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## Soil fatigue and its specificity towards pepper plants in greenhouses

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### Abstract

The aim was to confirm the depressive effect on pepper plants grown in non-disinfected soils and to ascertain the possible specificity of fatigue with the goal of establishing strategies for disinfecting soils with a broad spectrum of fumigants. Soil samples were taken from six greenhouses that had been used for different numbers of years to grow a monoculture of pepper and which received different disinfestation treatments (methyl bromide, 1,3-dichloropropene + chloropicrin, biosolarization) and one which received no treatment. Every soil sample was splitted in three fractions. The first fraction was disinfested with methyl bromide (MB), the second with steam at 120°C by autoclaving (A), and the third was not disinfected (ND). Pepper plants were cultivated in pots. Celery and lettuce were also cultivated in the same conditions to ascertain the degree to which soil fatigue was specific. In 87.5% of the 16 soil variants, the plants cultivated in the disinfected soil fractions (MB or A) were higher than those grown in the non-disinfected fractions. However, in the case of celery and lettuce, the plants cultivated in ND were higher than those cultivated in MB or A. The results show that the fatigue accumulated in the soil of the pepper monoculture was highly specific towards this crop, suggesting that rotation with other crops is an advisable agronomic practice in order to recover the soil productive capacity.

**Additional key words:** soil disinfestation; *Capsicum annuum*; celery; lettuce; monoculture.

### Introduction

In Campo de Cartagena (south-eastern Spain), pepper (*Capsicum annuum* L.) is grown as a monoculture in 95% of greenhouses (Lacasa & Guirao, 1997), a situation that has been prevalent for more than 25 years (Martínez *et al.*, 2009). The annual cycle lasts 9-10 months, from November-January to September-October. Many of these soils are contaminated by *Phytophthora* spp. (*capsici* and *nicotianae*) and/or *Meloidogyne* spp. (*incognita* and *javanica*) (Cenis & Fusch, 1988; Bartual *et al.*, 1991; Tello & Lacasa, 1997; Bello *et al.*, 2004; Guerrero *et al.*, 2013; Núñez-Zofio *et al.*, 2013).

From 1988 to 2005 greenhouse soils were disinfected annually with methyl bromide (MB) to control collar

and root rot and root-knot nematodes, and to increase production (Lacasa & Guirao, 1997) by counteracting the depressive effect of a repeated monoculture. However, MB was substituted in 2005 by the mixture 1,3-D+Pic (1,3-dichloropropene + chloropicrin) (Guerrero *et al.*, 2012), which remains in use.

The depressive effects on the plants of repeated monoculture were confirmed when MB stopped being used. Lacasa *et al.* (1999) and Guirao *et al.* (2004) found greenhouses where the above mentioned pathogens were absent but the harvest reduction was greater than 30% when monoculture had been practised for 18 years with no interruption (Lacasa *et al.*, 2002; Guerrero *et al.*, 2004b) and fell by 12% in those where the monoculture had been grown for two successive years. The depressive effects

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Abbreviations used: A (autoclaved); BS (biosolarization); CM (chicken manure); FSM (fresh sheep manure); MB (methyl bromide); ND (non-disinfected); PE (polyethylene, 0.05 mm); VIF (virtually impermeable film, 0.04 mm); 1,3-D+Pic (60.86% [w/w] 1,3-dichloropropene + 33.3% [w/w] chloropicrin).

