Chapter 21
Biodisinfestation with Organic Amendments for Soil Fatigue and Soil-Borne Pathogens Control in Protected Pepper Crops

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21.1 Soil Phytopathological Problems and Soil Fatigue in Protected Pepper Crops

Phytophthora root rot is a destructive disease for pepper plants (*Capsicum annuum* L.) worldwide (Wang et al. 2014). The mortality of pepper plants ranges between 30 and 40 % and in severe cases, even 100 % (Liu et al. 2008). As a consequence of the high plant mortality, relevant economic losses have been reported in pepper crops from Spain, not only in the Mediterranean region (Tello and Lacasa 1997) but also in areas characterised by a humid temperate climate, such as the Basque Country (Northern Spain) (Larregla 2003). The main causal agents of this disease in greenhouse pepper crops are the oomycetes *Phytophthora capsici* and *P. cryptogea* in Northern Spain and *P. capsici* and *P. parasitica* in South-eastern Spain. In the last region, the nematode *Meloidogyne incognita* is also a recurring and persistent problem that causes substantial crop damages (Tello and Lacasa 1997; Bello et al. 2004). In South-eastern Spain (Murcia and Alicante provinces) pepper occupies more than 90 % of the area dedicated to greenhouse crops and has been a monoculture for the last 20 years (Lacasa and Guirao 1997). The normal

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crop cycle lasts 9–10 months (November–December to September–October). Strategies recommended for management of phytophthora root rot involve integrated approaches that focus on cultural practices: reduced soil moisture, reduction of pathogen propagule in soil, utilisation of cultivars with resistance to the disease and the judicious fungicide applications (Ristaino and Johnston 1999). Until the year 2005, methyl bromide (MB) was used to disinfect soils to control both pathogens (Gilreath and Santos 2004) and to lessen the effects of fatigue caused by repeated monocultures (Martínez et al. 2011a). Since 2005, MB has been replaced by a mixture of 1,3-dichloropropene and chloropicrin, but these will also be banned by European legislation in the near future. The increasing demand for ecological foods produced by sustainable agricultural practices must be added to this forthcoming ban, meaning that non-chemical methods will have to be developed for controlling soil-borne plant pathogens and plant parasitic nematodes adapted for use in intensive horticulture.

In recent years, numerous alternatives for 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 (Guerrero et al. 2013) in intensive protected horticultural crops.

In this book chapter, we aim to review both the mechanisms involved in disease suppression and the organic amendment management strategies for the control of protected pepper crops soil-borne diseases and soil fatigue. Several disease suppression mechanisms following the addition of organic matter such as (1) the release of compounds that are toxic to the pathogens, (2) the stimulation of non-pathogenic microorganisms that inhibit or kill the pathogens and (3) the improvement of soil physical, chemical and biological properties will be explained.

21.2   Mechanisms Involved in Disease Suppression by Soil Organic Amendments

Several mechanisms have been identified as contributing to disease suppression following the addition of organic matter. These namely include the stimulation of non-pathogenic microorganisms that inhibit or kill the pathogens through competition (Lockwood 1988) or parasitism (Hoitink and Boehm 1999), the release of compounds that are toxic for the pathogens (Bailey and Lazarovits 2003) and the stimulation of the host plant's disease defence system (Zhang et al. 1996, 1998). Other indirect mechanisms that explain the ability of organic amendments to increase soil suppressiveness are: improvement of nutrition and vigour in the host plants and improvement of physicochemical and biological properties of soil. Some organic amendments are thought to work primarily by altering the structure of the microbial communities in the soil or by changing the physical and chemical properties of the soil.

21.2.1 Production of Biocidal Compounds

The application of animal manure leads to the generation of ammonia (NH_3), which is the mechanism most often implicated in killing soil pathogens (Tenuta and Lazarovits 2002), although other lethal molecules such as nitrous acid and volatile fatty acids (VFA) have also been reported (Tenuta et al. 2002; Conn et al. 2005). Tenuta and Lazarovits (2002) summarised that NH_3 is thought to kill cells by disrupting membranes, eliminating proton gradients across membranes, through the assimilation of NH_3 into glutamine and the exhaustion of the chemical energy of cells removing cytosolic NH_3. Accumulation of NH_3 and VFA derived from manure application have been described as mechanisms capable to kill soil pathogens (Conn et al. 2005), which is largely influenced by several factors that include moisture content, pH, soil organic matter content and quality, soil texture and buffering capacity and nitrification rate soil buffering capacity. These authors concluded that high VFA toxicity was achieved in acid soils (pH about 5.0) while high NH_3 toxicity was related with alkaline soils (pH about 7.5).

