



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

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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.

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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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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 CO₂ 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-floitation 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

behavior by adult female beetles on caged potato plants within the field plots.

Potato beetle larval infection bioassays were conducted on 8 August 2006 and 6 July 2007, at the peak of potato beetle populations in surrounding unsprayed potato fields (D. Henderson, personal observation). We utilized the technique of Armer et al. (2004), with slight modifications. Two 118 ml perforated buckets (perforated on the bottom and top to allow for water drainage and ventilation) were filled with soil from each plot. *S. feltiae* or *S. riobrave* nematodes were then sprayed in the field as described previously. After spraying the soil, including the soil-filled buckets, 10 fourth-instar *L. decemlineata* larvae were placed in each bucket. The buckets were capped and buried flush with the surrounding soil, and left in the field for 48 h to allow infection to take place. Thereafter, buckets were taken from the field and potato beetle larvae were re-collected and moved into individual Petri dishes. The petri dishes were maintained in the laboratory inside dark plastic containers (to maintain sufficient humidity levels) at 25 °C and observed for 5 days; any mortality during this time was recorded. After this time, remaining asymptomatic larvae were dissected using a Leica MZ95 dissecting microscope at 60× magnification (Leica Microsystems GmbH, Wetzlar, Germany) and recorded as infected if *Steinernema* spp. nematodes were observed in the haemocoel of the insects.

Colorado potato beetle oviposition trials were conducted on 20 July 2006 and 6 July 2007. Mesh sleeve cages (17 cm wide × 38 cm tall) were sewn to leave a sack-like opening at one end, allowing cages to be slid over individual potato stems. Sleeve cages were fitted over the stems of three randomly chosen potato plants in each experimental plot, each year. Three male and four female beetles were collected from nearby potato fields and released into the mesh sleeve cages, after which the open end was tied using a string to prevent escape of the beetles. The number of egg clutches (groups of ≥5 eggs were counted as clutches) was recorded for each of the three plants in each plot after 24 h.

2.2. Greenhouse experiment

In the greenhouse experiment we used tomato (*Lycopersicon esculentum* Mill.) rather than potato plants as hosts for *M. chitwoodi*, because the former were easier to score for infection. The experimental design was identical to that in the field, with two levels of mustard seed meal application (no mustard seed meal applied versus seed meal applied) crossed with three levels of *Steinernema* spp. application (no *Steinernema*, *S. feltiae* applied, or *S. riobrave* applied) to encompass a complete 2 × 3 factorial design. Each treatment was replicated 10 times per block, and we conducted three blocks separate in time, for a total of 180 replicates across the experiment (30 per treatment). The experiment was conducted in a greenhouse on the Washington State University campus in Pullman at a 16:8 (light:dark) photoperiod and an average temperature of 27 °C.

Plastic pots (0.5 l) were filled with 500 g of sterile 2:1 sand:soil mixture. *M. chitwoodi* eggs were inoculated into each pot at a rate of 2 eggs/g soil. At the same time as *M. chitwoodi* addition, mustard seed meal was applied to pots receiving this treatment by mixing 2.6 g/pot of *B. carinata* 'Biofence' seed meal into the soil. Also at this time, pots receiving *Steinernema* nematodes were applied with *S. feltiae* or *S. riobrave* at a rate of 7600 IJ/pot. *Steinernema* were applied by creating a small hole in the soil and pipetting in the nematodes. Ten days after these treatments, 5-week-old tomato seedlings (*L. esculentum* var. Rutgers Select) were transplanted into the pots. *Steinernema* spp. were reappplied at a rate of approximately 5100 IJ/pot 30 days after transplanting. After 2 months, the experiment was terminated and the roots of the tomato seedlings were stained with acid-fuchsin to allow nematodes to be

counted (Byrd et al., 1983), after which roots were dried overnight at 25 °C and weighed to allow calculation of *M. chitwoodi* females per gram dry root.

Colorado potato beetle larval infection was assayed in pots following the second *Steinernema* application, 30 days after tomato plants were transplanted, by placing 5 fourth-instar potato beetle larvae in cloth mesh bags and burying these bags 5 cm under the soil. Forty-eight hours later potato beetle larvae were retrieved from the soil, and incubated in plastic tubs for 5 days to observe for infection and mortality. After 5 days, asymptomatic beetle larvae were dissected and examined for *Steinernema* spp. infection, as described above for the field experiment.