were reduced after chemical disinfection (MB or 1,3-D+Pic) (Guerrero *et al.*, 2004a; Guirao *et al.*, 2004) or non-chemical disinfection means (biosolarization) using fresh sheep manure plus chicken manure as organic amendment (Guerrero *et al.*, 2004c). These depressive effects were interpreted as indicators of soil fatigue, as defined by Scotto-La Massese (1983) or Bouhot (1983): “the reduced development of certain crops when cultivated two or more times in the same soils”. These authors did not point to the cause or causes of the fatigue although Bodet (1983) and Meynard & Bouhot (1983) claimed that the depressive effects abated when the soils were disinfected with heat at 100°C, which suggests that in such soils the fatigue is related to biotic factors, in particular to microorganisms populations. Disinfection reduces the incidence of soilborne diseases and improves the health of plants (Katan, 2005), enabling consistently high levels of production to be obtained by reducing soil fatigue (Katan & Vanachter, 1990). Bouhot & Bonnel (1983) found no depressive effects on strawberry (*Fragaria vesca* L.) plants cultivated in a fresh soil but did so when they were repeatedly cultivated in the same soil, finding that in 74% of cases the limiting factor was microbiological in origin. The fungi isolated by Bouhot & Bonnel (1983) from the strawberry plants showing vegetative depression did not produce disease when they were inoculated; nor did they reproduce the symptoms, so that they were considered “weakness or subclinical pathogens” by Katan & Vanachter (1990). Disinfecting the soil with steam at 100°C reduces the depressive effect on plants. The involvement of microorganisms in soil fatigue and the depressive effect were considered by Messiaen *et al.* (1991) as complex, and they recommended the soil disinfection to lessen the effects on yields. Otto *et al.* (1994) considered that soil fatigue in apple (*Malus domestica* B.) orchards was related with the accumulation of Actinomycetes in this soil, which paralysed root development, although this relation was not considered so obvious by Zydlik *et al.* (2006). Martínez *et al.* (2009) found a relation between the increased density of *Fusarium* in the soil in pepper crops and reduced plant development and the reduced production of a pepper monoculture in non-disinfected soils. Disinfection of the soil with MB or by biosolarization reduced the density of *Fusarium*, increasing both plant development and yield (Martínez *et al.*, 2009, 2011).

In this work, we study the fatigue in the soils of greenhouses in Campo de Cartagena (Murcia, SE Spain) in which monocultures of pepper plants were grown in greenhouses. The aim was to confirm the depressive ef-

fect on pepper plants grown in non-disinfected soils and to ascertain the possible specificity of fatigue with the goal of establishing strategies for disinfecting soils with a broad spectrum of fumigants.

## Material and methods

### Soils background

Soils were taken from six greenhouses (named B, C, D, E, F and G), located in Campo de Cartagena (Murcia southeastern Spain), each representative of a pepper monoculture that had been grown for a different number of years (15, 16, 14, 2, 3 and 4 years, respectively, and with no fallow period between crops). Greenhouses size of 1,000 m<sup>2</sup> the smallest (E and F), and 3,100 m<sup>2</sup> the biggest (B, D and G).

The soils of greenhouses had also been subjected to different soil disinfection treatments: i) methyl bromide in greenhouse B, C and D; ii) non disinfection in E, F and G. In each greenhouse, three plots (size of 62 m<sup>2</sup> in E and F, 67 m<sup>2</sup> in B and D and 90 m<sup>2</sup> in G) were disinfected with: T1 = methyl bromide (MB); T2 = 1,3-D+Pic; T3 = biosolarization (BS) 7 kg m<sup>-2</sup> of fresh sheep manure (FSM) +3 kg m<sup>-2</sup> of chicken manure (CM); T4 = BS with 5 kg m<sup>-2</sup> FSM + 2.5 kg m<sup>-2</sup> CM; T5 = BS with 4 kg m<sup>-2</sup> FSM + 2 kg m<sup>-2</sup> CM; T6 = BS with 3 kg m<sup>-2</sup> FSM + 1.5 kg m<sup>-2</sup> CM; T7 = non-disinfected (ND) (Table 1), before last culture after which the soil was sampled from each plot.

### Soil sampling

The soil samples were taken when the crop had finished and the soils were being turned. Samples were taken at 25 cm depth in 12 points of each experimental plot, with 3 replicates per treatment and greenhouse. Each soil was sieved, and the field capacity was determined volumetrically.