The application of animal manure followed by soil plastic covering during spring period reduced inoculum survival of the fungal pathogen *P. capsici* and disease incidence in a greenhouse pepper crop in Northern Spain (Arriaga et al. 2011). Northern Spain is an area characterised by a temperate climate with annual mean temperature of 12 °C, a maximum mean temperature in summer of 25 °C and rainfall of 1200 mm per year.

Ammonia volatilisation, among other volatile compounds, and the increase in soil suppressiveness contributed to minimise *P. capsici* inoculum survival rate and subsequent greenhouse crop disease incidence, respectively. The use of fresh manure favoured NH_3 volatilisation as organic nitrogen (N_{org}) mineralisation was higher than in semicomposted manure, with more stable N_{org} content. Mean NH_3 concentration increased with fresh sheep manure and dry chicken litter (SCM) during biodisinfestation process compared with semicomposted mixture of horse manure and chicken litter (HCM) (Fig. 21.1).

NH_3 concentration increased significantly after manure amendment with respect to control plots (C) and also differed between SCM and HCM manure. Ammonia concentration from C plots averaged 3.9 mg NH_3 m^{-3}, while SCM averaged 14.8 mg NH_3 m^{-3} and 9.1 mg NH_3 m^{-3} in HCM. The highest NH_3 concentrations were reached at the beginning of the experiment in SCM and HCM treatments and decreased 45.0 % after 35 days of soil biodisinfestation (Fig. 21.1). The reduction of NH_3 concentrations could be related to adsorption of NH_3 or the increasing anaerobic conditions during manure decomposing process (Kirchmann and Witter 1989). The high water condensation observed on the plastic inner surface, which would trap volatilised NH_3 (Kroodsma et al. 1993), and the overall anaerobic conditions under plastic sheets might have reduced NH_3 accumulation (Kirchmann and Witter 1989).

P. capsici inoculum survival rate in infected plant residues was significantly different among treatments. The application of fresh SCM under plastic sheets
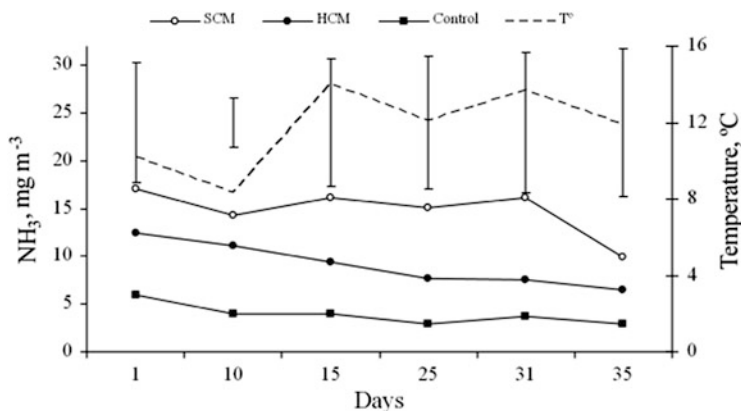
Fig. 21.1 Evolution of NH_3 concentration under the plastic sheets from non-amended (control), fresh manure (SCM) and semicomposted manure (HCM) amended plots during soil biodisinfestation starting on March 14, 2008, showing variation of air temperature throughout the biodisinfestation period. The *vertical bars* indicate least significance difference at 0.05 between treatments. Soil was tarped with 50-μm-thick (two million) transparent low density polyethylene plastic film. The greenhouse field experiment was located in Derio (Biscay) (Northern Spain). Reprinted from Journal of Crop Protection, 30(4), H. Arriaga et al. (2011), Gaseous emissions from soil biodisinfestation by animal manure on a greenhouse pepper crop, 412–419, Copyright (2015), with permission from Elsevier

reduced *Phytophthora* inoculum survival in relation to HCM and S treatments (Table 21.1) ($P < 0.05$). Biodisinfestation by SCM manure reduced by 50 % inoculum survival rate compared with C plots (61.1 %), while *Phytophthora* inoculum survived in 75.0 % and 94.4 % of plant residues in HCM and S treatments, respectively, which was significantly higher than survival reported in C plots. The higher NH_3 concentration in SCM contributed to reduce the inoculum survival rate of *P. capsici* (30.6 % and 75.0 % in SCM and HCM treatments, respectively). Inoculum survival rate was not reduced in solarised non-amended plots (94.4 %) as soil temperature at 15 cm depth did not exceed 33 °C under plastic sheets in S, SCM and HCM treatments (a temperature known to be insufficient to inactivate resistant propagules of *P. capsici* since this pathogen normally shows an optimum temperature range at 24–33 °C) (Erwin and Ribeiro 1996; Etxeberria et al. 2011). Additionally, warm soil temperatures and water condensation detected during the biodisinfestation process might have favoured the conditions for *Phytophthora* inoculum survival in S and HCM plots when compared with C plots. The lower inoculum survival rate in C plots was related to the higher water evaporation and the subsequent lower soil volumetric water content in these uncovered soils.