2.3. Statistical analyses

All analyses were conducted using SYSTAT software (version 11.0; SPSS, Chicago, IL). From the field experiment, marketable potato yields and levels of *M. chitwoodi* infection, and densities of *M. chitwoodi* and free-living nematodes in soil samples, were analyzed using a 2 × 2 × 3 factorial ANOVA with two levels of temporal block (2006, 2007), two levels of mustard treatment (mustard applied or not), and three levels of *Steinernema* spp. application (no *Steinernema*, *S. feltiae* applied, or *S. riobrave* applied). Analysis of tomato root infection by *M. chitwoodi* in the greenhouse experiment was similar, except that there were three temporal blocks (for a 3 × 2 × 3 factorial design). Individual treatment combinations were then compared to the synthetic-chemical (ethoprop) pesticide control using one-way ANOVA followed by Tukey's post hoc test. For the potato beetle infection and oviposition assays, analyses were similar except that there were just two levels of *Steinernema* spp. application (*S. feltiae* versus *S. riobrave*) within the multi-factorial ANOVA, and means for each treatment combination were then compared to the 'no mustard, no *Steinernema*' treatment, serving here as the control, using one-way ANOVA followed by Tukey's post hoc test.

3. Results

Because of the nearly identical experimental designs and close correspondence in results between the field and greenhouse experiments, we discuss results by responding species across field and greenhouse components of the project.

3.1. *Meloidogyne chitwoodi* infection rates and impacts

Yield of marketable tubers was significantly impacted by an interaction between the biofumigant and *Steinernema* spp. treatments ($P = 0.016$; Table 1; Fig. 1A and B). Mustard biofumigation and *Steinernema* application both appeared to improve yields when the sole control tactic, but potato production did not further benefit from their combination (Fig. 1A and B). Yields were higher in 2007 than in 2006, but other main and interactive effects were not statistically significant (Table 1). In 2006, marketable yields were higher in the ethoprop control than in all other treatments ($F = 6.05$, $df = 6, 28$, $P < 0.001$ for overall ANOVA; $P < 0.05$ for each of the individual comparisons). In 2007, only the treatment combination '*S. feltiae* + mustard seed meal applied' exhibited significantly lower yields than seen in the ethoprop control ($F = 2.44$, $df = 6, 28$, $P = 0.05$ for the overall ANOVA; $P = 0.036$ for this comparison, Tukey's post hoc test), again suggesting a negative interaction between biofumigation and the application of *S. feltiae*.

Consistent with the yield data, for tuber infection by *M. chitwoodi* there was a clear interaction between biofumigation and the *Steinernema* spp. treatments ($P = 0.003$; Table 1; Fig. 2A and B). Both nematode application and biofumigation alone decreased

Table 1

Statistical output for analysis of tuber infection index, marketable yield, densities of free-living and *Meloidogyne chitwoodi* nematodes in soil samples, and infection of Colorado potato beetles.

