Biological Control 48 (2009) 316–322

Contents lists available at ScienceDirect

Biological Control

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

Mustard biofumigation disrupts biological control by *Steinernema* spp. nematodes in the soil

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ARTICLE INFO

Article history:
Received 1 October 2008
Accepted 3 December 2008
Available online 24 December 2008

Keywords:
Biofumigant
Non-target effects
Colorado potato beetle
Plant-parasitic nematode
Entomopathogenic nematode
Leptinotarsa decemlineata
Meloidogyne chitwoodi

ABSTRACT

Mustard green manures or seed meal high in glucosinolates, which produce a natural biofumigant upon incorporation into the soil, form an alternative to synthetic fumigants. However, the non-target impacts of these biofumigants in the field are unclear. We examined the effectiveness of soil incorporation of *Brassica carinata* seed meal both in controlling the plant-parasitic Columbia root-knot nematode (*Meloidogyne chitwoodi*), and on the biological control exerted by the entomopathogenic nematodes *Steinernema feltiae* and *Steinernema riobrave* on root-knot nematodes and the Colorado potato beetle (*Leptinotarsa decemlineata*). Singly, both the seed meal and *Steinernema* spp. reduced root-knot nematode damage to potato tubers and increased marketable tuber yields. However, there was a negative interaction between the two bioagents such that their combination did not further improve suppression of plant-parasitic nematodes. Thus, mustard seed meal applications harmful to the target root-knot nematode also disrupted the ability of *Steinernema* spp. to act as biocontrol agents. Further, we observed modest disruption of the biological control of potato beetles following biofumigation. But, the potato beetles were less likely to lay eggs on potato plants grown in mustard-amended soil, suggesting a counteracting benefit of mustard application. Multiple, complementary controls must be integrated to replace the very effective pest suppression typical of synthetic soil fumigants. Our study suggests significant interference between biofumigation and biocontrol agents in the soil, presenting challenges in combining these two environmentally friendly approaches to managing plant-parasitic nematodes and other pests.

1. Introduction

Many traditional soil fumigants are damaging to the environment, are toxic to humans, and have negative effects on beneficial soil organisms (Ibekwe, 2004). The use of mustard (*Brassica* spp. and *Sinapis* spp.) green manures and seed meals provide promising alternatives to synthetic chemical fumigants (Brown and Morra, 1997). Mustards possess glucosinolate compounds in their seeds and foliage that upon soil-incorporation act as "biofumigants" (*sensu* Kirkegaard et al., 1993), hydrolyzing to form isothiocyanates and other volatile compounds toxic to many soil-borne pests (Brown and Morra, 1997). Mustard cover crops can be grown in-field prior to the crop and then tilled into the soil to achieve biofumigation, thus also providing the benefits for soil health associated with cover crops (McGuire, 2003). However, it is possible that the broad-spectrum toxicity of mustard biofumigants might harm non-target beneficial soil biota such as biological control agents or other pest antagonists (Bending and Lincoln, 2000; Ramirez et al., 2009). This means that the switch to mustard biofumigants might not eliminate all of the harmful non-target effects associated with synthetic chemicals, potentially complicating the integration of cultural and biological control. Achieving the highly effective pest control typical of synthetic fumigants will require the successful integration of multiple, complementary management tactics (Martin, 2003). Thus, antagonism between biofumigation and biological control could hinder movement away from synthetic fumigants.