Higher *P. capsici* inoculum inactivation observed in SCM was attributed to the effect of toxic volatile compounds generated from the decomposition of organic amendments. Soil pH of our experiment averaged 6.9 in SCM and HCM treatments, which might suggest that NH_3 contributed significantly to the reduction of *Phytophthora* inoculum survival in SCM. Moreover, Oka et al. (2007) summarised

Table 21.1 Infected plant rate (inoculum survival), disease incidence and crop yield in non-treated control (C), solarized (S), fresh sheep manure and dry chicken litter (SCM) and semicomposted mixture of horse manure and chicken litter (HCM) biodisinfestated plots

Treatment	Infected plant residues (%)	Disease incidence (%)	Crop yield (kg m^{-2})
C	61.1 ab	40.7 a	3.0 b
S	94.4 a	42.6 a	3.4 b
SCM	30.6 b	2.8 b	4.6 a
HCM	75.0 a	8.3 b	4.3 a

Soil was tarped with 50-μm-thick (two million) transparent low density polyethylene plastic film. The greenhouse field experiment was located in Derio (Biscay) (Northern Spain). Reprinted from Journal of Crop Protection, 30(4), H. Arriaga et al. (2011), Gaseous emissions from soil biodisinfestation by animal manure on a greenhouse pepper crop, 412–419, Copyright (2015), with permission from Elsevier
For each variable, values followed by the same letter are not significantly different according to Fisher's protected LSD test ($P < 0.05$). Mean values ($n = 3$)

that soil amendments with low C/N ratios have been reported to have fungicidal activity mainly through the release of NH_3. In our experiment, the amount of manure amended exceeded those rates applied by Oka et al. (2007), which would support that NH_3 was the main factor controlling inoculum survival of Arriaga et al. (2011).

21.2.2   Increase in Microbial Activity

Phytophthora disease incidence decreased significantly in biodisinfestated SCM and HCM plots compared with C and S treatments (Table 21.1). Plant disease incidence was reduced by 90 % in SCM and HCM plots in relation to inoculum survival rate observed in plant residues 4 months before. Of note, disease was only reduced by 33 % and 54 % in C and S plots, respectively. The application of high amounts of organic amendments contributes to the suppressive capacity of soils through enhanced activity and growth of edaphic microorganisms, which may play an important role in reducing disease incidence by an antagonistic mechanism (Hoitink and Boehm 1999). The significant reduction of disease incidence compared with the high inoculum survival rate could explain this phenomenon in SCM and HCM plots. Several authors have also reported the success of organic matter applications in the control of *Phytophthora* spp., suggesting that the competition for nutrients and antibiosis are the main mechanisms involved in *Phytophthora* spp. suppressiveness (Leoni and Ghini 2006).

21.2.3   Improvement of Plant Nutrition and Vigour

Soil organic matter management affects not only soil biological properties but also soil chemical and physical properties and plant nutrient status. All of them improve plant health and vigour (Stone et al. 2004) and thus may help the plants to overcome pathogen infection. The increase in soil-borne disease suppression by organic amendments may also be attributed to other effects such as increase in plant nutritional status and vigour (Hoitink et al. 1997).

Pepper fruit yield increased with manure amendment in SCM and HCM (Table 21.1), as the application of organic amendments improves soil quality, increasing the amount of plant-available nutrients and, in consequence, crop yield (Liu et al. 2008).

Highest values of crop vigour (plant height) were observed in plots amended with animal manures, and differences increased during crop development (Fig. 21.2) (Núñez-Zofío et al. 2010; Arriaga et al. 2011).

21.2.4   Improvement of Soil Physical and Chemical Properties

Detected differences in plant nutrient status have been generally found between nonamended and amended soils. This could be due to the improved nutrient content, water holding capacity and soil structure imparted to the soil by the amendments (Vallad et al. 2003).

The effects of repeated biodisinfestation with different organic amendments after three consecutive crop seasons improved soil physical properties through a reduction in soil bulk density and an increase in soil water infiltration (Table 21.2) (Núñez-Zofío et al. 2012). This management strategy provided an effective control of phytophthora root rot in protected pepper crops. Improvements in soil water properties that prevent water flooding are known to facilitate soil-borne pathogen control, mainly in the case of oomycetes (Liu et al. 2008).

