Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)
Available online at www.inia.es/sjar
http://dx.doi.org/10.5424/sjar/2013112-3275

Spanish Journal of Agricultural Research 2013 11(2), 485-493
ISSN: 1695-971-X
eISSN: 2171-9292

Evaluation of repeated biodisinfestation using *Brassica carinata* pellets to control *Meloidogyne incognita* in protected pepper crops

M. M. Guerrero-Diaz*, C. M. Lacasa-Martinez, A. Hernandez-Piñera, V. Martinez-Alarcon and A. Lacasa Plasencia

Departamento de Biotecnología y Protección de Cultivos. Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (IMIDA). 30150 La Alberca (Murcia), Spain

Abstract

The nematode *Meloidogyne incognita* is responsible for substantial losses in greenhouse-grown peppers in south-eastern Spain. This study evaluates the use of biodisinfestation (BS) (organic amendment + solarisation) as an alternative to using methyl bromide (MB) over three consecutive years to control the nematode in greenhouse conditions. *Brassica carinata* (BP) pellets or *B. carinata* (BP) + fresh sheep manure (M) were evaluated in treatments which began on two different dates (August and October) and the results were compared with MB-disinfested and untreated controls. During the third year, the gall index for BP was lower than that obtained for BP + M and in the August treatment than in the October treatment. The commercial crop of pepper fruit obtained with the biodisinfestation treatments begun in August was similar to or higher than that obtained with MB, and higher than that obtained with both October biodisinfestation treatments. The yield of the October biodisinfestation treatments was higher than that of the untreated one. In August of all the years studied, the accumulated exposure times were greater than the thresholds required to kill *M. incognita* populations at 15 cm depth. The incidence of the nematode did not correspond to the reduction achieved during solarisation, and seemed to increase during the crop cycle. Further studies should look at why high temperatures do not produce a sustained reduction in the populations of *Meloidogyne incognita.*

Additional key words: greenhouses; nematodes; organic amendments; soil.

Introduction

In the province of Murcia (south-eastern Spain) pepper (*Capsicum annuum* L.), is a crop that occupies more than 90% of the area dedicated to greenhouse crops and has been a monoculture for the last 20 years (Lacasa & Guirao, 1997). The normal crop cycle lasts 9-10 months (November-December to September-October). *Phytophthora* spp. (*P. capsici* and *P. parasitica*) and *Meloidogyne incognita* are recurring and persistent problems and, together, cause substantial crop spoilage (Tello & Lacasa, 1997; Bello *et al.,* 2004).

Until 2005, methyl bromide (MB) was used to disinfect soils to control both pathogens and to reduce the effects of fatigue caused by repeated monocultures (Martínez *et al.,* 2011). Since that time, MB has been replaced by a mixture of 1,3-dichloropropene and chloropicrin, but these, too, will shortly be banned by EU legislation. To this forthcoming ban must be added the growth in demand for ecological foods produced by sustainable agricultural practices, meaning that non-chemical methods must be sought for controlling soilborne plant pathogens and plant parasite nematodes adapted for use in intensive horticulture.

In recent years numerous alternatives to chemical disinfection have been studied, and of these, those based on organic amendments alone or in combination with solarisation seem to be the most promising ones. This method involves incorporating decomposable organic matter into the soil and subsequent humidification, covering with plastic for 2-15 weeks. Decomposition of the source of carbon in the humid or wet soil by anaerobic microorganisms reduces the oxygen content below the plastic (Messiha *et al.,* 2007) in a process that has been

* Corresponding author: mariam.guerrero@carm.es
Received: 27-06-12. Accepted: 04-04-13.

Abbreviations used: BP (*Brassica carinata* pellets); BS (biodisinfestation); GI (galling index); M (fresh sheep manure); MB (methyl bromide); VIF (virtually impermeable film).

called "biological disinfestation" (Blok *et al.,* 2000), "reductive soil disinfestation" (Katase *et al.,* 2009) or "soil reductive sterilization" (Yossen *et al.,* 2008). The anaerobic conditions increase the activities of the microorganisms in the soil, which have a suppressive effect on pathogens and parasites (Gamliel *et al.,* 2000; Bonanomi *et al*., 2007; Oka, 2010; Klein *et al.,* 2011).

The combined action of organic amendment and solarisation has demonstrated its effectiveness in the control of *Phytophthora capsici* in pepper crops. The process of soil disinfection was called "biodisinfestation" by Núñez-Zofio *et al*. (2011). In these processes, the effects of anaerobiosis and suppression are added to the effect of temperature and of the gases released during decomposition of the organic matter, such as ammonia and nitrous acid (Lazarovits, 2001; Tenuta & Lazarovits, 2002; Arriaga *et al.,* 2011). Although the characteristics of the OM are regarded as critical elements for the control of pathogens in general, the same characteristics do not seem to have a great impact on the control of some pathogens produced in anaerobic conditions (Bonanomi *et al.,* 2007, 2010).

