation crops. Given the satisfactory results obtained with these control methods against Fusarium wilt of carnation (29), reasonable control of *M. incognita* could be expected in soils moderately infested by this nematode where *F. oxysporum* f. sp. *dianthi* is the major phytopathological problem.

In all treatments and experiments conducted in this study, *M. incognita* J2 soil populations and root gall indices increased during the 9-month period after planting and, in most cases, were significantly and positively correlated with the incidence of dead plants

and stAUDPC and negatively correlated with commercial yields. Despite a higher rate of nematode reproduction at the end of each cropping cycle, a marked decrease in soil nematode population densities was observed in all plots during periods of host crop absence between experiments. A decline in nematode population densities between crops has been negatively correlated to accumulated soil temperature increases during this period (16).

The lower reductions of *M. incognita* J2 population densities, lower levels of disease control, and similarity in yields between nontreated plots and SS plots suggests that soil solarization alone is insufficiently effective for nematode control in carnation crops in coastal areas of southern Spain. The reason for this shortcoming is probably attributable to an insufficient temperature increase in the deeper soil layers and an insufficient duration of treatment. These results are consistent with previous reports (12,30,35) showing that the inactivation of *M. incognita* by means of soil solarization requires maximum mean temperatures of over 45°C (38), which only were occasionally reached at the 15-cm depth in experiment 2. This soil temperature is difficult to reach in this region at periods other than the optimal July-to-August solarization months which, unfortunately, coincide with the most appropriate planting season.

Poultry manure amendments combined with soil solarization achieved a noticeable reduction of nematode population density in the soil. Despite a 50% reduction in the dosages applied in experiments 2 and 3, the repeated applications to the same plots throughout the three experiments resulted in effective control of soil nematode populations in experiment 3. However, only moderate control of the pathogen was achieved in experiment 2. Although, in this experiment, both RPM and PPM treatments achieved reductions in nematode population densities at P₀, these were not maintained after 6 or 9 months of growth. Even though nematode soil populations were higher, the incidences of dead plants, stAUDPC, and flower yields were similar to those obtained from chemically fumigated plots. Therefore, we believe that a healthier root system, a better plant nutrient status, or more favorable environmental conditions may have contributed to higher rates of nematode reproduction in the second experiment.

Chemical soil fumigation with 1,3-dichloropropene + chloropicrin consistently achieved significant reductions in soil population densities of J2 nematodes, root gall indices, and the incidences of dead plants for at least 9 months in all three experiments in comparison with the nontreated controls. Consequently, the percentage of dead plants in this treatment was below 9% after the first 9 months of growth. This suggests that soils treated with 1,3-dichloropropene + chloropicrin at the preplanting stage may sustain acceptable yields of carnation stems for at least 9 months and

Table 4. Linear correlation coefficients between soil population densities of *Meloidogyne incognita*, incidence and severity of the root-knot nematode disease, and carnation 'Picaro' yields^y

Variables ^z	J2s post treatment			J2s 6 months			J2s 9 months		
	2005–06	2007–08	2009–10	2005–06	2007–08	2009–10	2005–06	2007–08	2009–10
GI6	0.7575 (0.0043)	0.1823 (0.5156)	0.8551 (0.0004)	0.5284 (0.0788)	0.6898 (0.0044)	0.9258 (0.0000)	0.5384 (0.0709)	0.7214 (0.0024)	0.9356 (0.0000)
GI9	0.7797 (0.0028)	0.2526 (0.3676)	0.8223 (0.0010)	0.4668 (0.1261)	0.7678 (0.0008)	0.8986 (0.0001)	0.4792 (0.1150)	0.7954 (0.0004)	0.9578 (0.0000)
DP6	0.2125 (0.5073)	0.6413 (0.0100)	0.6771 (0.0156)	0.6092 (0.0355)	0.2867 (0.3001)	0.8725 (0.0002)	0.6111 (0.0348)	0.3014 (0.2750)	0.8216 (0.0010)
DP9	0.5480 (0.0651)	0.8025 (0.0003)	0.7434 (0.0056)	0.7421 (0.0057)	0.6443 (0.0095)	0.9361 (0.0000)	0.7201 (0.0083)	0.6213 (0.0134)	0.8891 (0.0001)
stAUDPC	0.5059 (0.0934)	0.7753 (0.0007)	0.6866 (0.0137)	0.6598 (0.0196)	0.3883 (0.1526)	0.8797 (0.0002)	0.6284 (0.0286)	0.3770 (0.1660)	0.8317 (0.0008)
Y6	-0.4241 (0.1695)	-0.5623 (0.0291)	-0.7001 (0.0112)	-0.7323 (0.0066)	-0.7626 (0.0007)	-0.6683 (0.0175)	-0.7371 (0.0062)	-0.7689 (0.0008)	-0.7008 (0.0111)
Y9	-0.2682 (0.3994)	-0.7509 (0.0013)	-0.7809 (0.0027)	-0.4621 (0.1304)	-0.6266 (0.0124)	-0.9062 (0.0000)	-0.4832 (0.1115)	-0.6011 (0.0178)	-0.8782 (0.0002)