In general terms, biodisinfestation with non-composted and semicomposted manures increased the values of all soil chemical properties, except for pH (Table 21.3). Besides, non-composted manure was the only treatment that significantly increased P_2O_5, Cl^-, K^+ and Zn^{2+} contents. Significantly, higher values of Cu^{2+} content were found only in semicomposted manure-biodisinfected soils. However, no significant differences were observed between Brassica-treated plots and control soils (Table 21.3) (Núñez-Zofío et al. 2012).
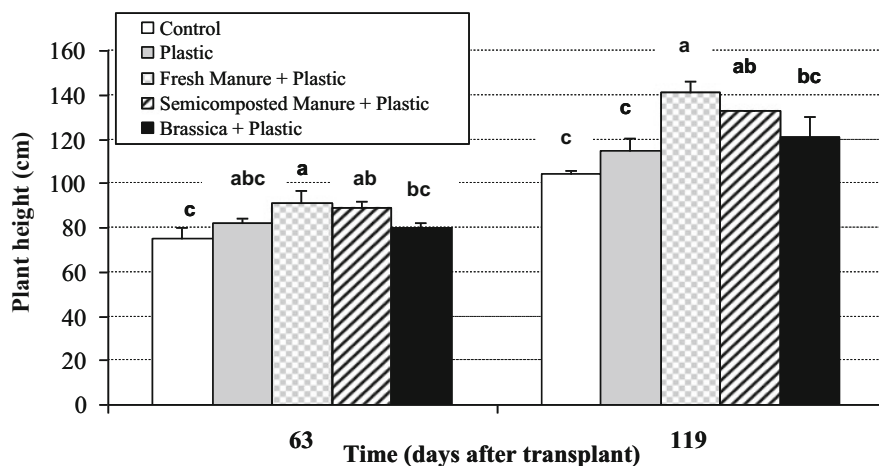
Fig. 21.2 Effect of treatments on pepper plant height, measured at the middle (63 days) and at the end (119 days) of crop development. Error bars represent standard error of the mean ($n = 3$) from three replicate plots. Different *letters* indicate significant differences ($P < 0.05$) (least significance difference = 9.71 and 17.72 cm at 63 and 119 days after transplant, respectively) according to Fisher's protected LSD test. Reprinted from Acta Horticulturae (ISHS), http://www.actahort.org/books/883/883_44.htm, 883, Núñez-Zofío et al. (2010), Application of organic amendments followed by plastic mulching for the control of *Phytophthora* root rot of pepper in Northern Spain, 353–360, with permission from International Society for Horticultural Science

Table 21.2 Effect of biodisinfestation treatments on soil physical properties

Treatments[a]	Bulk density (g cm^{-3})			Infiltration (cm)
	0–10 cm	10–20 cm	20–30 cm	
Control	1.35 a	1.32 a	1.38 a	42.81 c
Plastic-Mulched	1.28 ab	1.31 a	1.34 a	87.17 b
Non-composted	1.20 bc	1.21 ab	1.19 b	173.36 a
Semicomposted	1.13 c	1.17 b	1.27 ab	139.39 a
Brassica	1.28 ab	1.28 ab	1.35 a	182.98 a

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[a]Control: untreated soil, Plastic-Mulched: non-amended 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 ($n = 6$)

Table 21.3 Effect of biodisinfestation treatments on soil chemical properties

Variable	Treatments[a]				
	Control	Plastic-Mulched	Non-composted	Semicomposted	Brassica
OM[b] (%)	4.83 b	5.32 b	7.28 a	6.81 a	5.23 b
C_{org} (%)	2.80 b	3.09 b	4.22 a	3.95 a	3.03 b
N (%)	0.21 b	0.21 b	0.30 a	0.28 a	0.18 b
P_2O_5 (mg kg^{-1})	109.8 c	108.2 c	288.8 a	247.0 b	126.9 c
Cl^- (meq l^{-1})	0.94 c	0.91 c	9.17 a	3.18 b	1.61 bc
pH	6.87	6.71	7.10	6.89	6.95
EC[c] (dS m^{-1})	1.72 c	2.05 bc	3.84 a	3.09 ab	2.12 bc
K^+ (meq kg^{-1})	0.03 c	0.05 c	0.32 a	0.18 b	0.07 c
Ca^{2+} (meq kg^{-1})	1.18 c	1.42 bc	1.91 a	1.67 ab	1.37 bc
Mg^{2+} (meq kg^{-1})	0.17 b	0.20 b	0.31 a	0.29 a	0.20 b
Na^+ (meq kg^{-1})	0.09 b	0.08 b	0.16 a	0.13 ab	0.10 ab
CEC[d] (meq kg^{-1})	1.33 b	1.56 b	2.40 a	2.05 a	1.55 b
Cu^{2+} (mg kg^{-1})	1.65 b	1.58 b	1.69 b	2.85 a	1.50 b
Zn^{2+} (mg kg^{-1})	5.43 bc	5.86 bc	12.79 a	8.44 b	4.84 c

[a]Treatments: see Table 21.2. All values are expressed on a dry soil weight basis
[b]OM: organic matter content
[c]EC: electrical conductivity
[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 ($n = 3$)