The use of *Brassicaceae* in crop rotations or as green manure amendment in biofumigation treatments (Stapleton & Bañuelos, 2009) has been seen to reduce the incidence of some soilborne pathogens and plant parasites, including nematodes (Smolinska *et al.,* 2003; Larkin & Griffin, 2008) through their release of isothiocyanates. For example, improved pathogen and weed control has been achieved by using amendments obtained from by-products produced during the extraction of oil from *B. carinata* and *Sinapis alba* seeds (Lazzeri & Manici, 2000; Lazzeri *et al.,* 2004; Cohen *et al.,* 2005; Palmieri, 2005; Sachi *et al*., 2005).

Biodisinfestation (BS) using fresh sheep manure (M) as amendment in August was seen to provide similar results to those obtained using MB to control *Phytophthora* spp. in pepper crops (Guerrero *et al.,* 2004a). Production increased when the application was repeated more than two years (Guerrero *et al.,* 2004b, 2006; Candido *et al.,* 2005), but it seemed to have little effect when applied after the beginning of September (Guerrero *et al*., 2010).

Some growers are reluctant to use BS because they should be able to shorten the regular cycle by one or two months, which implies a substantial reduction in the crop yield (20-30%). The implementation of BS is one of the drawbacks of the method because BS must begin in August (Guerrero *et al.,* 2004a).

The objective of the present study was to evaluate the effect of BS using *B. carinata* pellets (BP) alone or combined with M, when applied in August or October for the control of *Meloidogyne incognita* as well as marketable yield in greenhouse grown pepper crops in a mild-warm climate. The experiment was carried out over three consecutive years and the treatments were repeated in the same plots.

Material and methods

Experimental design

The trials were carried out in a greenhouse (37° 46'41.93" N/0° 53' 55.31" W) located in Campo de Cartagena (Murcia, southeast Spain) over three consecutive crop seasons, from 2008 to 2010.

The soil was naturally contaminated by *M. incognita* (race 2, biovar pepper according to Robertson *et al.,* 2006). The experiment treatments were arranged in a completed randomized block design with three replicates, and a plot size of 52 m^2. The soil was a clay loam with an organic matter content of 2%, a total nitrogen content of 1.5 g kg^{-1}, pH = 7.8, a C/N ratio of 10.3 and electrical conductivity (EC) of 14.4 dS m^{-1}.

Two treatments were studied: (i) BP: solarisation + *B. carinata* pellets (0.3 kg m^{-2} as organic amend-ment); (ii) BP + M: solarization + a mixture of seed meal *B. carinata* pellets (0.3 kg m^{-2}) and fresh sheep manure (2.5 kg m^{-2}). Treatments were carried out for 6 weeks in both August and October (August and October treatments were performed in different experimental plots). Untreated (neither solarisation nor organic amendment) and MB-treated plots were included as reference controls.

Organic amendments were buried using a rotary tiller and a drip irrigation system (3 L h^{-1} emitters spaced 0.40 m apart in the same rows) was installed. Then, a transparent plastic (polyethylene, 0.05 mm) was spread over the BS treatments plots and the soil was watered (up to 25-30 cm) for 3 h day^{-1} on two consecutive days. The *B. carinata* pellets (BP) (Biofence Triumph Italia SPA) contained 6% N, 7% P, 2.4% K and 84.2% organic matter. The glucosinolate sinigrin constituted the *B. carinata* seed meal as well as the derived isothiocyanates which are effective against *Meloidogyne* nematodes (Lazzeri *et al.,* 2010). The applied manure had the following characteristics: pH 8.2 and EC 5.0 dS m^{-1}, organic C: 400 g kg^{-1}, total N: 25.0 g kg^{-1}, total P: 5.5 g kg^{-1} and total K: 26.1 g kg^{-1}.

In the treatments not including M, composted manure was added at 2.5 kg m^{-2} before planting. MB (98% *a.i.,* 2% chlorpicrin) was applied at a dosage of 30 g m^{-2} as a cold fumigation, using a volumetric dispenser after the soil was covered with virtually impermeable film (VIF, 0.04 mm thick) and maintained for one week.

In January, pepper plants were planted with a crop density of 25,000 plants ha^{-1} corresponding to 3 rows of 45 plants per row. The crop season ended in the first week of August. Pests were controlled according to Integrated Pest Management (CAARM, 2010).

Parameters studied

Soil temperature (at 15 and 30 cm depth) was monitored using sensors connected to a data-logger (H8-4 32K, Hobo Weather Stat., Onset Comp. Corp., Cape Cod, MA, USA) with continuous data recording and reading every 15 min.

Pepper fruits were harvested throughout the crop season and final marketable yield (kg m^{-2}) was determined.

The incidence of *M. incognita* was evaluated at the end of the crop season. Ten pepper plants of each experimental plot were pulled up in order to determine (i) the percentage of galled plants and (ii) the galling index according to Bridge & Page (1980) on a scale of 0-10.

Statistical analyses

The data were studied using ANOVA, Statgraphics Centurion XV.II. Differences among treatments were tested using the LSD test al 95% ($p < 0.05$). The data were transformed to achieve homoscedasticity and normality using the following transformations: log $(x + 1)$ for the crop yield and nodulation index, and the formula arcsine $\sqrt{(x)}$ for the percentage of galled plants.

