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Repeated biodisinfection controls the incidence of *Phytophthora* root and crown rot of pepper while improving soil quality

M. Núñez-Zofío[1], S. Larregla[1,] * and C. Garbisu[2, 3]

[1] *Department of Plant Production and Protection*
[2] *Department of Ecology and Natural Resources*
[3] *Soil Microbial Ecology Group. NEIKER-Tecnalia, Basque Institute of Agricultural Research and Development, Berreaga 1, 48160 Derio, Spain*

Abstract

Phytophthora root and crown rot is a plant disease responsible for important economic losses in protected pepper crops. A greenhouse experiment was carried out in a temperate climate region (northern Spain) to assess the effects of repeated biodisinfection after three consecutive crop seasons with different organic amendments (a non-composted mixture of sheep manure and chicken litter, a semicomposted mixture of horse manure and chicken litter, *Brassica carinata* dehydrated pellets plus *Sinapis alba* fresh green manure) on disease incidence, crop yield and soil quality. Biodisinfection treatments were found to improve soil water properties through reduction in soil bulk density and increased water infiltration. Biodisinfested soils showed higher values of physicochemical and microbial properties than control (untreated) and plastic-mulched soils. In plots treated with the non-composted or semicomposted mixture, the observed higher levels of microbial activity were strongly related with an increase in soil microbial biomass. *Brassica-Sinapis* treatment had a weaker effect on soil properties than animal manure-based treatments. However, highest counts of total bacteria, actinomycetes and *Pseudomonas* spp. were found in *Brassica-Sinapis*-treated soils. It was concluded that repeated biodisinfection for the control of *Phytophthora* root and crown rot in protected pepper crops located in temperate climate regions can improve soil quality and suppressiveness, as well as allow for a reduction in the dose of organic amendment needed for biodisinfection. Among the studied organic amendments, the semicomposted amendment was the best option in terms of reduction in disease incidence.

Additional key words: biofumigation; organic amendment; soil microbial properties; soilborne plant pathogens; solarization; suppressiveness.

Resumen

La reiteración de la biodesinfección controla la incidencia de la podredumbre radicular y del cuello del pimiento causada por *Phytophthora* y mejora la calidad del suelo

La podredumbre radicular y del cuello causada por *Phytophthora* es una enfermedad que genera importantes pérdidas económicas en cultivos de pimiento en invernadero. En una región de clima templado (norte de España) se realizó ensayo en invernadero para evaluar la reiteración de tratamientos de biodesinfección después de tres ciclos de cultivo consecutivos con diferentes enmiendas orgánicas (mezcla no-compostada de estiércol de oveja y gallinaza, mezcla semicompostada de estiércol de caballo y gallinaza, pellets deshidratados de *Brassica carinata* más *Sinapis alba* como abono verde fresco) sobre la incidencia de la enfermedad, la producción y la calidad del suelo. La biodesinfección mejoró las propiedades hídricas del suelo al reducir la densidad aparente y aumentar la infiltración. Los suelos biodesinfectados mostraron valores más altos en las propiedades físico-químicas y microbianas que los suelos control (no tratado) y acolchado con plástico. En las enmiendas no-compostada y semicompostada, los valores más altos de acti-

*Corresponding author: slarregla@neiker.net
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Abbreviations used: C_{mic} (microbial biomass carbon); C_{org} (organic carbon content); CD (colony development index); CEC (cation exchange capacity); EC (electrical conductivity); EP (ecophysiological index); FDA (fluorescein diacetate hydrolysis); N_{min} (potentially mineralizable nitrogen); OM (organic matter content); WSOC (water-soluble organic carbon).

vidad microbiana estuvieron fuertemente relacionados con un aumento en la biomasa microbiana. La biodesinfección con *Brassica-Sinapis* tuvo menor efecto sobre las propiedades del suelo que con ambas enmiendas animales. No obstante, los recuentos de bacterias totales, actinomicetos y *Pseudomonas* spp. fueron más altos en los suelos tratados con *Brassica-Sinapis*. La biodesinfección reiterada para controlar la podredumbre radicular y del cuello causada por *Phytophthora* en cultivos de pimiento en invernadero en regiones de clima templado, puede mejorar la calidad del suelo y su supresividad, así como reducir dosis de enmienda requerida. La enmienda semicompostada fue la mejor en disminuir la incidencia de enfermedad.

Palabras clave adicionales: biofumigación; calidad del suelo; enmienda orgánica; fitopatógenos telúricos; propiedades microbianas edáficas; solarización; supresividad.

Introduction

In northern Spain, *Phytophthora* root and crown rot, most frequently caused by the oomycete *Phytophthora capsici*, is a plant disease responsible for important economic losses in protected pepper crops. Control of this disease in our region has traditionally been carried out by means of pre-plant chemical fumigation and post-plant application of fungicides during crop development. However, increasing restrictions on the use of soil chemical disinfectants, owing to their adverse effects on human and environmental health (du Fretay *et al.*, 2010), have prompted the interest in the development of non-chemical methods for the control of soilborne pathogens.