### Treatments of soil samples

A fraction of each soil sample was disinfected with 98:2 MB (Brom-O-Gas from Dead Sea Bromide, Israel) at 60 g m<sup>-2</sup> under 0.05 mm thick polyethylene (PE) applied with a volumetric dispenser (Nolia

**Table 1.** Soil sample codes, age of pepper monoculture in each greenhouse, soil disinfection treatments in the past and treatment received prior to last crop and before sampling

Greenhouse	Soil code	Background	
		Previous treatments	Treatment before last crop
B	BT1	T1 <sup>a</sup> , 14 years	T1 <sup>a</sup>
	BT3	T1 <sup>a</sup> , 14 years	T2
	BT7	T1 <sup>a</sup> , 14 years	T7
C	CT1	T1 <sup>a</sup> , 15 years	T1 <sup>a</sup>
	CT2	T1 <sup>a</sup> , 15 years	T2
	CT7	T1 <sup>a</sup> , 15 years	T7
D	DT1	T1 <sup>a</sup> , 13 years	T1 <sup>a</sup>
	DT7	T1 <sup>a</sup> , 13 years	T7
E	ET1	T1 <sup>b</sup> , 2 years	T1 <sup>b</sup>
	ET3	T7, 2 years	T3
	ET4	T7, 1 year + T3, 1 year	T4
	ET5	T7, 1 year + T3, 2 years	T5
F	FT1	T1 <sup>b</sup> , 3 years	T1 <sup>b</sup>
	FT4	T7, 2 years + T3, 1 year	T4
	FT6	T7, 1 year + T3, 3 years	T6
	FT7	T7, 3 years	T7
G	GT7	T7, 4 years + T3, 1 year	T7

T1<sup>a</sup> = MB 60 g m<sup>-2</sup> under PE; T1<sup>b</sup> = MB 30 g m<sup>-2</sup> under VIF; T2 = 1,3-D+Pic; T3 = BS with 7 kg m<sup>-2</sup> FSM + 3 kg m<sup>-2</sup> CM; T4 = BS with 5 kg m<sup>-2</sup> FSM + 2.5 kg m<sup>-2</sup> CM; T5 = BS with 4 kg m<sup>-2</sup> FSM + 2 kg m<sup>-2</sup> CM; T6 = BS with 3 kg m<sup>-2</sup> FSM + 1.5 kg m<sup>-2</sup> CM; T7 = ND. MB = methyl bromide 98:2; PE = polyethylene (0.05 mm); 1,3-D+Pic = 60.86% (w/w) 1,3-dichloropropene + 33.3% (w/w) chloropicrin; ND = non-disinfected; VIF = very impermeable film (0.04 mm); BS = biosolarization; FSM = fresh sheep manure; CM = chicken manure.

McLean Co., Belmonoy Los Angeles, CA, USA) of 1.4 L capacity and 0.05 L precision. Another fraction was disinfected by autoclaved (A) at 120°C and 1 kg cm<sup>-2</sup> pressure for 1 hour (two 30-min sessions separated by 24 hours) in plastic bags containing 2 kg soil. The other fraction was not disinfected and acted as control. Ten aliquots (305 g) of disinfected or non-disinfected soil fractions were placed in 330 mL pots.

### Plants used as bioindicators and cultivation in climatic chamber

Five seeds of pepper cv. Sonar F1 (Clause Tezier Semances), celery (*Apium graveolens* L.) cv. Monterrey (Clause Tezier) or lettuce (*Lactuca sativa* L.) cv. Reina Verde (Seminis Petoseed) were sown in each pot after pre-germination in an incubation chamber in darkness at 25°C. The seeds were covered with a 0.3-0.4 mm layer

of vermiculite, disinfected in an autoclave at 120°C and 1 kg cm<sup>-2</sup> pressure for 1 h, as had been done with the soils. The pots containing the seeds were watered to field capacity and placed in a culture chamber at 23 ± 1°C, at 45-60% RH during the light and 85-100% during the dark (14:10 L:D photoperiod and 6,000 lux). The pots were watered 3 times per week according to the loss of soil humidity, as measured by a Hobbo volumetric probe (Onset Comp. Corp., Bourne, MA, USA), and fertilized with a macro and micro-nutrient solution (Bayfolan, Bayer CropScience, Spain) once a week. The plants remained in the climatic chamber for 8 weeks.