Results

Incidence of *Meloidogyne incognita*

In the first year the galling index (GI) of BS (BP and BP + M) was smaller than in the untreated control and the starting date of the treatment or amendment had no influence (Table 1). In the second year the GI of the August BS treatments was lower than that of the October BS treatments finding no differences between amendments in August, but in October there were differences.

In the third year there were differences in the GI between dates (August and October) and between both amendments. The GI of the October BP + M did not differ from that of the untreated control. The incidence was lower in the BP treatments than in the BP + M in both dates. The GI values of the August treatments were more similar to those of MB than those of the October treatments. The BP treatment beginning in August reduced the incidence of *Meloidogyne* in the third year of application (GI was 3.8 in the first year, and 2.6 in the third year), being more pronounced the reduction in the treatment with BP (31.58%) than with BP + M (14.6%). In the October treatments the incidence of *M. incognita* increased (50% in BP + M and 31% in BP), and also in the non-treated control (20%) and MB (150%).

The percentage of infested plants varied very little between the BS treatments and the non-treated control

Table 1. Incidence of *Meloidogyne incognita* (galling index, GI and % of galled plants)

Treatments[1]	First crop season		Second crop season		Third crop season	
	GI	% galled plants	GI	% galled plants	GI	% galled plants
Untreated	5.7 ± 0.96^{d}	100.0 ± 0^{b}	6.3 ± 0.79^{cd}	100.0 ± 0^{b}	7.3 ± 1.0^{e}	100.0 ± 0^{b}
MB	0.1 ± 0.51^{a}	6.6 ± 0.11^{a}	0.2 ± 0.77^{a}	6.6 ± 1.54^{a}	1.5 ± 0.70^{a}	43.3 ± 37.85^{a}
BP + M August	4.1 ± 0.88^{b}	100.0 ± 0^{b}	3.7 ± 1.64^{b}	93.3 ± 1.54^{b}	3.5 ± 1.87^{c}	96.7 ± 5.7^{b}
BP August	3.8 ± 1.69^{b}	93.3 ± 0.11^{b}	4.3 ± 2.19^{b}	86.6 ± 23.09^{b}	2.6 ± 1.54^{b}	86.7 ± 15.27^{b}
BP + M October	4.6 ± 1.76^{b}	93.3 ± 0.11^{b}	6.8 ± 1.14^{d}	100.0 ± 0^{b}	6.9 ± 2.42^{e}	100.0 ± 0^{b}
BP October	4.1 ± 1.12^{b}	100.0 ± 0^{b}	5.4 ± 0.98^{c}	100.0 ± 0^{b}	5.4 ± 2.42^{d}	100.0 ± 0^{b}

[1] MB: methyl bromide-treated plots to 30 g m^{-2}; BP + M: biodisinfestation with *Brassica carinata* pellets + fresh sheep manure; BP: biodisinfestation with *Brassica carinata* pellets. Mean values (n = 30) ± standard errors. For each parameter, values in each column followed by the same letter are not significantly different according to LSD test.

in all three years. However, this proportion increased in MB in the last year. In the August BS treatments there was a non-significant tendency for the percentage of infested plants to fall as time passed. In the plots with GI values below 4 no symptoms of yellowing, reduced growth, etc, were evident in the aerial part of the plants.

Effect on pepper crop yield

In the first year, differences were observed between BS dates but not between amendments, within a same date, being higher in August than in October (Table 2). Yield for the October treatments were similar to those obtained with MB but higher than those obtained in the non-treated control and lower than in the August BS treatments.

In the second year, there were no differences between dates or between both amendments (August and October) (Table 2), the production obtained with BS treatments being similar to that obtained with MB and higher than in the control.

In the third year, there were differences between dates but not between amendments (Table 2). The production obtained in October was lower than that obtained with MB, which was similar to that obtained in August. The production obtained with BS at any date was higher than that obtained with the control, as in the other years.

The August BS treatments provided similar levels of production or higher than MB every year, while the October BS treatment yields were no different from MB in two of the three years and always higher than the non-treated control.

Soil temperatures

In general, the daily evolution of the maximum, mean and minimum temperatures was similar for the same depth (15 and 30 cm) and treatment date (August and October).

Differences between depths and also between dates for the same treatment were observed and between the treatments and the untreated control. Table 3 depict the percentage of the total BS time that the soil temperatures were in given ranges above 30°C. Above 38°C the values are represented at 1°C intervals, as suggested by Wang & McSorley (2008).

In the August treatments the temperature at 15 cm always were above 25°C the first year, above 30°C the second year and above 32°C the third year, while at 30 cm the corresponding minimum temperatures were 28°C, 32°C, and 34°C, respectively. In the first year the percentage of time that the soil at 15 cm was above 42°C was higher for BP + M (24.44%) than for BP (0.3%), while at 30 cm the differences between the amendments were slight (0.6% for BP and 0.10% for BP + M). In the second year the percentage of differences between soils with temperatures above 42ºC were very small at 15 cm (1.12% for BP and 3.70% for BP + M), while at 30 cm the temperatures did not exceed 42°C in either amendment. In the third year the soil temperature frequently exceeded 42°C at 15 cm with both treatments (18.70% for BP, 23.28 % for BP + M) while at 30 cm the corresponding times were BP + M (22.41%) and BP (1.65%).