Soil biodisinfection through the application of organic amendments in combination with soil solarization (plastic mulching) (Gamliel *et al.*, 2000) appears promising for the control of soilborne plant pathogens in temperate climate areas, such as northern Spain, where solarization is *a priori* not a good option (Coelho *et al.*, 1999). A 25% of *P. capsici* oospores survived after 70 days in sterile moistened soil subjected to controlled conditions of daily cycling of 5 h at 35 °C and 19 h at 30 °C, under a temperature regime that closely reproduced common temperature profiles observed in solarization assays carried out in greenhouses in northern Spain (Etxeberria *et al*., 2011a,b). Actually, in a previous work, we found a reduction in *Phytophthora* root and crown rot disease incidence as a result of biodisinfection, in spite of soil temperatures during treatments which were not high enough to thermally inactivate *P. capsici* (Núñez-Zofío *et al.,* 2011). Biodisinfection with organic amendments can control soilborne pathogens through: increase in soil temperature, accumulation of toxic volatile compounds (*e.g*., ammonia) generated during organic matter (OM) decomposition, creation of anaerobic conditions in soil, and increase in soil suppressiveness due to higher levels of soil microbial activity (Gamliel *et al.*, 2000).

Apart from its intended effect on soilborne pathogens, biodisinfection may have an influence on soil quality (Ros *et al.*, 2008). In this respect, the impact of agronomic practices on soil quality has customarily been evaluated through the determination of soil physicochemical variables. However, soil biological properties, particularly those related to the biomass, activity and diversity of the soil microbial communities, are more and more used due to their sensitivity, rapid response, ecological relevance and integrative character (Mijangos *et al.*, 2006, 2009, 2010).

In the abovementioned work (Núñez-Zofío *et al.,* 2011), we observed that biodisinfection effectiveness was correlated with soil microbial activity. Then, we concluded that biodisinfection treatments were most likely leading to increased soil suppressiveness through a stimulation of the activity of soil microbial communities. On the other hand, it is critical to reduce the dose of organic amendment as much as possible in order to minimize its potential adverse impact on environmental quality, including the emission of greenhouse gases previously reported (Arriaga *et al.*, 2011). In this respect, we hypothesized that repeated biodisinfection might allow for a reduction in amendment dose.

The aim of this work was to assess, in a temperate climate region where solarization is frequently marginal, the effect of repeated (three consecutive crop seasons) biodisinfection with different organic amendments (a non-composted mixture of sheep manure and chicken litter, a semicomposted mixture of horse and chicken litter, and *Brassica carinata* commercial pellets) on *Phytophthora* root and crown rot disease incidence, crop yield, and soil quality and suppresiveness.

Material and methods

Experimental design

The experiment was established on March 2008 in a greenhouse located in Derio (Bizkaia province, north-

ern Spain; 43°17'18'' N, 2°53'5'' W). Before treatment application (see below), experimental plots were artificially infested as described in Arriaga *et al.* (2011). Briefly, pepper root balls containing mycelium, sporangia, zoospores and oospores of five different strains (see below) of *P. capsici* were buried at 10 cm soil depth using a completely randomized block design with three replicates and a plot size of 28.2 m^2 (6 × 4.7 m). The soil was a clay loam (16.2% sand, 36.6% silt, 47.2% clay) with pH 6.4, a 5.5% OM content, a 0.21% total nitrogen (N) content, a C-to-N ratio of 15.1, a phosphorus (P) content of 99.3 mg kg^{-1}, and an electrical conductivity of 2.15 dS m^{-1}.

The following treatments were carried out for 6 weeks in three consecutive crop seasons: (i) plastic-mulched: plots were covered with a 0.05 mm transparent polyethylene plastic film (Raisa film R, PTR-4,0-200, Arrigorriaga, Spain); (ii) non-composted: addition of a non-composted mixture of fresh sheep manure and dry chicken litter (2.33:1 w:w) followed by plastic mulching; (iii) semicomposted: addition of a semicomposted mixture of horse manure and chicken litter (1:1 v:v) followed by plastic-mulching; and (iv) Brassica: addition of defatted *Brassica carinata* seed meal dehydrated pellets (Biofence®) followed by plastic-mulching. A control treatment (neither plastic mulching nor amendments) was included in the experiment. Three biodisinfection treatments were applied in the same plots throughout the experiment (Fig. 1). Table 1 shows the composition of the amendments. Similarly, Table 2 shows the dosage and time of application of amendments. Due to the risks of greenhouse gas emissions (Arriaga *et al.*, 2011) and water pollution by nitrates (OJ, 1991), animal amendment dosage was progressively reduced so that, in the third application, an amount equivalent to 170 kg ha^{-1} of mineralized nitrogen was applied in each plot (limit established by the European Union 91/676/EEC Nitrates Directive).

Organic amendments were incorporated into soil to the 20 cm upper layer using a rotavator. The plots were then covered with a 0.05 mm transparent polyethylene plastic film and the soil was moistened to 60% field capacity with a spray irrigation system. In order to improve pathogen control effectiveness in Brassica plots after the second and third application of biodisinfection, in addition to the abovementioned 6-week