21.2.5   Improvement of Soil Biological Properties

Biodisinfected soils with non-composted and semicomposted manure showed significantly higher values of all enzyme activities when compared with control non-amended soils, whereas *Brassica* treatment significantly increased the values of dehydrogenase, β-glucosidase and acid phosphatase activity compared with control non-amended treatments (Table 21.4). Apart from the organic matter input, this increase of enzyme activities could also be attributed, at least partly, to a stimulatory rhizosphere effect caused by a *Sinapis alba* cover crop 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*-amended soil. No significant differences were found between control and plastic-mulched plots (Table 21.4). Significant positive correlations were obtained between organic matter content and the following

Table 21.4 Effect of biodisinfestation treatments on soil biological properties

Variable	Treatments[a]				
	Control	Plastic-Mulched	Non-composted	Semicomposted	Brassica
FDA[b] (mg F kg^{-1} h^{-1})	77.7 bc	64.0 c	129.8 a	114.7 a	93.5 b
Dehydrogenase (mg INTF kg^{-1} h^{-1})	4.8 b	4.8 b	10.5 a	9.7 a	9.0 a
Urease (mg N-NH$_4^+$kg^{-1} h^{-1})	23.8 b	17.2 b	56.8 a	46.9 a	26.1 b
β-glucosidase (mg NP kg^{-1} h^{-1})	39.3 c	39.1 c	64.5 a	64.6 a	51.2 b
Alkaline phosphatase (mg NP kg^{-1} h^{-1})	245.1 cd	210.3 d	456.5 a	393.7 b	280.2 c
Acid phosphatase (mg NP kg^{-1} h^{-1})	318.3 b	290.1 b	416.5 a	396.1 a	398.6 a
N$_{min}$ (mg N-NH$_4^+$ kg^{-1})	20.3 c	17.7 c	29.1 ab	28.8 b	33.4 a
WSOC (mg C$_{org}$ kg^{-1})	67.2 c	64.3 c	99.7 a	82.5 b	65.2 c
C$_{mic}$ (mg C kg^{-1})	277.8 c	241.5 c	463.8 a	401.5 b	296.7 c

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[a]Treatments: see Table 21.2. All values are expressed on a dry soil weight basis

[b]*FDA* fluorescein diacetate hydrolysis, *WSOC* water soluble organic carbon, *N$_{min}$* potentially mineralisable nitrogen, *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 ($n = 6$)

enzyme activities: FDA, dehydrogenase, urease, alkaline phosphatase and acid phosphatase.

Biodisinfested soils had significantly higher values of potentially mineralisable nitrogen than control and plastic-mulched soils, but higher values of water soluble organic carbon and microbial biomass carbon were obtained in manure-biodisinfected soils. Highest values were found in non-composted manure (Table 21.4). The higher values of enzyme activities obtained in non-composted and semicomposted manure-amended soils were concomitant with higher values of microbial biomass carbon, indicating that the higher levels of microbial activity were in this case due to an increase in microbial biomass. It was also observed both in non-composted and semicomposted manure-amended soils, an increase in potentially mineralisable nitrogen and water soluble organic carbon (indicators of biologically active N and C, respectively) (Núñez-Zofío et al. 2012).

Mandal et al. (2007) also found that the incorporation of farmyard manure increased enzyme activities in response to an increase in microbial biomass carbon and an improvement in the soil nutrient status.

The incorporation of fresh manure to the soil must be carried out with caution due to a possible increase in salt content which can alter soil properties and affect crop production and disease control (Litterick et al. 2004; Moral et al. 2009).

Núñez-Zofío et al. (2012) reported that significantly highest values of P_2O_5, K^+ and Cl^- were observed in non-composted soils when compared with semicomposted manure-amended soils. Although salinisation can negatively affect soil microbial properties (Rietz and Haynes 2003), Núñez-Zofío et al. (2012) found no differences in soil microbial properties between non-composted and semicomposted soils. In any event, composting provides a more stabilised product, thereby reducing the risk of soil salinisation, leaching and phytotoxicity (Moral et al. 2009).