The first year in October treatments the temperature was always lower than 38ºC at both depths and for both amendments except the second year at 15 cm for BP, where the soil was above this temperature for 8.9% of the time. The percentage of time when the soil was above 35°C was greater for BP + M than for BP at both depths the first year (17.07% and 3.5%, respectively, at 15 cm and 21.2% and 6.8%, respectively, at 30 cm). In the second year, the time with temperatures above 35°C was similar for both amendments at 15 cm (16.6% for BP + M and 25.3% for BP) at 30 cm (16.39% for BP + M and 23.58% for BP). In the third year, only the soils in BP + M at 15 cm exceeded 35°C (4.40% of the time).

In the untreated control the temperature was lower than for both treatments at both depths and dates during the three years of the experiment. Only in August of the first year did the soil temperature rise above 38°C (1.93% of the time).

Table 2. Pepper crop yield (kg m^{-2})

Treatments[1]	First season	Second season	Third season
Untreated	9.8[c]	10.3[b]	9.7[d]
MB	11.1[b]	11.9[a]	12.0[b]
BP + M August	12.7[a]	11.6[a]	12.1[ab]
BP August	12.6[a]	12.2[a]	12.7[a]
BP + M October	10.9[b]	11.8[a]	11.7[c]
BP October	10.9[b]	12.1[a]	11.1[c]

[1] See Table 1. Values followed by the same letter are not significantly different at significance level $p < 0.05$.

Discussion

In previous studies, we showed that BS with fresh sheep or poultry manure provided acceptable levels

Table 3. Percentage of hours accumulated for each range of soil temperatures at 15 and 30 cm depth in each treatment for 27 days in August and 40 days in October (first season), for 30 days in August and 41 days in October (second season) and for 44 days in August and 41 days in October (third season)

Temperature (°C)	15 cm depth						30 cm depth					
	Untreated August	Untreated October	BP August	BP October	BP + M August	BP + M October	Untreated August	Untreated October	BP August	BP October	BP + M August	BP + M October
First season												
< 30	11.31	81.6		68.3		11.93	86.7	56.71	25.6	71.8	4.7	5.2
30.1-35	54.58	18.4		28.2	32.93	71.0	12.75	43.29	14.98	21.4	26.11	73.6
35.1-38	32.18		57.36	3.5	34.82	17.07	0.55		20.2	6.8	41.9	21.2
38.1-39	0.53		26.2		6.42				21.66		27.29	
39.1-40	1.4		12.14		0.46				12.97		6.67	
40.1-41			3.02		0.76				2.39		0.56	
41.1-42			0.98		0.17				1.6		0.52	
42.1-43			0.3		7.38				0.6		0.10	
43-44					15.6							
44-45					1.46							
Second season												
< 30	100.0	100.0	15.24	12.03	1.28	9.0	100.0	100.0	0.39	5.23	2.3	11.9
30.1-35			17.89	62.67	12.86	74.4			1.33	71.19	17.5	71.71
35.1-38			35.4	16.4	35.46	16.6			3.23	23.58	33	16.39
38.1-39			10.06	8.9	11.46				25.52		29.15	
39.1-40			7.91		11.21				43.22		18.05	
40.1-41			12.38		24.03				26.31			
41.1-42			1.12		3.7							
Third season												
< 30	89.03	100.0		87.05		75.56	4.06	100.0		98.98		97.7
30.1-35	10.97		7.4	12.95		20.04	62.82			1.02		2.3
35.1-38			40.31		30.65	4.4	33.12		37.21		31.28	
38.1-39			9.53		25.41				36.7		15.71	
39.1-40			9.22		7.8				16.31		14.47	
40.1-41			7.94		6.4				3.5		10.45	
41.1-42			6.9		6.46				4.36		5.68	
42.1-43			6.19		6.05				1.65		4.22	
43.1-44			5.15		4.6						5.2	
44.1-45			4.39		4.5						3.54	
45.1-46			2.27		4.1						9.45	
46.1-47			0.7		4.03							

BP + M: biodisinfestation with *Brassica carinata* pellets + fresh sheep manure; BP: biodisinfestation with *Brassica carinata* pellets.

of *M. incognita* control (Guerrero *et al.,* 2004a, 2006) which improved as the treatments were prolonged over 3 to 7 years (Guerrero *et al.,* 2007). The results of the present study show that BS with seed mail *B. carinata* pellets, alone or in combination with fresh sheep manure, provides effective control of *M. incognita* when begun in August and ensures similar production levels to those obtained using MB. However this treatment was not effective when begun in October.