Table 1. Composition of amendments

Composition	Biofence®[a]	Semicomposted mixture[b]	Non-composted mixture	
			Fresh sheep manure	Dry chicken litter
pH (1:10)		7.7 ± 0.7	8.5 ± 0.2	7.6 ± 0.3
EC (25 °C, 1:10) (dS m^{-1})		6.0 ± 0.3	11.7 ± 0.5	7.5 ± 0.6
Humidity (%)		53.7 ± 3.4	65.2 ± 1.3	22.6 ± 7.2
OM[c] (%)	84.2	70.9 ± 5.5	71.5 ± 2.6	70.0 ± 2.4
Oxidable OM (%)		61.5 ± 0.7	63.9 ± 6.4	59.7 ± 3.2
Ashes (%)		29.1 ± 5.5	28.5 ± 2.6	30.0 ± 2.4
CaO (%)		6.2 ± 1.6	5.4 ± 0.7	10.1 ± 1.0
MgO (%)	0.9	0.9 ± 0.2	0.9 ± 0.1	1.1 ± 0.1
Na (%)		0.3 ± 0.0	0.5 ± 0.0	0.2 ± 0.1
K_2O (%)	2.6	3.8 ± 1.0	4.5 ± 1.1	3.8 ± 0.4
SO_3 (%)	4.4	0.5 ± 0.1	0.8 ± 0.2	0.6 ± 0.1
N (%)	6.0	1.3 ± 0.1	2.2 ± 0.1	4.1 ± 0.4
P_2O_5 (%)	7.0	3.3 ± 0.8	2.9 ± 0.5	4.3 ± 0.2
Fe (%)		0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
Mn (mg kg^{-1})		315.2 ± 59.2	213.6 ± 15.3	270.6 ± 27.8
Cu (mg kg^{-1})		102.8 ± 45.8	15.8 ± 1.0	28.9 ± 4.1
Zn (mg kg^{-1})		554.6 ± 290.6	792.1 ± 158.7	407.9 ± 89.4
B (mg kg^{-1})		37.3 ± 7.5	48.3 ± 4.8	52.5 ± 4.3
C/N ratio		26.9 ± 1.6	17.1 ± 2.7	8.5 ± 0.3

[a] Biofence®: defatted *Brassica carinata* seed meal dehydrated pellets. [b] The semicomposted mixture (horse manure and chicken litter; 1:1, v:v) was obtained, under aerobic conditions, from the composter after 25 days at 60 °C, immediately before the maturation phase. [c] OM: organic matter content. Values are expressed on a dry weight basis. Values are means ± standard error (n = 3).

treatment with *B. carinata* dehydrated pellets, treatments included the incorporation of *Sinapis alba* fresh green manure to the soil (Fig. 1). To that end, seeds (15 kg ha^{-1}) of *S. alba* var. Ludique were sown on Brassica-treated plots. At blooming, *S. alba* plants were harvested, mechanically chopped and, subsequently, incorporated to the soil using a rotavator. Brassica plots were then covered with the transparent plastic film for a further four weeks. In this way, Brassica plots were subjected to biodisinfection using organic amendment and plastic mulching twice: first, using *B. carinata* dehydrated pellets and, immediately before pepper planting, *S. alba* as fresh green manure. During these 4-week periods, the other plots were kept in fallow and, except for control plots, soil moisture was adjusted in all plots to that present in Brassica plots. Finally, all plots were plowed with a rotavator up to 20 cm depth, before crop planting.

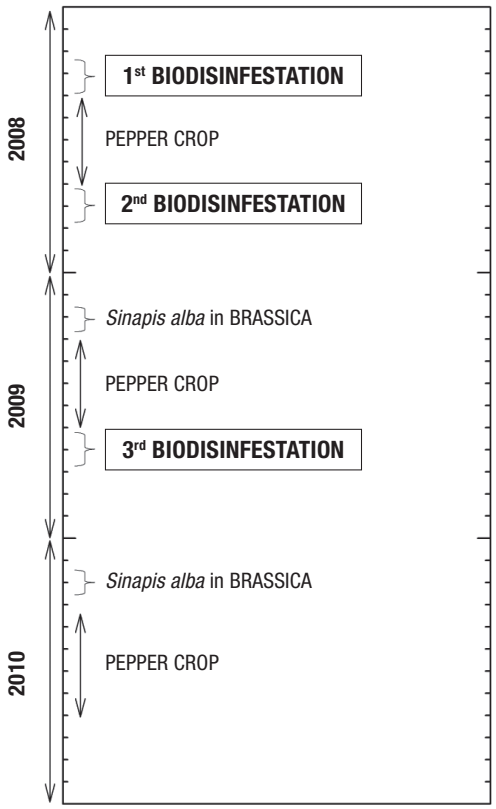

Figure 1. Chronogram of the experiment.

Soil properties

Physicochemical properties

After reiteration of biodisinfection treatments for three consecutive crop seasons, soil samples were randomly collected from each plot immediately before crop planting (March 18th, 2010). Each soil sample was made up from 20 sub-samples (core diameter: 2 cm; soil depth: 0-20 cm), which were then pooled to get a composite sample. After collection, the soil was sieved (< 4 mm) and air-dried at 30 °C for 48 h. Soil pH, electrical conductivity (EC), OM content, N content, organic C content (C_{org}), cation exchange capac-

Table 2. Dosage and time of application of soil amendments during biodisinfection treatments

Treatments	1st Biodisinfection (March 14th-Apr 22nd)	2nd Biodisinfection (Sept 12th-Oct 24th)	3rd Biodisinfection (Aug 5th-Sept 21st)
Non-composted			
Manure[a]	3.60 kg DW m^{-2}	2.70 kg DW m^{-2}	0.73 kg DW m^{-2}
Litter[b]	1.54 kg DW m^{-2}	1.16 kg DW m^{-2}	0.31 kg DW m^{-2}
Semicomposted[c]	5.14 kg DW m^{-2}	3.86 kg DW m^{-2}	1.04 kg DW m^{-2}
Brassica			
Biofence®[d]	0.3 kg DW m^{-2}	0.3 kg DW m^{-2}	0.3 kg DW m^{-2}
S. alba dosage	–[f]	4.5 kg FW m^{-2}	7.2 kg FW m^{-2}
S. alba date[e]	–[f]	Feb 19th-Mar 17th	Feb 18th-Mar 18th

