Scientia Horticulturae 197 (2015) 607–618

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Survival reduction of *Phytophthora capsici* oospores and *P. nicotianae* chlamydospores with Brassica green manures combined with solarization

Carmen María Lacasa[a], Victoriano Martínez[a], Ana Hernández[a], Caridad Ros[a], Alfredo Lacasa[a], María del Mar Guerrero[a,*], María del Carmen Rodriguez-Molina[b], Paula Serrano-Pérez[b], Santiago Larregla[c]

[a] *Biotecnología y Protección de Cultivos, IMIDA, C/Mayor s/n, 30150 La Alberca, Murcia, Spain*
[b] *CICYTEX, Instituto de Investigaciones Agrarias Finca La Orden-Valdesequera, 06187 Guadajira, Badajoz, Spain*
[c] *Plant Protection Department, Neiker-Tecnalia, C/Berreaga, 1, 48160 Derio, Vizcaya, Spain*

ARTICLE INFO

Article history:
Received 6 August 2015
Received in revised form 7 October 2015
Accepted 14 October 2015
Available online 29 October 2015

Keywords:
Phytophthora root rot
Sweet pepper crops
Greenhouses
Paprika pepper
Sinapis alba
Brassica carinata
Brassica nigra
Biofumigation
Biosolarization

ABSTRACT

Phytophthora capsici and *Phytophthora nicotianae* are the main soil-borne pathogens of greenhouse sweet pepper in Southeast (Murcia) and *P. nicotianae* of open field paprika pepper crops in West (Extremadura) of Spain. Both oomycete cause plant root rot. The effects of green manure amendment (*Brassica carinata*, *Brassica nigra*, *Sinapis alba*, non-amended as control), transparent plastic tarp (non-tarped = biofumigation, tarped = biosolarization) and depth (15 cm, 30 cm) were assessed for the survival of *P. capsici* oospores and *P. nicotianae* chlamydospores in two consecutive years. In Murcia, biosolarization for six weeks significantly reduced the viability of oospores (values for the two soil depths ranged from 4.0–12.2% viables in biosolarized soil to 21.2–26.0% in non-treated soil in 2010 and from 6.9–31.1% to 52.4–54.9% in 2011) and the infectivity of oospores (values for the two soil depths and the two years ranged from 0.0–11.1% of diseased plants in biosolarized soil to 8.3–44.4% in non-treated soil) and chlamydospores (0.0% to 0.0–11.1%). The reduction of inoculum viability was greater with *S. alba* and *B. nigra* (6.6 and 5.7% of viable oospores) than with *B. carinata* (18.9%) and higher than in the non-treated soil (21.2%). Despite the low soil temperatures in Extremadura (15–29 °C), *S. alba* further reduced chlamydospore infectivity when compared to *B. nigra* or non-amended soil in the second year (29.2, 52.1 and 45.9% of diseased plants respectively). Biosolarization with brassicas can be recognized as an effective method to reduce *Phytophthora* survival in soils of greenhouse pepper crops of Murcia and with better effects on infectivity of inoculum of *P. nicotianae* (0.0% diseased plants) than on *P. capsici* (0.0–11.1% diseased plants). Conversely, the results in Extremadura were not sufficiently consistent in the two year experiment thus, this procedure cannot be recommended for *Phytophthora* control in Extremadura open field.

1. Introduction

Phytophthora capsici Leonian is the main fungal soil pathogen in pepper (*Capsicum annuum* L.) crops worldwide (Erwin and Ribeiro, 1996; Lamour et al., 2012). *Phytophthora nicotianae* Breda de Haan is a polyphagous oomycete that causes losses in many herbaceous and woody crops (Erwin and Ribeiro, 1996). It is predominant in the greenhouses of Murcia (Southeast Spain) (Guerrero et al., 2013; Lacasa et al., 2013; Blaya et al., 2014) where about 2000 ha of sweet pepper are cultivated and of which, more than 100 ha are organic crops. *P. nicotianae* also causes *Phytophthora* root rot in open field paprika pepper crops of Extremadura (Western Spain) (Rodríguez et al., 2010), where about 1000 ha are cultivated (Bartolomé et al., 2006). Both species produce survival spores (oospores and chlamydospores) that are capable to persist in the soil and act as the main initial inoculum that causes primary infections in the next crop cycle (Erwin and Ribeiro, 1996).