^y Nematode soil population densities transformed to log (second-stage juvenile [J2] soil population densities + 1). In parentheses, P values of significance levels for the correlation coefficients.

^z Disease yield variables. Abbreviations: GI6 and GI9 = log (gall indices 6 or 9 months after treatment + 1); DP6 and DP9 = angular transformed data of percentage of dead plants 6 or 9 months after treatment; stAUDPC = standardized area under disease progress curve; Y6 and Y9 = accumulated carnation yields 6 or 9 months after treatment.

possibly for more than 1 year, even when root-knot nematodes are present in the soil. In addition, this chemical fumigation method has proven very satisfactory in the control of Fusarium wilt of carnation, frequently the most damaging and widespread disease of carnation worldwide (3).

For the control of root-knot nematode, our results demonstrated analogous degrees of effectiveness between the current standard chemical treatment and the two poultry manure soil amendment treatments. Nevertheless, at P₀, the higher root gall indices observed in poultry manure plots in comparison with chemically fumigated plots in experiments 1 and 2, suggest limitations to the long-term control of the disease, at least during the first years of treatment, highlighting the need for successive reapplications at the start of each new planting season.

It has been suggested that soil amendments may also have favorable effects on beneficial microorganisms which, in turn, could contribute to the long-term control of pathogens through suppressive mechanisms (34). Research is currently underway to ascertain what other mechanisms, apart from the toxic volatiles released through organic matter decomposition, may be involved in the control of soil pathogens (11,14,24,25,36). Furthermore, after application of organic soil amendments, an overall higher degree of plant vigor, due to an improvement of soil characteristics and higher levels of nutrients, may allow for reductions in fertigation during the growing season without affecting yields.

In recent years, the use of RPM has become more widespread and its efficacy as a method of pathogen control is being confirmed at a practical level by farmers themselves. Because PPM provided a degree of effectiveness similar to that of RPM, the pelletized form may be preferred by farmers due to both convenience and sanitary concerns. This is the first study to compare the effectiveness of RPM or PPM used in combination with solarization and with 1,3-dichloropropene chemical fumigation on *M. incognita* soil populations, root-knot galls, and carnation flower yields. Our results show that the implementation of RPM amendments for nematode control requires further research to optimize sustained effectiveness. Cost savings associated with local availability, lower transportation costs, and farm-waste product recycling are additional factors to be taken into account.