| Effect | df | F | P |
|---|-------|-------|--------|
| Marketable yield | | | |
| Year | 1, 48 | 83.45 | <0.001 |
| Biofumigant | 1, 48 | 0.54 | 0.465 |
| <i>Steinernema</i> | 2, 48 | 0.77 | 0.470 |
| Year * biofumigant | 1, 48 | 1.01 | 0.321 |
| Year * <i>Steinernema</i> | 2, 48 | 0.99 | 0.380 |
| Biofumigant * <i>Steinernema</i> | 2, 48 | 4.53 | 0.016 |
| Year * biofumigant * <i>Steinernema</i> | 2, 48 | 0.06 | 0.944 |
| Tuber infection | | | |
| Year | 1, 48 | 8.20 | 0.006 |
| Biofumigant | 1, 48 | 27.70 | <0.001 |
| <i>Steinernema</i> | 2, 48 | 6.03 | 0.005 |
| Year * biofumigant | 1, 48 | 3.94 | 0.053 |
| Year * <i>Steinernema</i> | 2, 48 | 1.70 | 0.194 |
| Biofumigant * <i>Steinernema</i> | 2, 48 | 6.80 | 0.003 |
| Year * biofumigant * <i>Steinernema</i> | 2, 48 | 1.97 | 0.150 |
| Free-living nematodes | | | |
| Year | 1, 48 | 8.99 | 0.004 |
| Biofumigant | 1, 48 | 1.16 | 0.286 |
| <i>Steinernema</i> | 2, 48 | 0.20 | 0.823 |
| Year * biofumigant | 1, 48 | 3.22 | 0.079 |
| Year * <i>Steinernema</i> | 2, 48 | 0.04 | 0.957 |
| Biofumigant * <i>Steinernema</i> | 2, 48 | 0.65 | 0.524 |
| Year * biofumigant * <i>Steinernema</i> | 2, 48 | 0.30 | 0.746 |
| <i>M. chitwoodi</i> | | | |
| Year | 1, 48 | 43.88 | <0.001 |
| Biofumigant | 1, 48 | 3.16 | 0.082 |
| <i>Steinernema</i> | 2, 48 | 0.43 | 0.655 |
| Year * biofumigant | 1, 48 | 0.27 | 0.606 |
| Year * <i>Steinernema</i> | 2, 48 | 0.17 | 0.847 |
| Biofumigant * <i>Steinernema</i> | 2, 48 | 0.46 | 0.633 |
| Year * biofumigant * <i>Steinernema</i> | 2, 48 | 0.24 | 0.786 |
| Infection of sentinel potato beetles | | | |
| Year | 1, 32 | 25.75 | <0.001 |
| Biofumigant | 1, 32 | 0.53 | 0.471 |
| <i>Steinernema</i> | 1, 32 | 0.94 | 0.339 |
| Year * biofumigant | 1, 32 | 1.47 | 0.234 |
| Year * <i>Steinernema</i> | 1, 32 | 0.21 | 0.652 |
| Biofumigant * <i>Steinernema</i> | 1, 32 | 0.11 | 0.741 |
| Year * biofumigant * <i>Steinernema</i> | 1, 32 | 4.65 | 0.039 |

tuber infection ($P \leq 0.005$ for both main effects, Table 1), but combining the two techniques yielded infection levels similar to when biofumigation alone was used (Fig. 2A and B). Overall *M. chitwoodi* infection levels were higher in 2007 than in 2006, but year did not interact significantly with any other effects in the model (Table 1). No *M. chitwoodi* infection was recorded for potatoes from the ethoprop control treatment in 2006, and so statistical comparison between that and the other treatments was not possible for that year. In 2007, *M. chitwoodi* infection levels were significantly lower in the ethoprop control than in the three treatments where mustard seed meal was not applied ($F = 12.03$, $df = 6, 28$, $P < 0.001$ for the overall ANOVA; $P < 0.05$ in Tukey's post hoc test for each of these individual comparisons).

Results from the greenhouse experiment, with tomato as the host plant, closely mirrored those from the field experiment. Densities of *M. chitwoodi* in tomato roots were again influenced by a strong interaction between mustard biofumigation and the *Steinernema* spp. treatments ($F = 65.27$, $df = 2, 162$, $P < 0.001$; Fig. 2C). Combining *S. feltiae* and mustard meal weakened the strong *M. chitwoodi* suppression that *S. feltiae* alone provided, whereas combining *S. riobrave* and mustard seed meal strengthened *M. chitwoodi* suppression (Fig. 2C). The three-way interaction between block, mustard application, and *Steinernema* spp. application was also significant ($F = 3.44$, $df = 4, 172$, $P = 0.010$), but was driven by

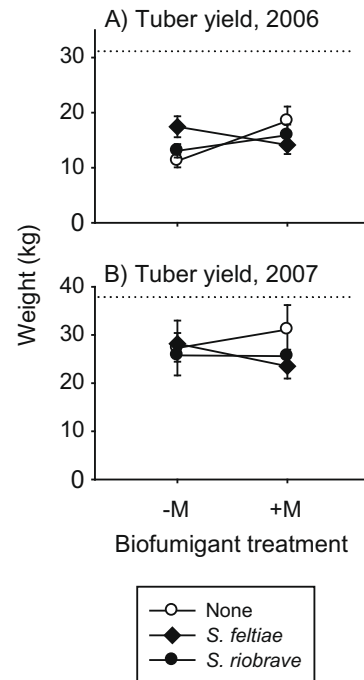


Fig. 1. From the field experiment, yield of marketable tubers in (A) 2006 and (B) 2007. Entomopathogenic nematode treatments: no entomopathogens applied (○); *Steinernema feltiae* applied (◆); *S. riobrave* applied (●). Biofumigation treatments: no mustard (–M) versus mustard seed meal applied before planting (+M). Dashed lines indicate mean treatment response in plots treated with the synthetic soil pesticide ethoprop. Data are means ± 1 SE.