Many species of the family *Brassicaceae* contain glucosinolates, which upon decomposition, give rise to isothiocyanates (Smolinska *et al.,* 1997; Kirkegard & Sarwar, 1998), which reduce the incidence of pathogens in the soil, whether buried as green manure (Pikerton *et al.,* 2000; Larkin & Griffin, 2008) or whether in the form of by-products of oil extraction from seeds (Lazzeri, *et al*., 2004; Palmieri, 2005; Sachi *et al*., 2005). The biofumigation effect of organic amendments on pathogens is due to, among other factors, toxic vola-

tile compounds, anaerobic conditions and the increasing suppressive capacity of the soil (Tenuta *et al.,* 2002; Everts *et al.,* 2006; Bonanomi *et al.,* 2007; Katase *et al.,* 2009; Arriaga *et al.,* 2011; Oka, 2010; Núñez-Zofio *et al*., 2011). The glucosinolate sinigrin of seed meal *B. carinata* pellets showed activity against *Meloidogyne* nematodes (Lazzeri *et al.,* 2010), but not all Brassicaceus pellets are effective to the nematode control, which must be taken into account when selecting the amendment to the management of *Meloidogyne* (Zasada *et al.,* 2009). The hydrothermal effect of solarization (Katan, 1981) has a direct effect on pathogens (Gamliel *et al*., 2004; Katan, 2005; Wang *et al.,* 2006) and an indirect effect since it speeds up the decomposition of the organic amendment applied. Combining soil solarization with sorghum green manure reduced the nematodes populations more that the untarped sorghum but the combination of solarisation with broccoli residues did not reduce nematodes populations compared with untarped broccoli (Zasada *et al.,* 2010).

In our case, the volatile compounds were not measured during the BS process but they may have been involved in reducing the incidence of *M. incognita* when compared the reduction obtained in October with BP and BP + M in second and third years. However, no such effect was observed in the second and third years with BP + M since the GI in October was similar to that of the control. Neither was nematode control improved when BP were mixed with M on both dates. An additive or synergic effect might have been expected between both amendments since M provided good control of *M. incognita* in pepper (Guerrero *et al.,* 2007; Ros *et al.,* 2008) as did the pellets alone (Guerrero *et al.,* 2009). However, the interaction seems to be negative. In our conditions and soils with a pH > 8.0, decomposition of the amendments generates NH_3, whose effect on fungi and nematodes, as described by Riegel & Noe (2000) and Everts *et al*. (2006), would explain the extent to which these pathogens are controlled by BS in similar conditions. The organic carbon may negatively influence the toxicity of glucosinolate-hydrolysis products (Morra, 2004). It seems that the organic matters of fresh sheep manure are not suitable to address the complex relationships than occur when using plant tissues as a source for pesticidal compounds.

It seems that the intrinsic water content of Brassicaceus tissues is insufficient to allow maximum hydrolysis of glucosinolates to isothiocyanates (Matthiessen, 2004). The addition of water performs the dual functions of boosting this process and of serving as a carrier for the isothiocyanates so formed into the soil. In our case the water absorption by fresh manure (M) could reduce the available water below the needs for hydrolysis of glucosinolates in pellets of *B. carinata* (BP). This might explain the lower efficiency of BP + M treatment compared to BP treatment in reducing the incidence of *Meloidogyne.*

Differences were observed in the soil oxygen content between the treatments (Guerrero, data not shown). The accumulated number of hours with oxygen levels below 5% or 1% was greater in BP + M than in the BP treatments and greater in August than in October for the same treatment. In the BP treatments the effect on the decrease of oxygen content disappeared after 3-4 weeks, while in BP + M it remained until the end of the solarization process. Anaerobiosis mechanisms may explain the differences in the incidence of *M. incognita* observed between the treatments and the control but not between BP and BP + M. Núñez-Zofío *et al.* (2011) observed differences between *B. carinata* pellets (BP) + *Sinapis alba* green manure and fresh manure (M) BS treatments for the control of *Phytophthora* sp. in pepper, but in the opposite direction as we observed for *M. incognita*. Núñez-Zofío *et al*. (2011) and Núñez-Zofío (2012) found greater numbers of bacteria, actinomycetes and *Pseudomonas spp.* in soils containing pepper crops biodisinfected with *B. carinata* pellets (BP) + *Sinapis alba* green manure than in soils disinfected with M or semi-composted sheep manure and the control, finding a positive correlation between total microbiota and the suppressive capacity of the soil after BS accompanied by a similar incidence of *Phyptophthora* spp. in the crop. We did not measure the microbiota but the effects on the incidence of the pathogen (*M. incognita*) differed between BP and BP + M the third year, which suggests that the possible effect in soil suppressiveness was not the main factor acting in the BS action mechanism.

Soil temperature seems to have been the main component of the effect of BS on *M. incognita*. In the August treatments of the third year more than 150 accumulated hours of temperatures above 42°C were recorded in BP at 15 cm depth and in BP + M at 15 and 30 cm; at this last depth the figure was 12.5 h for BP. Wang & McSorley (2008) found the accumulated exposure of more than 15 h to temperatures of 42°C were lethal for the eggs and juveniles 2 of *M. incognita.* In our case, this lethal exposure time was obtained for all the treatments and at both soil depths in the third