In Murcia greenhouses, pre-plant soil disinfestation with chemical fumigants has traditionally been used to control pepper root and crown rot (Guerrero et al., 2010, 2013). In Extremadura, soil

* Corresponding author.
E-mail addresses: mariam.guerrero@carm.es, mariam.guerrero2@gmail.com (M.d.M. Guerrero).

http://dx.doi.org/10.1016/j.scienta.2015.10.024
0304-4238/

disinfestation with 1,3-dichloropropene is a common practice in tobacco crops which precede pepper in the rotation systems. The ban on methyl bromide as a soil disinfectant and the limitations on the use of other chemical disinfectants (1,3-dichloropropene and chloropicrin are under review, European Directive 91/414/EEC Regulation 1107/2009 on Plant Protection Products) have created a need for the evaluation of other forms of soilborne pathogen control (Colla et al., 2012).

Biosolarization, through the combination of organic amendments and solarization, provides adequate control of soilborne pathogens (fungi, bacteria and nematodes) in warm climate regions (Ros et al., 2008). This is achieved through the use of fresh manure such as urban debris, non-harvestable plant residues or by-products of different agro-industrial processes (Coelho et al., 1999; Gamliel et al., 2000; Guerrero et al., 2010, 2013; Butler et al., 2012; Núñez-Zofío et al., 2011, 2012, 2013; Zanon et al., 2011).

The use of Brassica species as green manure or dehydrated seed meal amendments (Angus et al., 1994; Muditha et al., 2012) and as rotation crops fitted into farming systems (Rudolph et al., 2015), has become increasingly important as a biologically-based alternative for soilborne pest and disease control in cropping systems. The suppressive effects have been attributed to biocidal compounds released into the soil when Brassica tissues are decomposed, increased populations of antagonistic organisms following the incorporation of organic matter itself in the soil (Mazzola et al., 2001; Cohen et al., 2005), and Brassica performance as non-hosting (Stirling and Stirling, 2003) or trap crop for the pathogen (Thorup-Kristensen et al., 2003). Biocidal compounds include non-isothiocyanates (Brown and Morra, 1997) and isothiocyanates (Kirkegaard and Sarwar, 1998; Sarwar and Kirkegaard, 1998; Manici et al., 2000) derived from glucosinolates hydrolysis through the myrosinase enzymatic system of Brassicaceae, as well as non-glucosinolate sulfur-containing compounds (Bending and Lincoln, 1999) and other products of microbial decomposition of tissues such as fatty acids (Matthiessen and Kirkegaard, 2006). Kirkegaard et al. (1993) coined the term "biofumigation" to refer to the particular mechanism of isothiocyanate-related suppression of Brassica species. Suppressive effects of *Brassica juncea* on *Pythium* inciting apple replant disease lasted longer (several weeks) (Izzo and Mazzola, 2007) than those attributed to released isothiocyanates from residues decomposition (24–48 h) (Mazzola and Zhao, 2010).

Suppressive effects of *P. capsici* on a greenhouse pepper crop were observed when applying a green manure of *Sinapis alba* which favored increased soil bacterial microbiota (Núñez-Zofío et al., 2012) as opposed to the utilization of *Brassica carinata* pellets which did not reduce the disease in previous experiments with greenhouse pepper crops (Núñez-Zofío et al., 2011). Similarly, Gilardi et al. (2014) did not obtain a good control for *P. nicotianae* in tomato with *B. carinata* pellets.