differing strength of the mustard \times *Steinernema* interaction among blocks rather than a change in sign, as the interaction was consistently, negatively non-additive in all blocks.

3.2. Nematode densities in the soil

In the field experiment, densities of *M. chitwoodi* in soil samples were consistently lower in plots treated with mustard seed meal than in plots lacking this treatment, an effect that approached statistical significance (Table 1). Densities of *M. chitwoodi* in the soil were higher in 2007 than in 2006, but no other main and interactive effects were statistically significant (Table 1). Densities of free-living nematodes in the soil were higher in 2006 than in 2007 but mustard application, *Steinernema* spp. application, or the interactions among these effects and with year, had no significant impact on the densities of free-living nematodes (Table 1). No nematodes were found in soil samples from the ethoprop control plots in 2006, and thus statistical comparison with the other treatments is not possible for that year. In 2007, neither densities of *M. chitwoodi* ($F = 0.64$, $df = 6, 28$, $P = 0.70$), nor densities of free-living nematodes ($F = 2.32$, $df = 6, 28$, $P = 0.06$), significantly differed between the ethoprop control and the other treatments.

3.3. Potato beetle performance

In the field experiment, Colorado potato beetle infection by *S. riobrave* was disrupted by mustard application in 2007, but not in 2006, while infection by *S. feltiae* was not disrupted in either year, leading to a significant year \times mustard \times *Steinernema* species interaction ($P = 0.039$; Table 1; Fig. 3A and B). Overall infection rates were higher in 2006 than in 2007 ($P < 0.001$), but all other main and interactive effects were not statistically significant (Table 1). In the greenhouse experiment, compared to the field experiment, potato beetle infection assays were conducted relatively

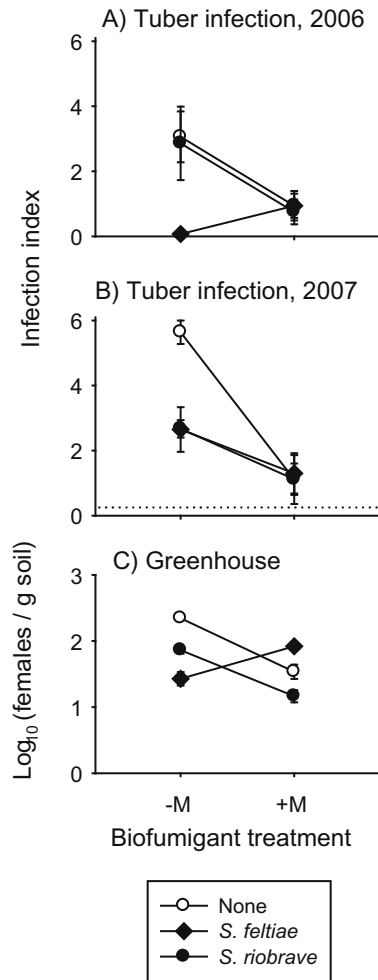


Fig. 2. From the field experiment *Meloidogyne chitwoodi* infection rating for a subsample of all harvested tubers in (A) 2006 and (B) 2007, and (C) from the greenhouse experiment root infection by *M. chitwoodi*. Entomopathogenic nematode treatments: no entomopathogens applied (○); *Steinernema feltiae* applied (◆); *S. riobrave* applied (●). Biofumigation treatments: no mustard (–M) versus mustard seed meal applied before planting (+M). Dashed lines indicate mean treatment response in field plots treated with the synthetic soil pesticide ethoprop; no infection was found in the ethoprop (Mocap®) control in 2006. Data are means ± 1 SE.

