Journal of Nematology 43(1):7–15. 2011.

Mustard seed meal mixtures: management of *Meloidogyne incognita* on pepper and potential phytotoxicity

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Abstract: Meals produced when oil is extracted from seeds in the Brassicaceae have been shown to suppress weeds and soilborne pathogens. These seed meals are commonly used individually as soil amendments; the goal of this research was to evaluate seed meal mixes of *Brassica juncea* (Bj) and *Sinapis alba* (Sa) against *Meloidogyne incognita*. Seed meals from Bj 'Pacific Gold' and Sa 'IdaGold' were tested alone and in combinations to determine rates and application times that would suppress *M. incognita* on pepper (*Capsicum annuum*) without phytotoxicity. Rates of soil application (% w/w) for the phytotoxicity study were: 0.5 Sa, 0.2 Bj, 0.25 Sa + 0.25 Bj, 0.375 Sa + 0.125 Bj, 0.125 Sa + 0.375 Bj, and 0, applied 0 – 5 weeks before transplant. Overall, 0.2% Bj was the least toxic meal to pepper seedlings. By comparison, 0.5% *S. alba* seed meal did not reduce lettuce (*Lactuca sativa*) seed germination at week 0, but all seed meal treatments containing *B. juncea* prevented or significantly reduced germination at week 0. The seed meals did not affect lettuce seed germination at weeks 1-5, but hypocotyl growth was reduced by all except 0.2% Bj at weeks 1, 4 and 5. *Brassica juncea* and Sa meals were tested for *M. incognita* suppression at 0.2, 0.15, 0.1 and 0.05%; mixtures were 0.1% Sa + 0.1% Bj, 0.15% Sa + 0.05% Bj, and 0.05% Sa + 0.15% Bj. All treatments were applied 2 weeks before transplant. The 0.2% Bj and 0.05% Sa + 0.15% Bj treatments overall had the longest shoots and highest fresh weights. Eggs per g root were lowest with 0.1 – 0.2% Bj amendments and the seed meal mixtures. The results indicate that Bj and some Bj + Sa mixtures can be applied close to transplant to suppress *M. incognita* populations on pepper; consequently, a seed meal mixture could be selected to provide activity against more than one pest or pathogen. For pepper, care should be taken in formulating mixtures so that Sa rates are low compared to Bj.

Key words: amendment, biofuel byproducts, *Brassica*, glucosinolate, management, *Meloidogyne incognita*, mustard seed meal, root-knot nematode, *Sinapis*.

Mustard seed meals are byproducts resulting from crushing seed to provide oil for the production of biodiesel. Because members of the Brassicaceae are planted as crops that can be incorporated into soil as green manures to suppress plant diseases, mustard seed meals have also been studied as soil amendments for management of weeds and soilborne pathogens (Brown and Morra, 1995, 2005; Cohen et al., 2005; Mazzola and Mullinix, 2005; Cohen and Mazzola, 2006; Vaughn et al., 2006; Mazzola et al., 2007, 2009; Rice et al., 2007; Boydston et al., 2008; Hoagland et al., 2008). This agricultural use of seed meals increases the economic viability of biodiesel production by providing an application for the meal byproduct, and supplies growers with a management tool for pests and pathogens (Cohen and Mazzola, 2004; Brown and Morra, 2005).

Mustard seed meals have also been investigated for management of plant-parasitic nematodes. Plant-parasitic nematodes suppressed by soil amendment with mustard seed meals include genera such as *Belonolaimus* (Cox et al., 2006), *Meloidogyne* (Rahman and Somers, 2005; Henderson et al., 2009; Lazzeri et al., 2009; Zasada et al., 2009), *Pratylenchus* (Mazzola et al., 2007, 2009; Yu et al., 2007; Walters et al., 2009; Zasada et al., 2009) and *Tylenchulus* (Walker, 1997). The biocontrol activity of the beneficial nematode *Steinernema* was also disrupted by application of mustard seed meals (Henderson et al., 2009). Possible mechanisms of action include production of toxins upon breakdown of glucosinolates (Lazzeri et al., 1993, 2004; Donkin et al., 1995; Buskov et al., 2002; Zasada and Ferris, 2003; Yu et al., 2005; Zasada et al., 2009), alteration in the bacterial community, and/or induction of plant systemic resistance by production of nitric oxide by soil bacteria (Mazzola et al., 2001, 2007; Cohen et al., 2005; Cohen and Mazzola, 2006).