Soil amendments with *B. juncea* and *Brassica napus* cover crops followed by mulching with black polyethylene plastic film (0.025 mm) reduced blight on squash caused by *P. capsici* under field conditions in Georgia (Southeastern USA) (Ji et al., 2012).

The cultivation of Brassicaceae cover crops in greenhouse pepper monocultures in Southeastern Spain has two aims: a cultural rotation and a green manure amendment for soil solarization. The green manure amendment was chosen given that solarization alone provides an insufficient control of *P. capsici* and *Meloidogyne incognita*, the main pathogens of greenhouse sweet pepper crops (Cenis and Fuchs, 1988; Guerrero et al., 2006). In Extremadura, paprika pepper is grown in open field during summer. Thus, biofumigation during these months as a supplement to solarization and as an appropriate method of control is not compatible with the crop cycle in this region. However, biofumigation in spring can be considered a more viable option. If the biofumigation is completed before the establishment of the crop and with material from the autumn-winter crop of Brassica with high glucosinolate content, it can be regarded as an adequate control for *Phytophthora* root and crown rot.

The aim of this study was to evaluate the effects of different Brassica cover crops used as green manure in biofumigation (non-tarped) and biosolarization (plastic-tarped) on the viability and infectivity of oospores of *P. capsici* as well as the infectivity of chlamydospores of *P. nicotianae* in pepper greenhouses in Southeastern Spain. In Western Spain, the effects of the same cover crops were evaluated on the infectivity of chlamydospores of *P. nicotianae* in open field.

2. Materials and methods

The present study was carried out in two sites: Murcia greenhouse and Extremadura open field. The experiments of biofumigation and biosolarization with Brassica green manures were conducted for two consecutive years in each site: 2010 and 2011 in Murcia and 2009 and 2010 in Extremadura.

The treatments of biofumigation and biosolarization were arranged in a randomized complete block design and comprised a factorial structure of three crossed factors: (i) amendment of Brassica green manure with four levels (Bc: *B. carinata*; Bn: *B. nigra*; Sa: *S. alba*; non-am: non-amended control); (ii) plastic tarped soil with two levels (P: plastic tarped, with transparent polyethylene plastic film 0.05 mm thick; NP: non-plastic); and (iii) depth of buried *Phytophthora* inoculum with two levels (15 cm; 30 cm).

The treatments of the experiment were overlaid in the same plots in both years in each site.

The effects of treatments were measured on the following variables: viability of *P. capsici* oospores buried at 15 and 30 cm and their infectivity in the Murcia greenhouse; infectivity of *P. nicotianae* chlamydospores in the Murcia greenhouse and the Extremadura open field.

2.1. Field sites

In (Murcia) Southeastern Spain, trials were conducted in a new 800 m^2 greenhouse of IMIDA research center (X: 685178; Y: 4,183226) located in Campo de Cartagena. The greenhouse was covered with a new three-layer transparent polyethylene film. The soil was a clay-loam with a pH of 7.8 and an organic matter content of 0.5%.

In Extremadura (Western Spain), trials were conducted on an open field plot of the research center La Orden (X: 702328, Y: 4303769), which contained a sandy-loam soil of pH 6.2 and an organic matter content of 0.7%.

2.2. Brassica crops

The brassicaceous green manure species used in this study were *S. alba, B. nigra* and *B. carinata.* The respective sowing rates were 15 kg ha^{-1}; 10 kg ha^{-1} and 12 kg ha^{-1}, as recommended by the supplier.

Trials in the Murcia greenhouse: six months before sowing Brassica, 40 t ha^{-1} composted sheep manure was added to the soil. The three brassicas were sown in the first week of June. It was watered by sprinkling and a solution of macro and micronutrients was added through the irrigation system every two weeks.

Trials in the Extremadura open field: before sowing Brassica crops, an organic compost (20 t ha^{-1}) was applied in all plots, and fertilized (except in control plots) with 600 kg ha^{-1} 8–15–15 (NPK). Brassicas were sown in the last half of October and irrigation was performed by sprinkling.