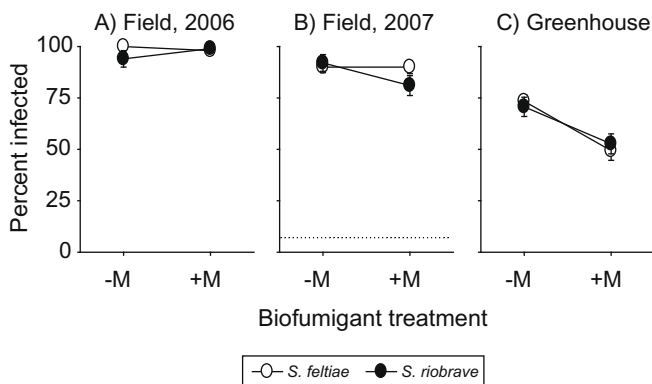


Fig. 3. Rates of entomopathogen infection of Colorado potato beetle larvae for the (A) 2006 and (B) 2007 blocks of the field experiment, and (C) across the three blocks of the greenhouse experiment. Entomopathogenic nematode treatments: *Steinernema feltiae* applied (○); *S. riobrave* applied (●). Biofumigation treatments: no mustard (–M) versus mustard seed meal applied before planting (+M). Dashed line indicates mean infection rate in controls where no entomopathogenic nematode was applied and no mustard applied; no background infection was recorded in controls in 2006 in the field, or in the greenhouse experiment. Data are means ± 1 SE.

soon after mustard seed meal application (30 days in the greenhouse versus 70 days later in the field), and perhaps for this reason disruptive effects were more apparent in the greenhouse than in the field. In the greenhouse, infection of potato beetle larvae by *Steinernema* was less common when mustard meal was also applied (Biofumigant main effect: $F = 12.85$, $df = 1, 108$, $P = 0.001$; Fig. 3C), an effect not altered by *Steinernema* species identity, block, or interactions among these effects ($P > 0.05$ for all other model terms). In 2006 in the field, and in the greenhouse experiment, no background infection of potato beetles was observed in the controls (Fig. 3A and C). In the field in 2007, all treatments receiving *Steinernema* applications exhibited higher potato beetle infection rates than were seen in the control treatment where *Steinernema* spp. were not applied ($F = 23.15$, $df = 4, 20$, $P < 0.001$; $P < 0.001$ for each individual comparison, Tukey's post hoc test).

In the field, Colorado potato beetle females laid ca. 50% fewer clutches of eggs when enclosed on potato plants grown in mustard-amended soil, compared to plants growing where mustard seed meal had not been applied ($F = 48.64$, $df = 1, 56$, $P < 0.001$; Fig. 4). Effects of year ($F = 2.74$, $df = 1, 56$, $P = 0.10$) and the year by mustard interaction ($F = 0.63$, $df = 1, 56$, $P = 0.43$) were not statistically significant.

4. Discussion

We found evidence that biofumigation interfered with biological control of the plant-parasitic nematode *M. chitwoodi*. Singly, both mustard biofumigant application and inundative application of *Steinernema* spp. biological control agents reduced *M. chitwoodi* infection of potato tubers in the field (Fig. 2A and B), and tomato roots in the greenhouse (Fig. 2C). However, the effects of these two control tactics were negatively non-additive, such that combining biofumigation and biological control did not further improve pest suppression. In the field experiment, biofumigation generally yielded relatively low levels of *M. chitwoodi* tuber damage, but entirely erased any additional benefits due to *Steinernema* application (Fig. 2A and B). This was seen most clearly for the tuber infection index data from the 2006 field season, wherein combining the biological control agent *S. feltiae* with biofumigation yielded tuber infection scores higher than those achieved by *S. feltiae* alone (Fig. 2A). Similarly, potato yield was impacted by the strong interaction between biofumigation and biological control, such that trends for higher marketable yields with seed meal or *Steinernema* spp. applied alone did not lead to higher yields when the two controls were combined (Fig. 1A and B). Earlier studies have demonstrated that mustard biofumigants can reduce damage caused by plant-parasitic nematodes (Mojtahedi et al., 1991; Laz-