21.3   Management of Soil-Borne Diseases with Organic Amendments in Protected Pepper Crops

Several reports from farmers show that plant diseases and the need for chemical control measures are reduced over time when practices that improve soil health are used. Research shows that soil management and microbial diversity are key factors in suppressing plant diseases. Organic amendments are quite diverse, including various types of organic materials such as animal manures, food processing wastes, crop residues and sewage sludge. Materials can be composted or uncomposted. The type of organic matter added to the soil could be of use as substrates, and their quality and quantity determine the types of organisms (both pathogens and natural occurring antagonists) that can profit the nutrients (Stone et al. 2004). It is well documented the use of specific organic amendments with suppressive effects against pathogens such as fungi, nematodes and bacteria. These amendments are primarily used for the control of diseases that these pathogens produce (Stone et al. 2004; Bonanomi et al. 2007, 2010; Oka 2010) although also are secondarily used to control soil fatigue caused by microorganisms that take advantage of plant weakness or subclinic pathogens (Manici et al. 2003; Martínez et al. 2011a, b; Mazzola and Manici 2012; Weerakoon et al. 2012; Guerrero 2013). Soil solarisation is an approach for soil disinfestation which uses passive solar heating of soil with plastic sheeting, usually transparent polyethylene (Stapleton 2000). The resulting soil temperature increase leads to decreased populations of pathogens. Soil solarisation and the application of organic amendments on soil have been described as a valid alternative to the use of chemical fumigants to reduce *Phytophthora* from pepper crops (Ristaino and Johnston 1999). In the technique known as Anaerobic Soil Disinfestation (also termed Biological Soil Disinfestation/Soil reductive sterilisation/Reductive Soil Disinfestation), organic amendments are applied in conjunction with soil tarping with an impermeable film for inducing anaerobiosis in order to generate toxic compounds (Blok et al. 2000; Momma 2008; Butler et al. 2012a, b). The advantage of anaerobic soil disinfestation when compared with soil solarisation is that the method does not require high solar radiation so it can be applied in cloudy areas or periods of low sunlight and, thus, a growing season is not lost (Baysal-Gurel et al. 2012).

21.3.1 Biofumigation

The term biofumigation was originally coined for that part of the suppressive effects of *Brassica* species on noxious soil-borne organisms (Kirkegaard et al. 1993) that arose quite specifically through liberation of isothiocyanates from hydrolysis of the glucosinolates that characterise the Brassicaceae (Kirkegaard and Matthiessen 2004). Since being coined, the initial term biofumigation has broadened its initial meaning and currently encompasses any beneficial effect arising from green manure or rotation crops and even composts (Matthiessen and Kirkegard 2006).

The use of Brassicaceae in crop rotations or as green manure amendment in biofumigation treatments (Stapleton and Bañuelos 2009) has proven to reduce the incidence of some soil-borne pathogens and plant parasites, including nematodes (Smolinska et al. 2003; Larkin and Griffin 2007), through their release of isothiocyanates. Improved pathogen and weed control has been achieved by using amendments obtained from by-products produced during the extraction of oil from *Brassica carinata* and *Sinapis alba* seeds (Palmieri 2005; Sachi et al. 2005; Lazzeri and Manici 2000; Cohen et al. 2005; Lazzeri et al. 2010). *Brassica carinata* (BP) pellets or *B. carinata* (BP) + fresh sheep manure (M) were evaluated for biodisinfestation treatments which began on two different dates (August and October), and the results were compared with MB-disinfested and untreated controls in greenhouse pepper crops in South-eastern Spain (Guerrero et al. 2013). During the third year, the gall index for BP was lower than that obtained for BP + M, and it was also lower in August than in October (Table 21.5).

The commercial crop of pepper fruit obtained in August biodisinfestations was similar or higher than the one obtained with MB, but higher than in October biodisinfestation treatments (Table 21.6). The yield of the October biodisinfestation treatments was higher than that of the untreated. In August of all the studied years, the accumulated exposure times were greater than the thresholds required to kill *M. incognita* populations at 15 cm soil depth. The incidence of the nematode did not correspond to the reduction achieved during solarisation and seemed to increase during the crop cycle.

21.3.2 Biosolarisation

The approach of combining soil solarisation together with the application of organic matter has been defined as biosolarisation (Ros et al. 2008) or biodisinfestation (de la Fuente et al. 2009). In this book chapter, the term biodisinfestation will be used in a more general sense than biosolarisation. Biodisinfestation will be applied for the combined use of an organic amendment and soil plastic tarping without implying soil heating (solarisation). Combining soil solarisation with the amendment of fresh organic residues elevates soil temperature by an additional

Table 21.5 Incidence of *Meloidogyne incognita* (galling index and % of galled plants) in a protected pepper crop greenhouse experiment located in South-eastern Spain

	First crop season		Second crop season		Third crop season	
Treatment	GI	% of galled plants	GI	% of galled plants	GI	% of galled plants
Untreated	5.7 d	100.0 b	6.3 cd	100.0 b	7.3 e	100.0 b
MB	0.1 a	6.6 a	0.2 a	6.6 a	1.5 a	43.3 a
BP+M August	4.1 b	100.0 b	3.7 b	93.3 b	3.5 c	96.7 b
BP August	3.8 b	93.3 b	4.3 b	86.6 b	2.6 b	86.7 b
BP+M October	4.6 b	93.3 b	6.8 d	100.0 b	6.9 e	100.0 b
BP October	4.1 b	100.0 b	5.4 c	100.0 b	5.4 d	100.0 b