### Measured variables: height and dry weight of plants

Several variables (plant height, fresh and dry weight, leaf length and width, among others) were measured

**Table 2.** Height and dry weight of pepper plants grown in pots with soils that received different greenhouse disinfection treatments prior to soil sampling. Every soil sample was splitted in three fractions: the first was disinfected with autoclave (A), the second with methyl bromide (MB), and the third was not disinfected (ND)

Greenhouse	Soil code	Height (cm)			Dry weight (g)		
		A	MB	ND	A	MB	ND
B	BT1	5.93a	5.34a	4.20b	0.07a	0.07a	0.05b
	BT3	5.81a	5.28a	3.93b	0.22a	0.21a	0.10b
	BT7	4.97a	4.85a	4.01b	0.14a	0.12b	0.12b
C	CT1	5.67a	5.55a	4.89b	0.22a	0.18b	0.10c
	CT2	7.22a	6.57b	5.73c	0.09a	0.09a	0.08b
D	DT1	8.28a	6.1b	3.62c	0.18a	0.17a	0.09b
	DT7	5.58a	4.14b	3.95b	0.17a	0.11b	0.11b
E	ET1	7.76a	6.84b	6.12c	0.27a	0.22b	0.17c
	ET3	7.02a	7.03a	5.82b	0.24a	0.21b	0.15c
	ET4	6.54b	8.11a	4.91c	0.36b	0.42a	0.29c
	ET5	5.58b	7.84a	4.84c	0.09b	0.12a	0.08c
F	FT1	6.12b	7.48a	4.93c	0.11a	0.10a	0.07b
	FT4	6.66a	6.1a	5.24b	0.08a	0.09a	0.02b
	FT6	5.58b	7.84a	5.33b	0.08b	0.12a	0.07b
	FT7	6.68a	7.14a	5.27b	0.10a	0.10b	0.07c
G	GT7	1.96a	2.00a	1.28b	0.04a	0.04a	0.02b

For each variable, values followed by the same letter are not significantly different according to LSD test ( $p < 0.05$ ).

in previous assays (Guerrero *et al.*, unpublished data). Plant height and dry weight were taken as being the most representative and constant variable. To obtain the dry weight, the washed plants were dried in a muffle oven at 75°C for 24 h until constant weight as measured in a Sartorius BL 1205 analytical balance with a precision of 0.1 mg. Measurements were made in the five plants per pot and 10 containers per treatment and 3 replicates, since each experiment was repeated three times in the same conditions.

## Experimental design

To demonstrate or evaluate the depressive effect of fatigue on pepper, soil samples of greenhouses B, C, D, E, F y G coded as BT1 BT3 BT7, CT1, CT2, DT1 DT7, ET1 ET3, ET4, ET6, FT1, FT4 FT5, FT7 and GT7 (Tables 1 and 2) were used. After disinfection with MB or by autoclaving, fractions of these soils were placed in containers, sown with pepper seeds and placed in a growth chamber.

To demonstrate the specificity of fatigue, besides pepper, celery was used, as in Bouhot *et al.* (1979a,b), and also lettuce, as indicators of soil fatigue: (i) in the case

of celery, soil samples from all greenhouses coded as BT1, CT1, CT2, CT7, DT1, DT7, ET1, ET3, ET4, ET5, FT1, FT4, FT5, FT7 and GT7 (Tables 1 and 3) were used; (ii) in the case of lettuce, soil samples of greenhouses C, E and F coded as CT1, CT2, CT7, ET1, ET3, ET4, ET5, FT1, FT4, FT6 and FT7 (Tables 1 and 4) were used.