[a] Manure = fresh sheep manure. [b] Litter: dry chicken litter. [c] The semicomposted mixture (horse manure and chicken litter; 1:1, v:v) was obtained, under aerobic conditions, from the composter after 25 days at 60 °C, immediately before the maturation phase. [d] Biofence®: defatted *Brassica carinata* seed meal dehydrated pellets. [e] Date of incorporation of *Sinapis alba* fresh green manure to the soil. DW: dry weight. FW: fresh weight. [f] Brassica treatment did not include the incorporation of *Sinapis alba* fresh green manure to the soil in the first biodisinfection.

ity (CEC) and content of macro- and micronutrients were determined according to standard methods (MAPA, 1994).

At crop planting (March 31st, 2010), two undisturbed soil cores at 3 depth layers (0-10 cm, 10-20 cm, 20-30 cm) were removed from each plot for the determination of soil bulk density following Grossman & Reinsch (2002). Similarly, in each plot, water infiltration rates were measured in duplicate using a double-ring infiltrometer (inner ring = 118 mm, outer ring = 300 mm) at different times ranging for 0 to 480 min. The rate of infiltration was determined according to the amount of water that infiltrated into the soil in the inner ring per surface area and unit of time, using Kostiakov's equations (Kostiakov, 1932).

Microbial properties

Microbial properties were determined in the same soil samples collected for physicochemical characterization. Immediately after collection, soils were sieved (<2 mm) and stored at 4 °C, for less than 1 month, until analysis.

Dehydrogenase activity (EC 1.1) was determined according to Taylor *et al*. (2002). β-glucosidase (EC 3.2.1.21), and alkaline and acid phosphatase (EC 3.1.3.1 and EC 3.1.3.2) activities were determined as described in Epelde *et al.* (2008). Urease (EC 3.5.1.5) activity was determined according to Kandeler & Gerber (1988), as described in Rodríguez-Loinaz *et al.* (2008). Fluorescein diacetate hydrolysis (FDA) was estimated according to Schnürer & Rosswall (1982).

Potentially mineralizable N (N_{min}) and water-soluble organic carbon (WSOC) were determined following Powers (1980) and Epelde *et al.* (2010), respectively. Microbial biomass carbon (C_{mic}) was determined using the fumigation-extraction method (Vance *et al.*, 1987).

Density of culturable microbial populations was determined using a 10-fold serial dilution plate-count technique in soil samples collected at the end of the winter fallow. Four selective media were used: (i) tryptic soy agar supplemented with cycloheximide at 100 μg mL^{-1} for total bacteria, (ii) potato dextrose agar with streptomycin sulfate at 100 μg mL^{-1} for fungi, (iii) Actinomycetes isolation agar for actinomycetes, and (iv) King's medium B for *Pseudomonas* spp. Plates were incubated (in triplicate) for 7 days at 25 °C. Total bacteria colonies appearing on plates were enumerated daily for 4 days and then on day 7 (*i.e*., 5 counts). Total bacteria data were used to calculate the colony development index (CD) (Kozdrój *et al.*, 2004) and the ecophysiological index (EP) (de Leij *et al.*, 1993). Both indices were calculated as described in Correa *et al.* (2009). Briefly, CD index was calculated as follows: $CD = (N_1/1 + N_2/2 + + N_3/3 + N_4/4 + N_7/7)$, where N = proportion of total bacteria appearing on each day. The EP index was calculated as follows: $EP = \sum(N_i \times \log_{10} N_i)$, where N_i = proportion of colonies on i^{th} day.

Soil suppressiveness

Since *P. capsici* was not able to grow onto cellophane, soil suppressiveness was determined using *Rhizoctonia solani* following Grünwald *et al.* (1997). A potate dextrose agar plug from a 7 day-old *R. solani* culture was placed on the centre of the cellophane surface. After 24 h incubation, the radial growth of the fungal colony was measured in two perpendicular directions and averaged. Each treatment was repeated nine times (three replicates per each soil sample from a plot and three plots per treatment). The relative reduction in *R. solani* growth was calculated as follows: relative growth = radial growth (cm) on non-autoclaved soil / radial growth (cm) on autoclaved soil.

Disease incidence

After reiteration of the three biodisinfection treatments, at crop planting (March 31st, 2010), pepper plants (*C. annuum* L., Derio variety) were planted in the experimental plots with a crop density of 33,670 plants ha^{-1} (corresponding to four rows of 18 plants per plot). Only data from the two inner rows of each four-row experimental plot were used. The incidence of *Phytophthora* root and crown rot of pepper was monitored weekly until the end of the crop season (October 20th, 2010). Plants were rated as "diseased" if wilted or when crown rot lesions were observed. Disease incidence was expressed as percentage of diseased plants at the end of the crop season (final disease incidence). Immature carnation petals were used as "vegetable traps" (Ricci, 1972) to confirm the presence of *P. capsici* in dead plants. Finally, pepper fruits were harvested twice a week throughout the crop season and total yield (kg m^{-2}) determined.