Two of the mustard seed meals that were active against nematodes in soil tests were *Brassica juncea* and *Sinapis alba*. Of these two meals, *B. juncea* seed meal exhibited higher nematotoxicity (Mazzola et al., 2009; Zasada et al., 2009). For example, rates of 2.5% and 10% *S. alba* (w/w in dry soil) seed meal were required for 100% suppression of *Meloidogyne incognita* and *Pratylenchus penetrans*, respectively, but only 0.5% *B. juncea* was needed for 100% suppression of both nematodes (Zasada et al., 2009). Efficacy of *S. alba* seed meal against nematodes was increased when the seed meal was ground to a small particle size; ground *S. alba* suppressed *P. penetrans* populations by 93%, compared to 37 – 46% suppression with the same seed meal applied as a pellet (Zasada et al., 2009).

Because chemistry varies with type of seed meal, the potential exists for a combination of *B. juncea* and *S. alba* to provide enhanced activity against pathogens and pests compared to seed meals applied alone. However, any seed meal combination needs to be selected to optimize nematicidal activity while minimizing phytotoxicity to the crop plant. The current study was conducted to compare the efficacy of individual seed meals and seed meal combinations for suppression of *M. incognita* on pepper (*Capsicum annuum*). The specific

Received for publication September 30, 2010.
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This paper was edited by Kris Lambert.

objectives of this study were to: 1) determine phytotoxicity of seed meals and seed meal mixtures to pepper seedlings and to lettuce (*Lactuca sativa*) seeds (the latter was included because lettuce seed bioassays are a standard means of testing for phytotoxicity); 2) determine application rates and times for treatments with *B. juncea* 'Pacific Gold' and *S. alba* 'IdaGold' seed meals that would suppress *M. incognita* on pepper without phytotoxicity; and 3) investigate whether nematode-suppressive activity could be retained or improved by mixing the two seed meals.

Materials and Methods

Meloidogyne incognita inoculum: Inoculum of *M. incognita* Race 1, originally isolated in MD, was grown on pepper 'PA-136' in greenhouse pots. Eggs for greenhouse experiments were obtained from the roots of 3-month-old pepper plants. Roots were rinsed and then immersed in 0.6% sodium hypochlorite for 1 min to release eggs from egg masses. The eggs were collected on a sieve following sugar centrifugation, rinsed in water, stored overnight at 4 °C and used the next day (Meyer et al., 2008).

Phytotoxicity of seed meals to pepper seedlings and lettuce seeds: The two seed meals tested were *Sinapis alba* 'IdaGold' and *Brassica juncea* 'Pacific Gold.' Seed meal treatments were placed into pots at week 0 (pepper seedling transplant day) and weeks 1, 2, 3, 4 and 5 prior to pepper seedling transplant (week 1 was 7 and 5 days prior to pepper transplant in Trial 1 and Trial 2, respectively). Steamed, air-dried soil (loamy sand; 16 sand:9 compost (volume:volume); 83.1% sand, 6.4% silt, 10.5% clay; pH 6.9; 0.8% organic matter) was placed into 16.5 x 14.9 cm sealable plastic bags, and seed meal that had been broken into smaller flakes with a mortar and pestle (< 7 mm and > 0.8 mm; most of the seed meal passed through a 2 mm mesh) was added at the appropriate rate for each treatment. The combined total weight of soil and mustard seed meal was 400 g per bag. Rates of mustard seed meal application (dry weight meal to dry weight soil) are listed in Table 1. The soil and seed meal were mixed, each bag received 48 mL water (70% water holding capacity of the soil), and the amended and nonamended soils were placed into 10-cm-diameter pots. Pots were watered 1–2 times per day, and 6-week-old pepper ('PA 136') seedlings that had been planted in starter mix (Premier Pro-mix®, Premier Horticulture Inc., Quakertown, PA) were transplanted into all treatments on the same day. The pots were arranged in a randomized complete block design and harvested 12 days later. The greenhouse was maintained at 24 – 29 °C and natural and supplemental lighting were combined for a 16-h daylength. At harvest, the number of viable plants, shoot lengths (from soil to growing tip) and shoot and root fresh weights were recorded. The experiment was conducted twice, with five seedlings per seed meal treatment/application time combination in each of the two trials; n = 10.

Table 1. Application rates of *Sinapis alba* and *Brassica juncea* seed meals tested for phytotoxicity and for suppression of *Meloidogyne incognita* populations.