## Statistical analysis

The homogeneity and homocedasticity of the data were checked. For the analysis of variance and comparison of the means (LSD test at 95%) the height and weight data were normalized using  $\log_{10}(x)$  transformation by means of the Statgraphic Centurion (Warrenton, VA, USA).

## Results

### Effect of soil fatigue in pepper

Disinfection with MB or by autoclaving of the soils fractions of different origin and which had received

**Table 3.** Height and dry weight of celery plants grown in pots with soils that received different greenhouse disinfection treatments prior to soil sampling. Every soil sample was splitted in three fractions: the first was disinfected with autoclave (A), the second with methyl bromide (MB), and the third was not disinfected (ND)

Greenhouse	Soil code	Height (mm)			Dry weight (g)		
		A	MB	ND	A	MB	ND
B	BT1	5.04b	4.40c	6.25a	0.14ab	0.13b	0.15a
C	CT1	8.05c	10.92b	14.13a	0.08b	0.13a	0.15a
	CT2	7.90b	8.10b	11.74a	0.09b	0.10b	0.14a
	CT7	9.15c	11.81b	14.15a	0.09b	0.13a	0.16a
D	DT1	5.96ab	5.74b	6.76a	0.12b	0.12b	0.15a
	DT7	4.23b	3.86b	5.53a	0.11b	0.11b	0.15a
E	ET1	5.85b	6.35b	7.91a	0.49c	0.71b	0.88a
	ET3	3.00c	4.34b	5.11a	0.13c	0.34b	0.44a
	ET4	3.16c	4.95b	5.82a	0.19c	0.33b	0.51a
	ET5	4.44b	4.06b	6.16a	0.27c	0.33b	0.40a
F	FT1	5.41b	5.80b	6.90a	0.10b	0.12a	0.13a
	FT4	3.03c	4.34ab	4.86a	0.03c	0.06b	0.09a
	FT6	4.44b	4.86b	6.16a	0.07b	0.07b	0.11a
	FT7	5.23c	6.20b	7.29a	0.11c	0.14b	0.16a
G	GT7	1.98b	2.01ab	2.76a	0.05b	0.04b	0.05a

For each variable, values followed by the same letter are not significantly different according to LSD test ( $p < 0.05$ ).

**Table 4.** Height and dry weight of lettuce plants grown in pots with soils that received different greenhouse disinfection treatments prior to soil sampling. Every soil sample was splitted in three fractions: the first was disinfected with autoclave (A), the second with methyl bromide (MB), and the third was not disinfected (ND)

Greenhouse	Soil code	Height (mm)			Dry weight (g)		
		A	MB	ND	A	MB	ND
C	CT1	9.74b	10.17b	12.22a	0.11b	0.12b	0.14a
	CT2	11.21b	10.94b	13.13a	0.16b	0.12c	0.19a
	CT7	9.80b	9.41b	10.65a	0.11b	0.09b	0.14a
E	ET1	8.90b	9.03b	11.69a	0.09b	0.08b	0.11a
	ET3	10.76a	10.70a	10.65a	0.11a	0.11a	0.11a
	ET4	9.61b	9.90b	11.35a	0.12b	0.11b	0.16a
	ET5	8.91b	9.23b	11.07a	0.09b	0.08b	0.11a
F	FT1	10.71a	10.65a	10.40a	0.16a	0.12a	0.12a
	FT4	9.82b	10.07b	11.41a	0.12b	0.11b	0.16a
	FT6	9.34b	9.42b	11.34a	0.91b	0.92b	0.12a
	FT7	8.91b	9.02b	11.81a	0.08b	0.09b	0.11a

For each variable, values followed by the same letter are not significantly different according to LSD test ( $p < 0.05$ ).

different treatments before planting led to higher plants with a greater dry weight than the ND fractions (Table 2).

Height in 87.5% of the plants grown in the MB or A disinfected fractions, was higher than in plants from the ND fraction, while 6.25% of the plants from the

MB disinfected fractions had the same height as ND control plants, while another 6.25% of the fraction disinfected in the autoclave had a similar height to the ND control plants.