Statistical analysis

Data were analysed using Statistical Analysis Software, SAS Institute. Pearson's correlation analysis was done using SPSS for Windows. Data on final disease incidence, and soil physicochemical and microbial properties were submitted to one-way ANOVA: treatment means were compared by the Waller-Duncan's Bayesian *K*-ratio *t* test ($p < 0.05$). Prior to analysis, data were checked for homogeneity and homocedasticity, and transformed when needed. To that end, data on final disease incidence were transformed according to the following equation: arcsin $\sqrt{(x/n)}$, where x = number of diseased plants and n = number of plants in each plot, and then submitted to one-way ANOVA; data on microbial counts were normalized using $\log_{10}(x + 1)$ transformation, where x is the average number of colonies per gram of dry weight soil.

Results

Physicochemical soil properties

The non-composted treatment decreased bulk density at 0-10 and 20-30 cm, in relation to control plots (Table 3). The semicomposted treatment decreased soil bulk density at 0-10 and 10-20 cm. On the other hand, non-composted, semicomposted and Brassica treatments increased soil water infiltration as compared to plastic-mulched and control plots (Table 3).

At the end of the winter fallow, non-composted and semicomposted treatments increased, in general terms, the values of all soil physicochemical parameters, except for pH (Table 4). Besides, non-composted was the only treatment that significantly increased the values of P_2O_5, Cl^-, Na^+ and Zn^{2+} content in relation to control plots, and significantly higher values of Cu content were found only in semicomposted soils. However, no significant differences were observed between Brassica-treated plots and control soils (Table 4).

Microbial soil properties at the end of the winter fallow

Immediately before pepper crop planting, non-composted and semicomposted soils showed significantly higher values of all enzyme activities than control soils, whereas Brassica treatment significantly increased the values of dehydrogenase, β-glucosidase and acid phosphatase activity as compared to control plots (Table 5). No significant differences were found between control and plastic-mulched plots (Table 5). Significant positive correlations were obtained between OM content and enzyme activities: FDA ($r = 0.746$, $p = 0.001$), dehydrogenase ($r = 0.570$, $p = 0.033$), urease ($r = 0.826$, $p < 0.001$), alkaline phosphatase ($r = 0.788$, $p < 0.001$) and acid phosphatase ($r = 0.632$, $p = 0.012$).

Biodisinfested soils had significantly higher values of N_{min} than control and plastic-mulched soils, but higher values of WSOC and C_{mic} were obtained in non-composted and semicomposted plots *versus* all the other plots (highest values were found in non-composted soils) (Table 5).

Pertaining to microbial population densities, higher counts of total bacteria, actinomycetes and *Pseudomonas* spp. were found in Brassica-amended soils as compared to all the other soil treatments (Table 6). Counts of total bacteria, actinomycetes and *Pseudomonas* spp. in non-composted and semicomposted plots were also higher

Table 3. Effect of biodisinfection treatments on soil bulk density and infiltration

Treatments[a]	Bulk density (g cm^{-3})			Infiltration (cm)
	0-10 cm	10-20 cm	20-30 cm	
Control	1.35 ± 0.05 a	1.32 ± 0.02 a	1.38 ± 0.07 a	42.81 ± 8.41 c
Plastic-mulched	1.28 ± 0.03 ab	1.31 ± 0.05 a	1.34 ± 0.05 a	87.17 ± 26.37 b
Non-composted	1.20 ± 0.04 bc	1.21 ± 0.05 ab	1.19 ± 0.03 b	173.36 ± 12.43 a
Semicomposted	1.13 ± 0.04 c	1.17 ± 0.03 b	1.27 ± 0.01 ab	139.39 ± 27.19 a
Brassica	1.28 ± 0.03 ab	1.28 ± 0.04 ab	1.35 ± 0.03 a	182.98 ± 46.39 a

[a] Control: untreated soil; Plastic-mulched: plastic-mulched soil; Non-composted: non-composted manure amended soil + plastic-mulched; Semicomposted: semicomposted manure amended soil + plastic-mulched; Brassica: *B. carinata* dehydrated pellets + *S. alba* fresh green manure amended soil + plastic-mulched. For each variable and depth, values followed by the same letter are not significantly different according to Waller-Duncan's *K*-ratio *t* test ($p < 0.05$). Mean values ± standard errors (n = 6).