year, at 15 cm the second year and at both depths the first year. However, the GI differed between treatments the second and third year. The time with temperatures above 38°C exceeded 400 h the second year at 30 cm in BP and at 15 and 30 cm in BP + M and only at 15 cm in BP the third year. Although Wang & McSorley (2008) suggested that 390 h at 38°C were necessary to kill all J2, this would still not explain the differences in GI between the treatments. Exposure of J2 and eggs to sublethal periods (< 13 h) of temperatures above 42.5°C may have an accumulative effect (Heald & Robinson, 1987), while temperatures that vary between 22°C and 42°C considerably reduce *M. incognita* J2 populations and eggs (Wang & McSorley, 2008). However, a reduction in the populations as a result of solarization is not always reflected in the adequate control of the damage caused to crops, which, according to Wang & McSorley (2008) would be related to low temperatures at greater depths of the soil. Populations remaining in the deep soil would recolonized and infest the damaged roots (Hewlet & Dickson, 1991), as occurred in the untreated control of long term crops (Chellemi *et al.,* 1993). In our case, the 7-month crop cycle would have facilitated the multiplication of *M. incognita* and reinfestation of the plants, particularly in the autumn treatments, when the temperature never exceeded 38°C at either depth in any of the years.

Despite the fact that solarization only has a short term impact on nematode populations (Wang & McSorley, 2008) such an effect can be improved in conjunction with organic amendments, both in warmer and cooler periods of the year. The action of the volatile compounds released during the decomposition of BS amendments, the possible increase in the microbial community, and the concomitant suppressive effect of the soil and the reduction of the soil oxygen content, together, would explain the damage caused to nematodes by the August treatment. This reduced incidence of *M. incognita* enabled yields of pepper fruit similar to those obtained by MB. While in Autumn BS of greenhouse soils used for growing pepper in southeastern Spain cannot necessarily be recommended for the total control of *M. incognita*, such treatment can be used to alleviate the effect of soil fatigue due to microbiological causes (Martínez *et al.,* 2011). The use of cultivars or plants grafted on rootstock that carry genes resistant to *M. incognita* may be considered as a complement to BS for the control of soil pathogens (Guerrero *et al.,* 2012).

Acknowledgments

This research was financially supported by the National Institute for Agricultural and Food Research and Technology (INIA) of the Spanish Ministry of Science and Innovation (projects RTA 2008-00058-C03 and RTA 2011-00005-C03).

References

Arriaga H, Núñez-Zofío M, Larregla S, Merino P, 2011. Gaseous emissions from soil biodisinfestation by animal manure on a greenhouse pepper crop. Crop Prot 30: 412-419.

Bello A, López-Pérez JA, García-Álvarez A, Arcos SC, Ros C, Guerrero MM, Guirao P, Lacasa A, 2004. Biofumigación con solarización para el control de nematodos en cultivo de pimiento. In: Desinfección de suelos en invernaderos de pimiento (Lacasa A, Guerrero MM, Oncina M, Mora JA, eds). Publicaciones de la Consejería de Agricultura, Agua y Medio Ambiente, Región de Murcia (Spain). pp: 105-129.

Blok WJ, Lamers JG, Termorshuizen AJ, Bollen GJ, 2000. Control of soilborne plant pathogens by incorporating fresh organic amendments followed by tarping. Phytopathology 90: 253-259.

Bonanomi G, Antignani V, Pane C, Scala F, 2007. Suppression of soilborne fungal diseases with organic amendments. J Plant Pathol 89: 311-324.

Bonanomi G, Antignani V, Capodilupo M, Scala F, 2010. Identifying the characteristics of organic soil amendments that suppress soil-borne plant diseases. Soil Biol Biochem 42: 136-144.

Bridge J, Page SLJ, 1980. Estimation of root-knot infestation levels in roots using a rating chart. Trop Pest Manage 26: 296-298.

Candido V, Miccolis V, Basile M, D'Addabbo T, Gatta G, 2005. Soil solarization for the control of *Meloidogyne javanica* on eggplant in Southern Italy. Acta Hort 698: 195-199.

CAARM, 2010. Normas técnicas de producción integrada en cultivo de pimiento. Consejería de Agricultura y Agua de la Región de Murcia. BORM 134: 32348-32364.

Chellemi DO, Olson SM, Scott JW, Mitchell DJ, McSorley R, 1993. Reduction of phytoparasitic nematodes on tomato by solarization and genotype. J Nematol 25: 800-805.

Cohen MF, Yamasaki H, Mazzola M, 2005. *Brassica napus* seed meal soil amendment modifies microbial community structure, nitric oxide production and incidence of *Rhizoctonia* root rot. Soil Biol Biochem 37: 1215-1227.

Everts KL, Sardanelli S, Kratochvil RJ, Armentrout DK, Gallagher LE, 2006. Root-knot and root-lesion nematode suppression by cover crops, poultry litter, and poultry litter compost. Plant Dis 90: 487-492.

Gamliel A, Austerweil M, Kritzman G, 2000. Non-chemical approach to soilborne pest management – organic amendments. Crop Prot 19: 847-853.

Gamliel A, Gadkar V, Zilberg V, Beniches M, Rabinowich E, Manor H, Wininger S, Kapulnik Y, 2004. Effect of solarization intensity on the control of pink root of chives, and the response of the crop to AM fungal application. Symbiosis 37: 233-247.