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Mean values ($n = 30$). For each variable, values followed by the same letter are not significantly different according to Fisher's protected LSD Test ($P < 0.05$)

GI galling index, *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

Table 21.6 Pepper crop yield (kg m^{-2}) in a protected pepper crop greenhouse experiment located in south-eastern Spain

Crop yield (kg m^{-2})	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

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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. For each variable, values followed by the same letter are not significantly different according to Fisher's protected LSD Test ($P < 0.05$)

1–3 °C, in addition to the generation of toxic volatile compounds (Biofumigation) which enhance the vulnerability of soil pathogens (Gamliel and Stapleton 1993; Gamliel et al. 2000; Bello et al. 2004; Stapleton and Bañuelos 2009). Several mechanisms are involved: (1) accumulation of toxic volatile compounds generated during organic matter decomposition; (2) creation of anaerobic conditions in the soil and (3) increase in soil suppressiveness due to high levels of microbial activity
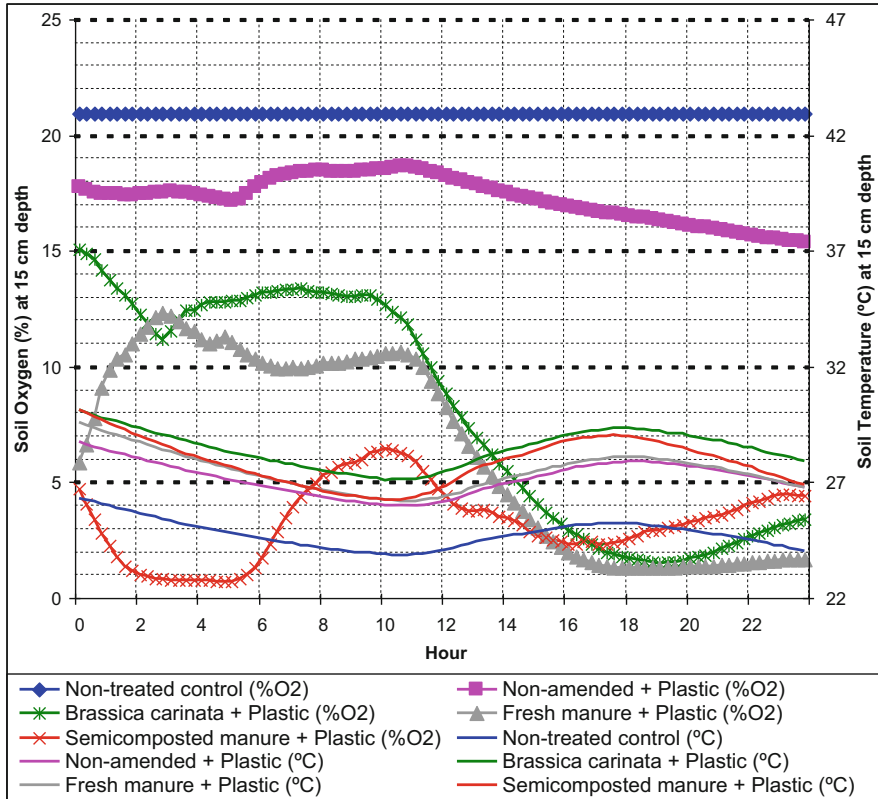
Fig. 21.3 Hourly temperatures (°C) and oxygen volumetric content (%) continuously recorded at 15 cm soil depth during biodisinfestation treatments with different organic amendments on August 7, 2009. The greenhouse field experiment was located in Derio (Biscay) (Northern Spain). Soil was tarped with 50-μm-thick (two million) transparent low density polyethylene plastic film from August 6 to September 22, 2009 (Larregla S; unpublished data)

(Gamliel et al. 2000). In these processes, the effects of anaerobiosis and temperature (Fig. 21.3) are added to the effect of gases and bioactive compounds released during organic matter decomposition (Lazarovits 2001; Tenuta and Lazarovits 2002; Arriaga et al. 2011).

Biosolarisation (BS) using fresh sheep manure (M) as amendment in August provided similar results to those obtained using MB for *Phytophthora* spp. control of protected pepper crops in South-eastern Spain (Guerrero et al. 2004a). Production increased when the application was repeated more than 2 years (Guerrero et al. 2004b, 2006; Cándido et al. 2005), but it seemed to have little effect when applied after the beginning of September (Guerrero et al. 2010).