There was 93.5% correspondence between the plant height and dry weight in the response to disinfection by any means regardless of the treatments received in the distant past (seven soils had been disinfected with MB for more than 13 consecutive years, two for more than 2 years and nine soils had not been disinfected with chemicals) and in the recent past (six soils were disinfected chemically before cultivation and sampling, six by biosolarization and four were not disinfected).

In all cases soil disinfection led to better plant development, as measured by plant height and dry weight. In contrast, in the non-disinfected fractions, plants showed smaller development, as well as yellowing leaves and a smaller size.

The way in which the soil fractions were disinfected influenced plant height in almost 50% of the soils (7 out of 16), MB producing higher plants than autoclave disinfection in two soils (ET4 and ET5) and autoclave disinfection producing higher plants in five soils (CT2, DT1, DT7, FT1 and FT5), with no response being showed with the disinfection treatments received in the greenhouses in the past: three disinfected chemically, three by biosolarization and one non-disinfected.

### Effect on other indicator species

#### *Effect on celery*

When celery was used as indicator the results were contrary to those obtained for pepper. For all the soils the plants grown in the ND fraction were higher than those grown in the MB and A fractions (Table 3), regardless of the past treatments (six soils had been disinfected with MB for more than 13 consecutive years, two for more than 2 years and seven soils had not been disinfected with chemicals) and in the recent treatments (five soils were disinfected chemically before cultivation and sampling, six by biosolarization and four were not disinfected). In 13.6% of the soils the height of plants grown in ND fractions was similar to that of plants grown in the MB disinfected fraction and in 6.6% it was similar to the height of plants grown in the autoclave-disinfected fraction.

#### *Effect on lettuce*

The results obtained with lettuce were similar to those obtained with celery. In 81.8% of the fractions disinfected with MB the lettuce plants were smaller than those obtained in the ND fraction (Table 4), regardless of previous treatments (three soils had been disinfected with MB for more than 13 consecutive years, one for 3 years, another one for 2 years and six soils had not been disinfected with chemicals) and the recent treatments (four soils were disinfected chemically before cultivation and sampling, five by biosolarization and two were not disinfected). In 18.2% of the soils the height of plants grown in ND fractions was similar to that of plants grown in the MB disinfected fraction and autoclave-disinfected fraction, the same being true for plant dry weight. The disinfection mode (MB or A) did not affect plant development.

## Discussion

Soil fatigue resulting from repeated monocultures has been associated with individual and multiple abiotic or biotic causes, in long term woody crops such as apple (Zydlik & Pacholak, 2008; Styla & Sawicka, 2009), herbaceous crops like clover or cereals (Bodet, 1983; Sørengaard & Møller, 2005) and horticultural crops (Bouhot *et al.*, 1979a,b; Louvet, 1980; Bouhot & Bonnel, 1983).

The contributors to fatigue may have several origins: physical (through deterioration of the soil characteristics); chemical (deficiencies in nutrient availability or accumulation); toxic (accumulation of allelopathic substances emitted by the crop); or microbiological (accumulation of pathogens, competitors for nutrients or liberators of substances with harmful or sublethal effects for plants) (Borner, 1960; Kimber, 1973; Bouhot *et al.*, 1979a; Otto *et al.*, 1994; Cebolla & Maroto, 2004; Zydlik *et al.*, 2006; Zydlik & Pacholak, 2008).

In vegetable crops it is frequent to find geographical areas dedicated to monocultures of a given crop, with the result that pathogen levels in the soil greatly increase (Louvet, 1980; Katan, 2005) accompanied by increased incidence of soil diseases and decreased yields. However, the yields of horticultural monocultures frequently decline even in the absence of primary soil pathogens (Bouhot *et al.*, 1979b). The presence of microorganisms (particularly fungus) with subclinical pathogenic effects were considered by Bouhot &

Bonnel (1983) to be the cause of vegetative depression in plants and reduced yields in strawberry.