Phytotoxicity test with pepper and lettuce[a] (% seed meal weight to weight soil)	*M. incognita* suppression on pepper[b] (% seed meal weight to weight soil)
Single meal amendments	
0.5% *S. alba*	
0.2% *B. juncea*	0.2% *S. alba*
	0.2% *B. juncea*
	0.15% *S. alba*
	0.15% *B. juncea*
	0.1% *S. alba*
	0.1% *B. juncea*
	0.05% *S. alba*
	0.05% *B. juncea*
Meal combination amendments	
0.25% *S. alba* + 0.25% *B. juncea*	0.1% *S. alba* + 0.1% *B. juncea*
0.375% *S. alba* + 0.125 % *B. juncea*	0.15% *S. alba* + 0.05% *B. juncea*
0.125% *S. alba* + 0.375% *B. juncea*	0.05% *S. alba* + 0.15% *B. juncea*
No meal	
Nonamended	Nonamended ± *M. incognita*

[a]The phytotoxicity test was conducted with pepper seedlings and with germinating lettuce seeds.
[b]Suppression of *M. incognita* was studied on pepper plants in a separate experiment from the phytotoxicity test conducted with lettuce and pepper.

For lettuce seed germination trials, 20 g of soil were removed from each of three pots/treatment and placed on filter paper in a Petri dish. The next day, 8 ml water and lettuce seeds (10 or 20) were added to each Petri dish. The dishes were placed at a 45° angle and incubated for 5 days at 26 °C. After 5 days, number of seeds germinated, hypocotyl length, and root length were measured from 10 seeds per Petri dish. The experiment was conducted twice; n = 60.

Suppression of M. incognita on pepper by seed meals. Based on the results of the phytotoxicity tests, 2 weeks was selected as the time for seed meal application prior to transplanting, and individual seed meals were applied at rates ≤ 0.2%. All seed meal mixtures were applied for a combined total of 0.2%. The seed meal treatments are listed in Table 1.

Greenhouse conditions for the nematode suppression studies were as listed above. Mustard seed meal treatments and steamed, air-dried soil (loamy sand; 16 sand:9 compost; 82.9% sand, 5.3% silt, 11.8% clay; pH 7.3; 0.8% organic matter) were mixed in plastic bags. The combined total weight of soil and mustard seed meal was 400 g per bag. Each *M. incognita* treatment received 5,000 eggs (collected as described above) in 5 ml water per bag, plus an additional 43 ml water. The control without nematodes received 48 ml water per bag. The eggs and/or water were mixed into the soil/mustard seed meal mixtures and in nonamended soil, and then the soils were placed into 10-cm-diameter pots

in the greenhouse. The treatments were watered 1–2 times per day, as needed. Two weeks later, one 6-week-old pepper seedling that had been planted in Premier Pro-mix® starter mix was transplanted into each pot. After transplant, the pots were arranged in a randomized complete block design. Plants were fertilized as needed with Osmocote® Plus 15N-9P-12K fertilizer (Scotts-Sierra Horticultural Products Co., Marysville, OH). Plants were harvested 5 weeks after transplant. At harvest, shoot lengths and fresh weights were recorded as described above. Eight pots were used per treatment in each trial, and the experiment was conducted twice.

Following harvest, roots were removed from pots and soil was rinsed from the roots. Root fresh weights were recorded. Total numbers of galls per root system were counted and root galling index values were assessed as follows (Daulton, 1959): 0 = free from galls; 1 = less than five galls; 5 = trace to 25 galls, 10 = 26 to 100 galls; 23 = more than 100 galls. Roots were stored at 4 °C until eggs were extracted and counted. To extract eggs, roots were cut into pieces and blended on low speed in 0.6% sodium hypochlorite for 1 min, and then poured onto nested 60 / 500 (250 μm / 25 μm) mesh sieves. The eggs were rinsed with water, collected from the 500-mesh sieve and stored at 4 °C until counting.

Statistical methods: For the pepper seedling phytotoxicity study, the characteristics analyzed were numbers of live plants, shoot lengths, and shoot and root fresh weights. Shoot length and shoot and root fresh weight were $\log_{10}(x+1)$-transformed to meet the assumptions of analysis of variance (ANOVA). For the lettuce seed phytotoxicity study, the characters of interest were seed germination, hypocotyl length, and root length. The data were not log transformed for analysis. To determine effects of seed meals on pepper plants and suppression of *M. incognita*, data analyzed were shoot lengths, shoot and root fresh weights, root gall indices, and number of *M. incognita* eggs/g root. The data were not log transformed prior to analysis. Data were analyzed with the statistical package JMP (SAS Institute, Cary, NC). Differences among treatments were determined by ANOVA, and means were compared using Tukey Kramer's adjustment for multiple comparisons ($P \leq 0.05$). Data presented are nontransformed means, with ± standard errors (SE) in the figures.