Table 4. Effect of biodisinfection treatments on soil physicochemical properties

Variable	Treatments[a]				
	Control	Plastic-mulched	Non-composted	Semicomposted	Brassica
OM[b] (%)	4.83 ± 0.39 b	5.32 ± 0.17 b	7.28 ± 0.61 a	6.81 ± 0.10 a	5.23 ± 0.18 b
C_{org} (%)	2.80 ± 0.22 b	3.09 ± 0.10 b	4.22 ± 0.36 a	3.95 ± 0.06 a	3.03 ± 0.10 b
N (%)	0.21 ± 0.02 b	0.21 ± 0.01 b	0.30 ± 0.03 a	0.28 ± 0.02 a	0.18 ± 0.02 b
P_2O_5 (mg kg^{-1})	109.8 ± 11.4 c	108.2 ± 12.3 c	288.8 ± 25.5 a	247.0 ± 14.6b	126.9 ± 6.2 c
Cl^- (meq L^{-1})	0.94 ± 0.46 c	0.91 ± 0.38 c	9.17 ± 1.31 a	3.18 ± 0.94 b	1.61 ± 0.36 bc
pH	6.87 ± 0.26	6.71 ± 0.12	7.10 ± 0.03	6.89 ± 0.07	6.95 ± 0.19
EC[c] (dS m^{-1})	1.72 ± 0.44 c	2.05 ± 0.80 bc	3.84 ± 0.36 a	3.09 ± 0.21 ab	2.12 ± 0.33 bc
K^+ (meq kg^{-1})	0.03 ± 0.01 c	0.05 ± 0.01 c	0.32 ± 0.03 a	0.18 ± 0.01 b	0.07 ± 0.01 c
Ca^{2+} (meq kg^{-1})	1.18 ± 0.15 c	1.42 ± 0.12 bc	1.91 ± 0.03 a	1.67 ± 0.03 ab	1.37 ± 0.12 bc
Mg^{2+} (meq kg^{-1})	0.17 ± 0.02 b	0.20 ± 0.02 b	0.31 ± 0.01 a	0.29 ± 0.01 a	0.20 ± 0.02 b
Na^+ (meq kg^{-1})	0.09 ± 0.01 b	0.08 ± 0.01 b	0.16 ± 0.01 a	0.13 ± 0.03 ab	0.10 ± 0.02 ab
CEC[d] (meq kg^{-1})	1.33 ± 0.18 b	1.56 ± 0.09 b	2.40 ± 0.05 a	2.05 ± 0.03 a	1.55 ± 0.12 b
Cu^{2+} (mg kg^{-1})	1.65 ± 0.17 b	1.58 ± 0.09 b	1.69 ± 0.11 b	2.85 ± 0.20 a	1.50 ± 0.23 b
Zn^{2+} (mg kg^{-1})	5.43 ± 0.18 bc	5.86 ± 0.53 bc	12.79 ± 2.06 a	8.44 ± 0.66 b	4.84 ± 0.42 c

[a] Treatments: see Table 3. All values are expressed on a dry soil weight basis. [b] OM: organic matter content; [c] EC: electrical conductiviyty ; [d] CEC: cation exchange capacity; For each variable, values followed by the same letter are not significantly different according to Waller-Duncan's *K*-ratio *t* test ($p < 0.05$). Mean values ± standard errors (n = 3).

than those of the control. No significant differences among treatments were found with regard to fungi. Lowest and highest values of the CD index were obtained in non-composted and semicomposted plots, respectively, whereas non-composted was the only treatment that resulted in higher values of the EP index (Table 6).

Soil suppressiveness

Higher values of *R. solani* growth were observed in autoclaved *versus* non-autoclaved biodisinfested soils (as reflected by the relative growth values). The relative growth of *R. solani* was lower in biodisinfested soils than in controls. Semicomposted treatment resulted in lower values of relative growth of *R. solani* as compared to plastic-mulched plots (Fig. 2).

Disease incidence and crop yield

Semicomposted was the only treatment that significantly reduced disease incidence as compared to control and plastic-mulched plots (Fig. 3). Similarly, higher

Table 5. Effect of biodisinfection treatments on soil microbial properties

Variable	Treatments[a]				
	Control	Plastic-mulched	Non-composted	Semicomposted	Brassica
FDA[b] (mg F[c] kg^{-1} h^{-1})	77.7 ± 7.4 bc	64.0 ± 1.8 c	129.8 ± 4.0 a	114.7 ± 10.7 a	93.5 ± 4.8 b
Dehydrogenase (mg INTF[d] kg^{-1} h^{-1})	4.8 ± 0.6 b	4.8 ± 0.3 b	10.5 ± 1.1 a	9.7 ± 0.2 a	9.0 ± 1.6 a
Urease (mg N-NH_4^+ $kg^{-1}h^{-1}$)	23.8 ± 0.9 b	17.2 ± 1.6 b	56.8 ± 7.2 a	46.9 ± 5.0 a	26.1 ± 3.3 b
β-Glucosidase (mg NP[e] kg^{-1} h^{-1})	39.3 ± 3.0 c	39.1 ± 2.8 c	64.5 ± 1.9 a	64.6 ± 2.5 a	51.2 ± 3.5 b
Alkaline phosphatase (mg NP kg^{-1} h^{-1})	245.1 ± 21.4 cd	210.3 ± 9.6 d	456.5 ± 20.0 a	393.7 ± 5.4 b	280.2 ± 36.7 c
Acid phosphatase (mg NP kg^{-1} h^{-1})	318.3 ± 32.5 b	290.1 ± 15.6 b	416.5 ± 16.2 a	396.1 ± 21.8 a	398.6 ± 12.2 a
N_{min} (mg N-NH_4^+ kg^{-1})	20.3 ± 2.0 c	17.7 ± 3.1 c	29.1 ± 1.5 ab	28.8 ± 3.6 b	33.4 ± 3.4 a
WSOC (mg C_{org} kg^{-1})	67.2 ± 5.9 c	64.3 ± 2.6 c	99.7 ± 5.7 a	82.5 ± 1.8 b	65.2 ± 3.1 c
C_{mic} (mg C kg^{-1})	277.8 ± 17.0 c	241.5 ± 14.6 c	463.8 ± 5.0 a	401.5 ± 44.8 b	296.7 ± 24.2 c

[a] Treatments: see Table 3. All values are expressed on a dry soil weight basis. [b] FDA: fluorescein diacetate hydrolysis; [c] F: fluorescein sodium salt; [d] INTF: iodonitrotetrazolium formazan; [e] NP: nitrophenol; N_{min}: potentially mineralizable nitrogen; WSOC: water soluble organic carbon; C_{mic}: microbial biomass carbon. For each variable, values followed by the same letter are not significantly different according to Waller-Duncan's *K*-ratio *t* test ($p < 0.05$). Mean values ± standard errors (n = 6).