Guerrero MM, Lacasa A, Ros C, Bello A, Martínez MC, Torres J, Fernández P, 2004a. Efecto de la biofumigación con solarización sobre los hongos del suelo y la producción: fechas de desinfección y enmiendas. In: Desinfección de suelos en invernaderos de pimiento (Lacasa A, Guerrero MM, Oncina M, Mora JA, eds). Publicaciones de la Consejería de Agricultura, Agua y Medio Ambiente, Región de Murcia (Spain). pp: 208-238.

Guerrero MM, Ros C, Martínez MA, Barceló N, Martínez MC, Guirao P, Bello A, Contreras J, Lacasa A, 2004b. Estabilidad en la eficacia desinfectante de la biofumigación con solarización en cultivos de pimiento. Acta Hort 42: 20-24.

Guerrero MM, Ros C, Martínez MA, Martínez MC, Bello A, Lacasa A, 2006. Biofumigation vs biofumigation plus solarization to control *Meloidogyne incognita* in sweet pepper. IOBC-WPRS Bulletin 29: 313-318.

Guerrero MM, Martínez MA, Ros C, Bello A, Fernández P, Martínez MC, Lacasa A, 2007. Eficacia de la biosolarización como desinfectante del suelo en invernaderos de pimiento. Acta Hort 48: 451-454.

Guerrero MM, Lacasa CM, Ros C, Martínez V, Fenoll J, Torres J, Beltrán C, Fernández P, Bello A, Lacasa A, 2009. Pellets de brasicas como enmiendas para biosolarización de invernaderos de pimiento. Acta Hort 54: 424-429.

Guerrero MM, Ros C, Lacasa C, Martínez V, Lacasa A, Fernández P, Martínez MA, Núñez M, Larregla S, Díez-Rojo MA, Bello A, 2010. Effect of biosolarization using pellets of *Brassica carinata* on soil-borne pathogens in protected pepper crops. Acta Hortic 381: 337-344.

Guerrero MM, Lacasa CM, Hernández A, Martínez V, Martínez MA, Ros C, 2012. Biosolarización e injerto para el manejo integrado de los patógenos del suelo en cultivos de pimiento en invernadero. Acta Hort 60: 321-326.

Heald CD, Robinson AF, 1987. Effect of soil solarization on *Rotylenchuslus reniformis* in the lower Rio Grande Valley of Texas. J Nematol 19: 93-103.

Hewlet TE, Dickson DW, 1991. Population dynamics of *Meloidogyne arenaria* race 1 in peanut. J Nematol 25: 532-533.

Katan J, 1981. Solar heating (solarization) of soil control of soilborne pets. Annu Rev Phytopathol 19: 211-236.

Katan J, 2005. Soil disinfestation: one before methyl bromide phase out. Acta Hortic 698: 19-25.

Katase M, Kubo C, Ushio S, Ootsuka E, Takeuchi T, Mizukubo T, 2009. Nematicidal activity of volatile fatty acids generated from wheat bran in reductive soil disinfestation. J Nematol 39: 53-62.

Kirkegaard JA, Sarwar M, 1998. Biofumigation potential of brassicas – I. Variation in glucosinolate profiles of diverse field-grown brassicas. Plant Soil 201: 71-89.

Klein E, Katan J, Gamliel A, 2011. Soil suppressiveness to *Fusarium* dissease following organic amendments and solarization. Plant Dis 95: 1116-1123.

Lacasa A, Guirao P, 1997. Investigaciones actuales sobre alternativas al uso del bromuro de metilo en pimiento en invernaderos del campo de Cartagena. In: Posibilidades de alternativas viables al bromuro de metilo en pimiento en invernadero (López A, Mora JA, eds). Jornadas 11, Publicaciones de la Consejería Medio Ambiente, Agricultura y Agua, Región de Murcia (Spain). pp: 21-36.

Larkin RP, Griffin TS, 2008. Control of soil potato diseases using *Brassica* green manure. Crop Prot 26: 1067-1077.

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.

Lazzeri L, Manici LM, 2000. The glucosinolate-myrosinase system: a natural and practical tool for biofumigation. Acta Hort 532: 89-95.

Lazzeri L, Leoni O, Manici LM, 2004. Biocidal plant dried pellets for biofumigation. Ind Crop Prod 20: 59-65.

Lazzeri L, Leoni O, Mancini L, Palmieri S, Patalano G, 2010. Use of seed flour as soil pesticide. Patent number US7749549B2.

Martínez MA, Martínez MC, Bielza P, Tello J, Lacasa A, 2011. Effect of biofumigation with manure amendments and repeated biosolarization on *Fusarium* density in pepper crops. J Ind Microbiol Biot 38(10): 3-11.

Matthiessen J, 2004. The importance of plant maceration and water in achieving high ITC levels in soil. Proc First Int Symp on Biofumigation: A Possible Alternative to Methylbromide? (Lazzeri L, Matthiessen J, Morra MJ, Palmieri S, Rollin P, eds). Italian Ministry of Agricultural and Forestry Politics and Italian Ministry of Environmental and Territory. p: 13.

Messiha N, Van Diepeningen A, Wenneker M, Van Beuningen A, Janse J, Coehen T, Termorshuizen A, Van Bruggen A, Blok W, 2007. Biological soil disinfestation (BSD), a new control method for potato brown rot, caused by *Ralstonia solanacearum* race 3 biovar 2. Eur J Plant Pathol 117: 403-415.