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Literature Cited

- Ajwa, H. A., Trout, T., Mueller, J., Wilhelm, S., Nelson, S. D., Soppe, R., and Shatley, D. 2002. Application of alternative fumigants through drip irrigation systems. *Phytopathology* 92:1349-1355.
- Baptista, M. J., de Souza, R. B., Pereira, W., Carrijo, O. A., Vidal, M. C., and Charchar, J. M. 2006. Solarização do solo e biofumigação no cultivo protegido de tomate. *Hortic. Bras.* 24:47-52.
- Basallote-Ureba, M. J., Vela-Delgado, M. D., Macías, F. J., López-Herrera, C. J., and Melero-Vara, J. M. 2010. Soil chemical treatments for the control of Fusarium wilt of carnation in Spain. *Acta Hortic.* 883:175-179.
- Chellemi, D. O. 2002. Non chemical management of soilborne pests in fresh market vegetable production systems. *Phytopathology* 92:1367-1372.
- Cho, M. R., Kim, J. Y., Song, I. C., Ko, J. Y., Na, S. Y., and Yi, M. S. 1996. Screening of carnation cultivars for resistance to *Meloidogyne incognita*. *J. Nematol.* 28:639-642.
- Conn, K. L., Tenuta, M., and Lazarovits, G. 2005. Liquid swine manure can kill *Verticillium dahliae* microsclerotia in soil by volatile fatty acids, nitrous acid, and ammonia toxicity. *Phytopathology* 95:28-35.
- Coosemans, J. 2005. Dimethyl disulphide (DMDS): a potential novel nematicide and soil disinfectant. *Acta Hortic.* 698:57-63.
- Díez, J. A., and Dusenbery, D. B., 1989. Preferred temperature of *Meloidogyne incognita*. *J. Nematol.* 21:99-104.
- Environmental Protection Agency (EPA). 2010. Pesticide Product Registrations; Conditional Approval of Paladin DMDS. Federal Register (75) 195:62387-62388.
- European Commission. 2007. Commission Decision of 20 September 2007 concerning the non-inclusion of 1,3-dichloropropene in Annex I to Council Directive 91/414/EEC and the withdrawal of authorizations for plant protection products containing that substance. *OJEU-L* 249:11-13.
- Gamliel, A., Austerweil, M., and Kritzman, G. 2000. Non-chemical approach to soilborne pest management-organic amendments. *Crop Prot.* 19:847-853.
- Gamliel, A., and Katan, J. 2009. Control of plant disease through soil solarization. Pages 196-220 in: *Disease Control in Crops: Biological and Environmentally Friendly Approaches*. D. Walters, ed. Blackwell Publishing Ltd., Edinburgh.
- Gamliel, A., and Stapleton, J. J. 1993. Characterization of antifungal volatile compounds evolved from solarized soil amended with cabbage residues. *Phytopathology* 83:899-905.
- Gamliel, A., and Stapleton, J. J. 1993. Effect of soil amendment with chicken compost or ammonium phosphate and solarization on pathogen control, rhizosphere microorganisms, and lettuce growth. *Plant Dis.* 77:886-891.
- González-Torres R., Melero-Vara J. M., Gómez-Vázquez J., and Jiménez-Díaz, R. M. 1993. Use of soil solarization and fumigation to control Fusarium wilt of watermelon in plastic houses. *Plant Pathol.* 42:858-864.
- Goodell, P. B., and Ferris, H., 1989. Influence of environmental factors on the hatch and survival of *Meloidogyne incognita*. *J. Nematol.* 21:328-334.
- Greco, N., and Esmenjaud, D. 2004. Management strategies for nematode control in Europe. Pages 33-43 in: *Nematology Monographs and Perspectives 2*. Proc. Fourth Int. Congr. Nematol. 2002. R. Cook and D. J. Hunt, eds. Tenerife, Spain. Brill. Leiden.
- Gullino, M. L., Camponagara, A., Gasparrini, G., Rizzo, V., Clini, C., and Garibaldi, A. 2003. Replacing methyl bromide for soil disinfection—the Italian experience and implications for other countries. *Plant Dis.* 87:1012-1021.
- Hartman, K. M., and Sasser, J. N. 1985. Identification of *Meloidogyne* species on the basis of differential host test and perineal-pattern morphology. Pages 69-77 in: *An Advanced Treatise on Meloidogyne*, vol. II. Methodology. K. R. Barker, C. C. Carter, and J. N. Sasser, eds. North Carolina State University, Raleigh.
- Hussey, R. S., and Barker, K. R. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. *Plant Dis.* 57:1025-1028.