Table 6. Effect of biodisinfection treatments on soil microbial (total bacteria, actinomycetes, *Pseudomonas* spp., fungi) population densities (CFU g^{-1} of dry soil). Colony development (CD) and ecophysiological (EP) indices calculated from total bacteria counts

Variable	Treatments[a]				
	Control	Plastic-mulched	Non-composted	Semicomposted	Brassica
Total bacteria ($\times 10^6$)	28 ± 4.3 c	30 ± 5.5 c	44 ± 3.4 b	48 ± 5.1 b	89 ± 11 a
Actinomycetes ($\times 10^6$)	22 ± 3.8 d	37 ± 6.5 cd	58 ± 6.5 b	44 ± 9.3 bc	110 ± 17.0 a
Pseudomonas spp. ($\times 10^6$)	32 ± 4.3 d	43 ± 5.3 cd	51 ± 4.3 bc	58 ± 3.8 b	110 ± 16.0 a
Fungi ($\times 10^4$)	20 ± 2.3 a	25 ± 4.0 a	18 ± 1.9 a	16 ± 1.2 a	22 ± 2.2 a
CD index	52.99 ± 2.54 b	56.23 ± 3.40 b	43.26 ± 2.17 c	64.10 ± 2.92 a	58.39 ± 2.31 b
EP index	0.58 ± 0.02 b	0.56 ± 0.04 b	0.66 ± 0.01 a	0.54 ± 0.03 b	0.55 ± 0.02 b

[a] Treatments: see Table 3. CFU: colony forming units. For each variable, values followed by the same letter are not significantly different according to Waller-Duncan's *K*-ratio *t* test ($p < 0.05$). Mean values ± standard errors (n = 9).

values of disease incidence were found in plastic-mulched plots *versus* semicomposted plots.

Average values of total yield (kg m^{-2}) at the end of the crop season were: control = 6.6 ± 0.9, plastic-mulched = 9.2 ± 0.8, non-composted = 9.7 ± 0.1, semicomposted = 10.5 ± 0.7 and Brassica = 8.5 ± 0.9. Significantly higher values of crop yield were obtained in semicomposted and non-composted plots, as compared to control plots, respectively leading to 59 and 47% increases in final crop yield.

Discussion

Biodisinfection appears a good alternative to chemical soil disinfection, even when soil temperatures reached during treatments are not high enough to thermally inactivate plant pathogens (Núñez-Zofío *et al*., 2011). As abovementioned, biodisinfection effectiveness for soilborne pathogen control is due to a variety of mechanisms: increase in soil temperature, accumulation of toxic volatile compounds, creation of anaerobic conditions and increase in soil suppressiveness. Among them, the generation of toxic volatile compounds (*e.g*., NH_3) has been studied in great detail (Tenuta & Lazarovits, 2002; Tenuta *et al*., 2002).

On the other hand, Oka (2010) indicated that the requirement of large amounts of organic amendments for effective pathogen control is an important limitation of this technique, not only because of the potential phytotoxic effects of amendments but also for the high levels of greenhouse gas emission released during the decomposition of OM, as reported by Arriaga *et al.* (2011). The reduction in disease incidence observed here after repeated application of biodisinfection treatments, especially in semicomposted manure-treated

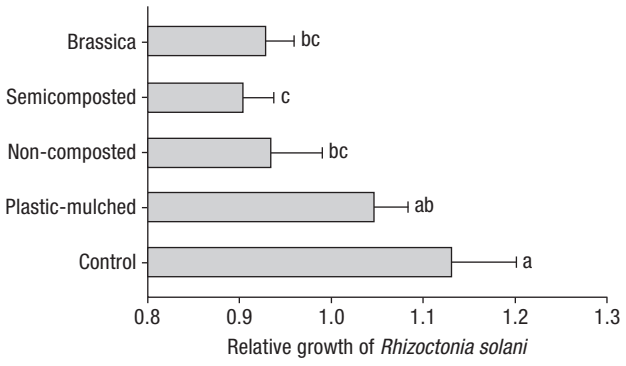

Figure 2. Effect of treatments on soil suppressiveness. Mean values with the same letter are not significantly different according to Waller-Duncan's *K*-ratio *t* test ($p < 0.05$). Error bars represent standard error (n = 9).

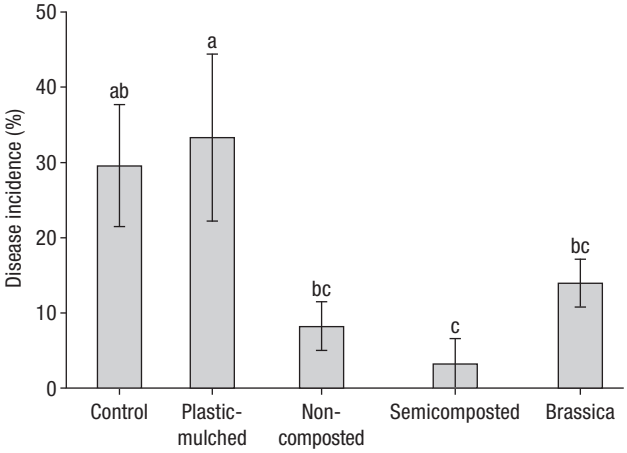

Figure 3. Effects of soil treatments on *Phytophthora* root and crown rot of sweet pepper cv. Derio. Mean values with the same letter are not significantly different according to Waller-Duncan's *K*-ratio *t* test ($p < 0.05$). Error bars represent standard error (n = 3).

plots, indicates that the progressively lower doses of organic amendment used in this study (as compared to those used in previous studies; see Arriaga *et al*., 2011; Núñez-Zofío *et al*., 2011) can also result in effective pathogen control.