Greenhouses in South-eastern Spain cultivated by the monoculture of pepper for more than 15 years, had higher yields when soils were disinfected than in non-disinfected soils, even in soils that were free of the main pathogens normally found, *Phytophthora capsici* and *Meloidogyne incognita* (Guirao *et al.*, 2004). Our study shows that soil fatigue in protected pepper crops is reduced by disinfection (MB or autoclaving), independently of the years spent in monoculture and the soil background. The principal cause of this fatigue is considered to be biotic and related with the extent to which the soils are contaminated with *Fusarium* sp. (Martínez *et al.*, 2009, 2011). Similar effects were observed by Bouhot *et al.* (1979b) after disinfecting soils used to cultivate strawberry, while Cebolla & Maroto (2004) found similar response for citrus and apple crops in which micotoxins segregated by some fungi were considered the causes of vegetative depression in the trees. Some isolates of *Fusarium oxysporum* and *Aspergillus* sp. from the soils of greenhouses used to grow pepper led to deficiencies in pepper plant development, either directly or when crude extracts of the fungus were added to the substrate (Martínez *et al.*, 2009).

Zydlik & Pacholak (2008) proposed using strawberry for diagnosing the level of soil fatigue in replantation of apple, suggesting that soil fatigue is of a generalist nature, in this case within the same family of Rosaceae. In contrast, Bouhot *et al.* (1979a) concluded that soil fatigue required specific studies of the soil-plant relation since soils showing signs of fatigue in the case of celery did not do so in the case of parsley (*Petroselinum crispum* Mill.), which manifested no symptoms of vegetative depression (Bouhot, 1983).

In our study, it was observed that celery and lettuce plants showed a better development in the non-disinfected fractions of soils that had been dedicated to pepper cultivation, regardless of the greenhouse of origin and the disinfection treatments previously received. This indication of soil fatigue specificity might be explained by the elimination of microorganisms which release substances that are harmful to pepper but not to celery or lettuce. The pepper specific fatigue in greenhouse soils used for pepper monocultures leads to vegetative depression (reduced height) and an increasing loss of yield, as seen in the greenhouses of Campo de Cartagena where the soils have been disinfected with MB for more than 15 consecutive

years (Guirao *et al.*, 2004) or by means of biosolarization during two consecutive crop cycles (Guerrero *et al.*, 2004c). The elimination of microorganisms that might have been beneficial to celery and lettuce by disinfecting the soil fractions with MB or by autoclave might explain the lower degree of plant development compared with that observed in the ND soil fractions.

Despite the fact that monoculture usually increases soil-borne plant pathogens, there may still be some cases where evidence suggests the contrary, such as disease decrease induced by several years of consecutive cultivation. An explanation would be that the host plant, when grown in monoculture, can have a profound influence on the interaction with a pathogen. A case of this phenomenon is take-all decline of wheat (*Triticum aestivum* L.) produced by the fungus *Gaeumannomyces graminis* var. *tritici*, which has been observed after two or more years of monoculture in USA, The Netherlands and Australia (Cook & Rovira, 1976; Cook, 1993; Weller *et al.*, 2002). The increased of the fluorescent *Pseudomonas* spp. that produce the antifungal metabolite 2,4-diacetylphloroglucinol, seems to cause the reduction of the incidence of the *G. graminis* (Weller *et al.*, 2002).

The contribution of organic matter by biosolarization did not improve the development of pepper plants cultivated in the ND fractions compared with the plants grown in the MB or autoclave-disinfected fractions. Biosolarization in greenhouses used to grow pepper improves the physical and chemical characteristics of the soil (Fernández *et al.*, 2005). Neither the disinfection of soils in the greenhouse had effect on plants grown in the MB or A-disinfected fractions or on plants grown in the non-disinfected fractions. This might mean that fatigue accumulates significantly during the 9 or 10 months of the crop cycle.

In summary, the results showed that the fatigue accumulated in greenhouses used for pepper monocultures is highly specific towards pepper and points to the advisability of rotating crops as a strategy for recovering the productive capacity of the soils.

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