In the Columbia Basin of east-central Washington State, USA, the Columbia root-knot nematode (*Meloidogyne chitwoodi* Golden, O'Bannon, Santo and Finley) is among the most economically damaging pests of potato (*Solanum tuberosum* L.) and other crops (O'Bannon et al., 1982). Nematode feeding induces blemishes on the tubers, and when 10% or more of the tubers are blemished, the crop is considered unmarketable (Ingham et al., 2000). Increasingly, regional potato growers are transitioning to the planting of mustard (*Brassica* spp. and *Sinapis* spp.) green manures, grown and tilled into the soil in fields preceding potato crops, as a more environmentally benign alternative to synthetic soil fumigants for plant-parasitic nematode control (McGuire, 2003). However, any harmful effects of mustard biofumigants on beneficial soil organisms could be problematic in this system. For example,

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doi:10.1016/j.biocontrol.2008.12.004

entomopathogenic nematodes such as *Steinernema feltiae* (Filipjev) and *Steinernema riobrave* (Cabanillas, Poinar & Raulston) have been considered as biological controls against Colorado potato beetle (*Leptinotarsa decemlineata* Say), a major herbivorous insect pest of potato in the region (Berry et al., 1997; Ramirez et al., 2009). Recently, it has been reported that *Steinernema* spp. also exert biological control on plant-parasitic nematodes (Grewal et al., 1997; Jagdale et al., 2002; Lewis et al., 2001; Perez and Lewis, 2001, 2004). If mustard green manures are harmful to *Steinernema* nematodes, it may be difficult to combine biofumigation and biological control for the integrated management of nematode and insect pests of potato.

Here, we report on field and greenhouse experiments examining the use of *Brassica carinata* (A. Braun) seed meal, soil-incorporated before planting, to control Columbia root-knot nematode on potato. Within a fully factorial design, we also applied *S. feltiae* or *S. riobrave* as biological control agents against Columbia root-knot nematode and Colorado potato beetle. We recorded the effects of these treatments on the target pests, non-target nematode species, and the host plant.

2. Materials and methods

2.1. Field experiment

The field experiment was conducted at Washington State University's Irrigated Agriculture Research and Extension Center in Prosser, Washington. We conducted a factorial manipulation of mustard seed meal application (mustard seed meal applied versus not applied) and *Steinernema* spp. application (no nematodes, *S. feltiae* applied, or *S. riobrave* applied), for a complete 2×3 factorial design with six unique treatment combinations. We also included a conventional treatment control, using an application of the synthetic chemical soil pesticide ethoprop (this chemical is toxic to both insects and nematodes; Mocap®, Rhone-Poulenc, Inc., Troy, NY), as a seventh treatment. The experiment was conducted in two temporal blocks, the first in 2006 and the second in 2007, with five replicates of each treatment in each year, for a total of 70 field plots across the 2-year experiment. Replicate plots were 2.4×6 m with 0.30 m inter-row spacing and 3 rows per plot, planted with Russet Burbank potatoes on 15 June 2006 (block 1) and 1 May 2007 (block 2). Soil at the site is Quincy loamy sand (Rasmussen, 1971), and irrigation was provided by solid set sprinklers. Plots in the 2 years were located in two different, nearby fields, with both fields known to harbor robust populations of *M. chitwoodi* (E. Riga, unpublished data). In both years, Colorado potato beetle densities were very high in surrounding research plots, threatening complete defoliation of our experimental plots. Thus, in 2006, plots were sprayed with the insecticides spinosad (on 21 June, 7 and 14 July, and 19 August) and carbaryl (on 5 and 12 August) at the label rates. In 2007, plots were sprayed with carbaryl (on 14 and 28 July) and acetamiprid (on 25 August and 9 September) at the label rates. Fertilizer was applied (402.5 kg actual nitrogen/ha; 113.25 kg actual phosphorus/ha; 85 kg actual potassium/ha; 45.35 kg actual sulfur/ha; 2.25 kg actual boron/ha) to all plots prior to potato planting, on 10 June 2006 and 11 May 2007.