Morra MJ, 2004. Controlling soil-borne plant pests using glucosinolate containing tissues. Proc First Int Symp on Biofumigation: A Possible Alternative to Methylbromide? (Lazzeri L, Matthiessen J, Morra M J, Palmieri S, Rollin P, eds). Italian Ministry of Agricultural and Forestry Politics and Italian Ministry of Environmental and Territory. p: 7.

Núñez-Zofío M, Larregla S, Garbisu C, 2011. Application of organic amendments followed by soil plastic mulching reduces the incidence of *Phytophthora capsici* in pepper crops under temperate climate. Crop Prot 30: 1563-1572.

Núñez-Zofío M, 2012. Evaluación de la biodesinfección para el control de *Phytophthora capsici* en el cultivo de pimiento en invernadero. Doctoral thesis. Univ del País Vasco, Spain.

Oka Y, 2010. Mechanisms of nematode suppression by organic soil amendments: a review. Appl Soil Ecol 44: 101-115.

Palmieri S, 2005. Biofumigation: a new technology based on the use of sulphur-containing metabolites in *Brassica* plants. Proc Annual Meeting Assoc Advancement of Industrial Crops, Murcia (Spain), Sept 17-21. pp: 201-213.

Pikerton JN, Ivors KL, Miller ML, Moore LW, 2000. Effect of soil solarization and cover crops on populations of selected soilborne plant pathogens in Western Oregon. Plant Dis 84: 952-960.

Riegel C, Noe JP, 2000. Chicken litter soil amendment effects on soilborne microbes and *Meloidogyne incognita* on cotton. Plant Dis 84: 1275-1281.

Robertson L, López-Pérez JA, Bello A, Díez-Rojo MA, Escuer M, Piedra-Buena A, Ros C, Martínez C, 2006. Characterization of *Meloidogyne incognita, M. arenaria* and *M. hapla* populations from Spain and Uruguay parasitizing pepper (*Capsicum annuum*). Crop Prot 25: 440-445.

Ros M, García C, Hernández MT, Lacasa A, Fernández P, Pascual JA, 2008. Effects of biosolarization as methyl bromide alternative for *Meloidogyne incognita* control on quality of soil under pepper. Biol Fertil Soils 45: 37-44.

Sachi S, Odorizzi S, Lazzeri L, Marciano P, 2005. Effect of *Brassica carinata* seed meal treatment on the *Trichoderma harzianum* T39-*Sclerotinia* species interaction. Acta Hort 698: 289-292.

Smolinska U, Morra MJ, Kundsen GR, Brown PD, 1997. Toxicity of glucosinolate degradation products from *Brassica napus* seed meal toward *Aphanomyces euteiches* f. sp. *pisi.* Phytopathology 87: 77-82.

Smolinska U, Morra MJ, Kanudsen GR, James RL, 2003. Isothiocyanate produced by *Brassicaceae* species as inhibitors of *Fusarium oxysporum*. Plant Dis 87: 407-412.

Stapleton J, Bañuelos GS, 2009. Biomass crops can be used for biological disinfestation and remediation of soil and water. Calif Agr 63: 41-46.

Tello J, Lacasa A, 1997. Problemática fitosanitaria del suelo en cultivos de pimiento en el Campo de Cartagena. In: Posibilidades de alternativas viables al bromuro de metilo en pimiento en invernadero (López A, Mora JA, eds). Jornadas 11, Publicaciones de la Consejería Medio Ambiente, Agricultura y Agua, Región de Murcia (Spain). pp: 11-17.

Tenuta M, Lazarovits G, 2002. Ammonia and nitrous acid from nitrogenous amendments kill the microsclerotia of *Verticillium dahliae*. Phytopathology 92: 255-264.

Tenuta M, Conn KL, Lazarovits G, 2002. Volatile fatty in liquid swine manure can kill microsclerotia of *Verticillium dahliae*. Phytopathology 92: 548-552.

Wang KH, McSorley R, 2008. Exposure time to lethal temperatures for *Meloidogyne incogita* suppression and its implication for soil solarisation. J Nematol 40(1): 7-12.

Wang KH, McSorley R, Kokalis-Burelle N, 2006. Effects of cover cropping solarization, and soil fumigation on nematode comunities. Plant Soil 286: 299-243.

Yossen V, Zumelza G, Gasoni L, Kobayashi K, 2008. Effect of soil reductive sterilization on Fusarium wilt in greenhouse carnation in Cordoba, Argentina. Australas Plant Path 37: 520-522.

Zasada IA, Meyer SLF, Morra MJ, 2009. *Brassicaceous* seed meals as soil amendments to suppress the plant-parasitic nematodes *Pratylenchus penetrans* and *Meloidogyne incognita*. J Nematol 41(3): 221-227.

Zasada IA, Halbrent JM, Kokalis-Burelle N, La Mondia J, McKerny MV, Noling JW, 2010. Managing nematodes without methyl bromide. Ann Rev Phytopathol 48: 311-328.