21.4 The Problem of Soil Fatigue in Greenhouse Pepper Monocultures and its Control with Organic Amendments

Soil fatigue has been defined as: "the reduced development of certain crops when cultivated two or more times in the same soils" (Scotto-La Massese 1983; Bouhot 1983). Soil fatigue can be caused by one or a combination of several factors of physical, chemical or biological nature. Biotic component of soil fatigue usually includes certain microorganisms that take advantage of plant weakness or subclinic pathogens that tend to accumulate in soil with crop repetition (Manici et al. 2003; Martínez et al. 2011a, b; Mazzola and Manici 2012; Weerakoon et al. 2012; Guerrero 2013; Guerrero et al. 2014). These microorganisms have been isolated from plants showing vegetative depression, when they were repeatedly cultivated in the same soil, but did not produce disease when inoculated, nor reproduced the symptoms of depression, so that they were considered "weakness or subclinical pathogens" by Katan and Vanacher (1990).

In greenhouse pepper monocultures, soil fatigue appears in soils without primary pathogens (*Phytophthora capsici* or *P. parasitica, Meloidogyne incognita*) after the second year of crop repetition (Guerrero 2013). The depressive effect on plant development and the loss of production are related to the proliferation of species of *Fusarium* (Martínez et al. 2009, 2011a). Soil fatigue's specific depressive effect on the pepper plots is mitigated by soil disinfection (Guerrero 2013) and the reduction of the population densities of *Fusarium solani, F. oxysporum* and *F. equiseti* (Martínez et al. 2009). Soil disinfection through the use of chemical disinfectants (methyl bromide or chloropicrin) have less durable effects than when an organic amendment is used (fresh sheep manure + poultry manure), either alone (biofumigation) or when the soil is covered with plastic (biosolarisation) (Martínez et al. 2011b) (Table 21.7).

When biosolarisation is repeated, its effectiveness against soil fatigue increases (Martínez et al. 2009), either by providing direct action against fungal microbiota and/or increasing plant health through the improvement of soil chemical and physical characteristics (Fernández et al. 2005). Increase in macro- and micronutrients, increase in water infiltration capacity and decrease in apparent density and compaction are among the improvements in soil characteristics that may be mentioned. The use of biosolarisation combined with organic amendments in pepper greenhouses influences the soil physical characteristics, specifically in relation to the control of *Phytophthora capsici* or *P. parasitica*. These fungal pathogens are found in greater numbers in compact clay soils than in well-ventilated soils with adequate drainage. Even so, the control of the disease (root rot) can also be attributed to the effects of temperature, the released gases in the amendments bio-decomposition (Guerrero et al. 2010; Lacasa et al. 2010) and the suppressiveness connected with bacterial microorganisms (Núñez-Zofío et al. 2011).

Table 21.7 Changes on soil *Fusarium* spp. communities (colony-forming units per gramme of soil) isolated at first and in the last soil sampling of two growing seasons at two pepper greenhouses in Southeast of Spain

Soil treatments	Greenhouse CH				Greenhouse E			
	Year 1		Year 2		Year 1		Year 2	
	First	Last	First	Last	First	Last	First	Last
Control	1652.0[a] b	558.7 a	25.7 a	1097.5 b	1635.1 ns	1874.5 ns	463.0 ns	427.5 ns
Methyl bromide	110.4 a	481.4 b	23.9 a	1356.3 b	187.6 a	1587.2 b	131.5 a	716.0 b
Biofumigation	1481.5 ns	2047.2 ns	3157.2 ns	3608.6 ns	54.6 a	204.7 b	[b]	
Biosolarisation	45.7 ns	52.1 ns	105.5 a	1483.5 b	0.0 a	9.5 b	2.1 a	432.5 b

Reprinted from Bulletin IOBC/WPRS (International Organisation for Biological Control/West Palaeartic Regional Section), https://www.iobc-wprs.org, 71, Martínez et al. (2011b), Long-term effects of the application of organic amendments on soil fungal communities in pepper crops, 81–84, with permission from International Organisation for Biological Control

[a]Values are the means of three replicates. Mean values at first and in the last of the same growing season with the same letter do not differ significantly, according to Fisher's protected LSD test (*P* < 0.05). The √(*x* + 0.5) transformation was performed to normalise data; *ns* not differ significantly

[b]There were not biofumigation assays

21.5   Conclusions

Biosolarisation provides an effective and stable strategy for soil-borne pathogens control and the mitigation of soil fatigue in protected pepper monocultures.

Biosolarisation reiteration improves soil chemical, physical and biological properties with a subsequent increase in *Phytophthora* control effectiveness and crop yield.

However, field studies to establish types and rates of organic amendments should be carried out in different horticultural pathosystems in order to optimise pest, soil and crop responses when organic amendment incorporation is combined with soil plastic tarping at moderate soil temperatures. In-depth knowledge of several mechanisms that are contributing to control of soil-borne pathogens is needed. Future research should focus on the complexity of relationships among microbial communities for the establishment of soil management strategies towards a sustainable plant disease control.

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