- Johnson, P. W., and McKeen, C. D. 1973. Vertical movement and distribution of *Meloidogyne incognita* (Nematoda) under tomato in a sandy loam greenhouse soil. *Can. J. Plant Sci.* 53:837-841.
- Kaplan, M., and Noe, J. P. 1993. Effects of chicken-excrement amendments on *Meloidogyne arenaria*. *J. Nematol.* 25:71-77.
- Katan, J. 1981. Solar heating (solarization) of soil for control of soilborne pests. *Annu. Rev. Phytopathol.* 19:211-236.
- Lazarovits, G. 2001. Management of soil-borne plant pathogens with organic soil amendments: a disease control strategy salvaged from the past. *Can. J. Plant Pathol.* 23:1-7.
- Lazarovits, G., Conn, K. L., Abbasi, P. A., and Tenuta, M. 2005. Understanding the mode of action of organic soil amendments provides the way for improved management of soilborne plant pathogens. *Acta Hortic.* 698:215-224.
- López-Pérez, J. A., Roubtsova, T., and Ploeg, A. 2005. Effect of three plant residues and chicken manure used as biofumigants at three temperatures on *Meloidogyne incognita* infestation of tomato in greenhouse experiments. *J. Nematol.* 37:489-494.
- MARM. 2010. Anuario de Estadística del Ministerio de Medio Ambiente y Medio Rural y Marino 2009. Madrid.
- Martini, P., Moreno, E., and Garibaldi, A. 1991. Resistenza del garofano a patogeni diversi di *Fusarium oxysporum* f. sp. *dianthi*. *Culture Protete* 10:97-102.
- Melero-Vara, J. M., López-Herrera, C. J., Prados-Ligero, A. M., Vela-Delgado, M. D., Navas-Becerra, J. A., and Basallote-Ureba, M. J. 2011. Effects of soil amendment with poultry manure on carnation Fusarium wilt in greenhouses in southwest Spain. *Crop Prot.* 30:970-976.
- Nico, A. I., Jiménez-Díaz, R. M., and Castillo, P. 2003. Solarization of soil in piles for the control of *Meloidogyne incognita* in olive nurseries in southern Spain. *Plant Pathol.* 52:770-778.
- Piedra Buena, A., García-Álvarez, A., Díez-Rojo, M. A., Ros, C., Fernández, P., Lacasa, A., and Bello, A. 2007. Use of pepper crop residues for the control of root-knot nematodes. *Bioresour. Technol.* 98:2846-2851.
- Plaza, B. M., Jiménez, S., Vela, M. D., Pérez, M., and Lao, M. T. 2008. El sector ornamental en Andalucía. *Horticultura* 204:12-19.
- Roberts, P. A., Van Gundy, S. D., and McKinney, H. E. 1981. Effects of soil temperature and planting date of wheat on *Meloidogyne incognita* reproduction, soil populations, and grain yield. *J. Nematol.* 13:338-345.
- Stapleton, J. J., and Duncan, R. A. 1998. Soil disinfection with cruciferous amendments and sublethal heating: effects on *Meloidogyne incognita*, *Sclerotium rolfsii* and *Pythium ultimum*. *Plant Pathol.* 47:737-742.
- Stapleton, J. J., and Heald, C. M. 1991. Management of phytopathogenic nematodes by soil solarization. Pages 51-60 in: *Soil Solarization*. J. Katan and J. E. Devay, eds. CRC Press, Boca Raton, FL.
- Tenuta, M., and Lazarovits, G. 2004. Ammonia and nitrous acid from nitrogenous amendments kill microsclerotia of *Verticillium dahliae*. *Phytopathology* 92:255-264.
- United Nations Environment Programme. 2006. Resolution on methyl

- bromide. Annex XV of the Report of the Fourth Meeting of the Parties. Page 441 in: Handbook for the Montreal Protocol on Substances that Deplete the Ozone Layer, 7th ed. United Nations Environment Programme, Nairobi.
38. Wang, K. H., and McSorley, R. 2008. Exposure time to lethal temperatures for *Meloidogyne incognita* suppression and its implication for soil solarization. *J. Nematol.* 40:7-12.
 39. Whitehead, A. G., and Hemming, J. R. 1965. A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Ann. Appl. Biol.* 55:25-38.
 40. Zeck, W. M. 1971. A rating scheme for field evaluation of root-knot nematode infestations. *Pflanzenschutz Nachr. Bayer (Ger. Ed.)* 24:141-144.
 41. Zijlstra, C., Donkers-Venne, D. T. H. M., and Fargette, M. 2000. Identification of *Meloidogyne incognita*, *M. javanica* and *M. arenaria* using sequence characterised amplified region (SCAR) based PCR assays. *Nematology* 2:847-853.