The incorporation of organic amendments is known to improve soil properties and fertility (Bonanomi *et al.*, 2007). After repeated biodisinfection, non-composted and semicomposted treatments decreased soil bulk density causing an improvement in soil water properties, as reflected by the higher infiltration values, in agreement with Martens & Frankenberger (1992). Improvements in soil water properties that prevent water flooding are known to facilitate soilborne pathogen control, mainly in the case of Oomycetes (Liu *et al.*, 2008).

As expected, non-composted and semicomposted plots presented higher contents of soil OM and macro/micronutrients than non-amended soils. By contrast, no differences in physicochemical properties were observed between Brassica and control and plastic-mulched soils, most likely due to the lower level of OM applied in Brassica treatment, as compared to non-composted and semicomposted treatments. The incorporation of fresh manure to the soil must be carried out with caution due to a possible increase in soil salt content which can alter soil properties and affect crop production and disease control (Litterick *et al.*, 2004; Moral *et al.*, 2009). In our study, significantly highest values of P_2O_5, K^+ and Cl^- were obtained in non-composted than in semicomposted soils. Although salinization can negatively affect soil microbial properties (Rietz & Haynes, 2003), we found no differences in soil microbial properties between non-composted and semicomposted soils. In any event, composting provides a more stabilized product, thereby reducing the risk of soil salinization, leaching and phytotoxicity (Moral *et al.*, 2009).

Enzyme activities are closely related to OM content (Beyer *et al.*, 1993). At the end of the winter fallow, positive correlations were obtained between OM content and enzyme activities. The higher values of enzyme activities obtained in non-composted and semicomposted plots were concomitant with higher values of C_{mic}, indicating that the higher levels of microbial activity were in this case due to an increase in microbial biomass. Mandal *et al.* (2007) also found that the incorporation of farmyard manure increased enzyme activities in response to an increase in C_{mic} and an improvement in the soil nutrient status. Similarly, we observed an increase in N_{min} and WSOC (indicators of biologically active N and C, respectively) in non-composted and semicomposted soils. By contrast, Brassica treatment had a weaker effect on soil microbial properties, probably due to the lower level of OM applied. However, in Brassica-amended soils, an increase in dehydrogenase, β-glucosidase and acid phosphatase activity was observed in relation to non-amended plots. Apart from the OM input, this increase of enzyme activities could also be attributed, at least partly, to a stimulatory rhizosphere effect caused by *S. alba* plants during the winter season. This rhizosphere effect could also be responsible for the higher values of microbial population densities (total bacteria, actinomycetes and *Pseudomonas* spp.) detected in Brassica soils.

Higher counts of total bacteria, actinomycetes and *Pseudomonas* spp. were also obtained in non-composted and semicomposted plots, compared to non-amended plots. Interestingly, significant differences were however obtained regarding CD and EP indices between these two treatments: the higher CD values in semicomposted soils indicate a greater proportion of r-strategists; in contrast, the higher EP values in non-composted soils suggest a more even distribution of the bacterial community structure (Correa *et al.*, 2009). Although a change in the composition and diversity of microbial communities during OM decomposition has been related with disease suppression (van Bruggen & Semenov, 2000), in our experiment, no differences in soil suppressiveness and disease incidence were observed between non-composted and semicomposted plots. Boehm *et al.* (1997) found a predominance of oligotrophs in conducive substrates to *Pythium ultimun* as well as a higher predominance of copiotrophic taxa, like *Pseudomonas* spp., in suppressive soils. We found a negative correlation between *Pseudomonas* spp. populations and relative growth of *R. solani* ($r = –0.708$, $p = 0.003$), suggesting a *Pseudomonas*-induced suppressiveness against *R. solani* growth. Pseudomonads populations have been proposed as good indicators of soil suppressiveness (Janvier *et al.*, 2007; Bonanomi *et al.*, 2010).

A positive correlation was found between disease incidence and relative growth of *R. solani* ($r = 0.698$, $p = 0.006$). Higher values of relative growth of *R. solani* correspond to a soil with a lower suppressive capacity (Grünwald *et al.*, 1997). The semicomposted treatment was the only one that reduced both relative growth of *R. solani* and disease incidence,

as compared to non-amended plots. Then, the improvement in soil physicochemical and microbial properties is most likely responsible for the lower values of disease incidence. As expected, a significant negative correlation was obtained between crop yield and disease incidence ($r = –0.818$, $p < 0.001$). Nevertheless, despite the improvement in soil properties, no significant reduction in disease incidence was obtained in non-composted soil treatments, which could be explained by their higher salt content with concomitant negative effects on *Phytophthora* spp. control (Hoitink *et al.*, 1993). Several authors recommend the use of composted manures for pathogen control, due to the controversial results obtained with fresh manures (Aryantha *et al.*, 2000; Litterick *et al.*, 2004). In any case, although differences were not statistically significant, values of disease incidence in non-composted and Brassica biodisinfection treatments were lower than in control and plastic-mulched treatments.

In this study, we have found that repeated biodisinfection for the control of *Phytophthora* root and crown rot in protected pepper crops located in temperate climate regions can improve soil quality and suppressiveness, as well as allow for a reduction in the dose of organic amendment needed for biodisinfection. Among the studied organic amendments, the semicomposted amendment was the best option in terms of reduction in disease incidence.

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