The *B. carinata* seed meal that we applied was a commercial product ("Biofence", Triumph Italia, Livorno, Italy) produced from *B. carinata* selection ISCI 7 using a proprietary partial de-fatting method that limits glucosinolate and myrosinase degradation (Lazzeri et al., 2002). The chemical composition of the mustard seed meal has previously been characterized and found to contain 163.4 μmol/g of glucosinolates, 98% of type 2-propenyl glucosinolate (sinigrin) and a sufficient level of myrosinase enzyme to catalyze glucosinolate hydrolysis (Leoni et al., 2004). Seed meal (supplied by High Performance Seed Company, Moses Lake, WA) was applied to plots receiving this treatment at a rate of 2.5 tons/ha (4.42 kg/plot), 15 days before potatoes were planted on 30 May 2006 (block 1) and on 15 April 2007 (block 2). The seed meal was broadcast applied and tilled 15 cm deep with a tractor-mounted rototiller, and the mustard application was followed immediately by approximately 5 cm of irrigation. Synthetic soil pesticide control plots were treated with ethoprop (Mocap® 6 EC; 18.3 l/ha; 13.47 kg active ingredient/ha); ethoprop was broadcast applied using a CO_2 pressurized backpack sprayer and then incorporated 15.2 cm deep using a tractor and a rototiller at potato pre-plant.

On the same day as potato planting, entomopathogenic nematodes were applied to plots receiving that treatment. For *S. feltiae* we applied strain 75 (Nemasys®), and for *S. riobrave* we used strain 355 (BioVector®) (Becker Underwood, Littlehampton, UK), applied at the label rate of 7.5 billion infective juveniles (IJ)/ha, mixed in 2.3 l of water per plot and applied using a backpack sprayer. The entomopathogenic nematodes were reapplied, using the same methodology and application rate, on 8 August 2006 and 6 July 2007. Nematodes were applied after 17:00 h to avoid ultraviolet light and heat damage (Smits, 1996).

Potato plots were harvested on 30 October 2006 and 15 October 2007. Middle rows of each plot were dug with a potato harvester, bagged into burlap sacks, and put into cold storage (4 °C) until processing (within 2–4 weeks). Twenty potato tubers were randomly chosen from each plot for a more detailed assessment of *M. chitwoodi* infection levels. These tubers were peeled and inspected under a magnifying lens with light for presence of female *M. chitwoodi* in the potato cortex; *M. chitwoodi* are easily identified by the presence of glistening white pear-shaped female bodies or by characteristic 1-mm-diameter necrotic spots in the vascular ring. The number of females per tuber was counted and each potato was assigned an infection rating using the six point infection index scale advocated by Bridge and Page (1980): 0 = 0 females, 1 = 1–3 females, 2 = 4–5 females, 3 = 6–9 females, 4 = 10–50 females, 5 = 100–200 females, and 6 = 200+ females. Remaining tubers were weighed, counted, and sorted using a Lectro Tek® Singulator (Lectro Tek, Inc., Wenatchee, WA), and separated into culls (unmarketable tubers) and two marketable grades, #1 and #2 tubers. Through this process culls are identified by misshapen, undersized or diseased tubers; #1 tubers are not less than 5.7 cm in diameter or 113 g in weight, clean, firm, well shaped and are free from freezing, disease and internal defects; and #2 tubers weigh a minimum of 113 g, not seriously misshapen and free from damage resulting from freezing and disease (USDA, 2008).

For each plot, on two sampling dates each year (30 May 2006 and 1 May 2007; 30 October 2006 and 15 October 2007), three soil samples were collected to the depth of 30.5 cm using a 2.5 cm diameter soil core sampler. These soil samples were taken from each of three randomly selected locations in the center row of each plot and combined and put into cold storage (4 °C). Within 1–2 weeks, total nematodes were extracted from 250 cc of the homogenized field soil by a centrifugal–flotation technique (Byrd et al., 1966) using a series of 500, 400, and 35 μm pore-sieves. Extracted plant-parasitic nematodes were identified to species level while free-living nematodes were enumerated.

Colorado potato beetles are attacked by *Steinernema* spp. nematodes when the fourth-instar beetle larva burrows into the soil to pupate. Because our fields had to be treated with insecticide due to high numbers of beetles moving in from surrounding, untreated potatoes, we were not able to compare ambient beetle densities among the plots. Instead, within the field experiment we conducted assays looking at the infection rates of sentinel potato beetle larvae in soil from our field plots, and compared oviposition