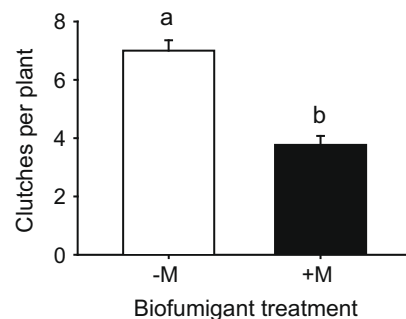


Fig. 4. From the field experiment, oviposition by Colorado potato beetle females on potato plants growing in soil not amended with mustard seed meal (–M) versus plants growing in soil where mustard seed meal was applied before planting (+M). Data are means ± 1 SE. Different lower case letters above bars indicate significant differences at $P < 0.001$.

zeri et al., 2004), and that *Steinernema* species can be effective antagonists of plant-parasitic nematodes under field conditions (Grewal et al., 1997). The results presented here expand upon this earlier work by presenting evidence that these two natural controls for plant-parasitic nematodes interfere with one another, in trials encompassing entire cropping cycles in the field, and that these interactive effects significantly impact crop production.

Steinernema spp. are entomopathogens that reproduce within host insects. Thus, application of *Steinernema* spp. for control of plant-parasitic nematodes should also benefit control of insect pests with belowground stages, such as the Colorado potato beetle. We found evidence, although somewhat equivocal, that biofumigation using *B. carinata* seed meals could also disrupt potato beetle control by *Steinernema*. In the 2007 field experiment, infection of potato beetles by *S. riobrave* was reduced in plots receiving biofumigation (Fig. 3B), and a similar disruptive effect was recorded for both *Steinernema* species in the greenhouse experiment (Fig. 3C). However, no harmful effects were noted in the field in 2006, and the magnitude of disruption of *S. riobrave* activity in the 2007 field experiment was relatively small (ca. 10% reduction in potato beetle infection), suggesting that biofumigation was less harmful to potato beetle than *M. chitwoodi* biological control. This may partially result from our ability to time *Steinernema* application to coincide with peak potato beetle densities. It is possible that increasing the time between biofumigant application and the application of *Steinernema* biological control agents could also reduce disruption of the biological control of plant-parasitic nematodes. As an additional complication, female potato beetles were less likely to oviposit on potato plants grown in plots receiving mustard seed meal, suggesting a deterrent effect of either the soils directly or through an indirect change in plant chemistry following soil amendment. Regardless of the mechanism, lower rates of egg-laying on plants grown in biofumigant-treated soil would have the tendency to offset any disruption of beetle control due to direct negative effects of mustard biofumigants on *Steinernema*.

The specific mechanism through which mustard biofumigant products disrupt the activity of plant-parasitic nematodes and other pests remains controversial (Chitwood, 2002), but our results suggest that these biofumigants also are either toxic to, or disrupt the foraging efficiency of, beneficial *Steinernema* species. Consistent with this conclusion, survey work in production potato fields also indicates that the use of mustard biofumigants reduces activity of insect-attacking *Steinernema* and *Heterorhabditis* spp. nematodes (Ramirez et al., 2009). Also controversial is the precise mechanism through which *Steinernema* spp. and other entomopathogenic nematodes suppress populations of plant-parasitic nematodes. The exact mechanism is unclear, but suppression of plant-parasitic nematodes could be a direct effect due to competition for space (Bird and Bird, 1986) or the release of allelochemicals by *Steinernema* or their bacterial symbionts (Hu et al., 1995; Grewal et al., 1999), or an indirect effect mediated by apparent competition as dense *Steinernema* spp. populations support higher densities of nematode antagonists such as nematode-trapping fungi (Ishibashi and Choi, 1991). In various studies live or dead *Steinernema* nematodes, extracts of the nematodes and host insect cadavers, and the nematode's symbiotic bacteria alone suppressed plant-parasitic nematode populations and/or damage (Grewal et al., 1999; Jagdale et al., 2002; Shapiro-Ilan et al., 2006; Molina et al., 2007; Jagdale and Grewal, 2008). With so much uncertainty as to the precise individual modes of action of mustard biofumigants and *Steinernema* biological control agents, it is difficult to speculate on the mechanism(s) through which mustard biofumigation disrupted the activity of *S. feltiae* and *S. riobrave* in the experiments reported here. Disruption may have resulted from direct toxic effects of the biofumigant on *Steinernema*, indirect effects mediated by other community members (e.g., Cohen and Mazzola, 2006), or both factors.

However, it is notable that disruption of *Steinernema* activity in the greenhouse was detected 30 days after the soil incorporation of mustard seed meal, longer than toxic breakdown products are likely to remain active (Morra and Kirkegaard, 2002). This suggests a mode of action other than direct toxicity in at least some cases.

We found some evidence that the two *Steinernema* species differed in their interaction with mustard biofumigation. In the first year of the field experiment and in the greenhouse, although not in the second year of the field experiment, *S. feltiae* exerted stronger suppression of root-knot nematodes than did *S. riobrave*. This meant that the impact of biofumigant application was generally to weaken the relatively strong impact of *S. feltiae*, but to strengthen the relatively weak impact of *S. riobrave*. It is somewhat discouraging that biofumigation harmed *S. feltiae* in our field trails, as this species is relatively resistant to the negative effects of mustard exposure compared to other entomopathogenic nematode species (Ramirez et al., 2009). Both *Steinernema* species exerted strong control of Colorado potato beetle, but the only substantial disruption of insect control in the field was recorded for *S. riobrave* in 2007 (beetle mortality due to entomopathogens was unaffected by biofumigation in the field in 2006, and disruptive effects of biofumigation were similar on both species in the greenhouse experiment). It is unclear why mustard biofumigation disrupted *S. feltiae* activity against plant-parasitic nematodes, but not against Colorado potato beetle. Most likely it will be necessary to learn more about the specific mechanisms leading to disruptive effects of entomopathogenic nematodes on plant-parasitic nematodes, and of mustard biofumigants on the entomopathogens, before these complex and variable results can be better understood.

In many vegetable crops, including potato, processors and other end-users tolerate very little pest damage. For *M. chitwoodi* on potato in the northwestern United States, for example, the treatment threshold is just one nematode per 250 cc soil (Cram et al., 2007). This extreme sensitivity to damage provides a major challenge when looking for replacements for the highly effective synthetic soil fumigants, which commonly reduce pest damage by soil pests to nearly undetectable levels (Martin, 2003). Most likely, no single control tactic can replace the very efficient pest control that these chemicals provide, necessitating the integration of multiple cultural and biological controls to achieve full pest suppression (Stirling and Pattison, 2008). However, our results demonstrate the type of challenges that may face efforts to combine multiple bioagents for the control of plant-parasitic nematodes. In general, control exerted by either mustard biofumigation or *Steinernema* biological control agents alone fell short of that seen in the synthetic pesticide (ethoprop) control. However, had the effects of these two control options been additive, we would have seen control in treatments combining both biofumigation and biological control that approached that seen with ethoprop treatment. For example, looking at tuber infection in the 2007 block of the field experiment, infection index scores were reduced >50% by *Steinernema* spp. and >80% by *B. carinata* seed meal. Under a multiplicative-risk model [i.e., taking into account that a single prey cannot be killed twice (Sih et al., 1998)], we would predict ca. 90% reduction in infection scores with both control tactics combined, or a mean infection score of 0.55, which is very close to the observed mean score of 0.23 in the ethoprop control.

The activity of entomopathogenic nematodes can be reduced due to predation by nematode-trapping fungi and other intraguild predators (Jaffee et al., 2007), by intensive tillage regimes (Millar and Barbercheck, 2002), and by rotational practices that include plants with nematicidal properties (Ramirez et al., 2009). Such negative effects on biological control are particularly troubling in organic and other less chemically intensive farming systems, wherein diverse integrated controls are needed to replace synthetic chemical inputs (Zehnder et al., 2007). In our experiments,

interference between biofumigation and biological control prevented the successful combination of these two tactics to reproduce the very effective control typical of synthetic soil pesticides. Such negative interactions present challenges in combining these environmentally friendly practices to improve suppression of plant-parasitic nematodes and other soil pests. It may be necessary to apply biofumigants and entomopathogenic nematodes sufficiently far apart in time that interference is reduced. Such an approach would be particularly effective when long-lived soil insects are present in the soil before the crop is planted, such that entomopathogenic nematodes can be applied before biofumigation. Alternatively, it may be possible to find species of entomopathogenic nematode resistant to the harmful effects of particular biofumigants (e.g., Ramirez et al., 2009).

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