Results

Phytotoxicity of seed meals to pepper seedlings and lettuce seeds: Pepper seedling viability. Viability in the nonamended control (0% seed meal) was 100% at all transplant times (Table 2). At week 0 all pepper seedlings transplanted into mustard seed meals died, regardless of rate or combination. At week 1, 0.5% *S. alba* seed meal resulted in death of all pepper seedlings, and the two seed meal mixtures containing the higher rates of *S. alba*, 0.25 and 0.375%, also caused death of most of the seedlings (10% to 30% viable, respectively). Treatment with *B. juncea* seed meal alone or with the low *S. alba* + high *B. juncea* (0.125% + 0.375%) mixture was not toxic to pepper transplanted one week after application. When pepper was transplanted at weeks 2 and 3, only 0.5% *S. alba* seed meal caused significant seedling death compared to the control. By week 5, only 0.5% *S. alba* and 0.375% *S. alba* + 0.125% *B. juncea* resulted in less than 100% viability, although this was not significantly lower than the control. Plant viability in all other treatments was 100%.

Pepper seedling shoot length and root and shoot fresh weight. At weeks 1-5, the control and 0.2% *B. juncea* seed meal had the longest pepper seedling shoots

Table 2. Viability of pepper seedlings and germination of lettuce seeds in soil amended with *Sinapis alba* and *Brassica juncea* seed meals.

Rates (% w/w)[a]	Week[b]	Viable pepper seedlings (%)[c]	Germinated lettuce seeds (%)
0.5% *Sinapis alba*	0	0 d	67 a
	1	0 d	69 a
	2	30 bcd	76 a
	3	30 bcd	65 a
	4	80 ab	77 a
	5	80 ab	80 a
0.2% *Brassica juncea*	0	0 d	5 b
	1	100 a	83 a
	2	100 a	86 a
	3	100 a	78 a
	4	100 a	78 a
	5	100 a	77 a
0.25% *S. alba* +0.25% *B. juncea*	0	0 d	0 b
	1	10 cd	73 a
	2	70 ab	83 a
	3	70 ab	68 a
	4	70 ab	68 a
	5	100 a	78 a
0.375% *S. alba* +0.125% *B. juncea*	0	0 d	21 b
	1	30 bcd	83 a
	2	60 abc	80 a
	3	60 abc	74 a
	4	80 ab	78 a
	5	70 ab	65 a
0.125% *S. alba* +0.375% *B. juncea*	0	0 d	0 b
	1	90 a	84 a
	2	80 ab	77 a
	3	100 a	78 a
	4	100 a	78 a
	5	100 a	82 a
Nonamended control	0	100 a	72 a
	1	100 a	84 a
	2	100 a	90 a
	3	100 a	88 a
	4	100 a	88 a
	5	100 a	83 a

[a]Amended and nonamended soils were placed into pots in the greenhouse 0 to 5 weeks prior to pepper seedling transplant and to lettuce seed germination in Petri dishes.

[b]Week 0 = pepper seedlings transplanted the day that amended and nonamended soil was placed into pots.

[c]Values are the means from two trials, with five replicates of each treatment per trial for pepper seedlings (n = 10), and thirty replicates of each treatment per trial for lettuce seeds (n = 60). Within a column, values followed by the same letter are not significantly different ($P < 0.05$) according to Tukey's adjustment for multiple comparisons. Significance letters are not comparable between columns.

(Fig. 1A) and the highest root fresh weights (Fig. 1B). *Sinapis alba* seed meal at 0.5% had the most phytotoxic effect on pepper shoot lengths and root fresh weights (Figs. 1A, 1B). Treatment with the three seed meal mixtures resulted in a trend toward intermediate shoot lengths, and some phytotoxicity on roots.

Shoot fresh weights showed a trend similar to that of shoot lengths (data not shown). To summarize briefly, the control and 0.2% *B. juncea* seed meal had the greatest shoot fresh weights. Low shoot fresh weights were recorded from the 0.5% *S. alba* seed meal at 3, 4 and 5 weeks, and from a few other treatments applied 1 and 2 weeks prior to transplant. The control and 0.2% *B. juncea* seed meal at 1 and 2 weeks were the only treatments that resulted in plants with greater shoot fresh weights than the three treatments containing *S. alba*. As with shoot lengths, the three seed meal mixtures had intermediate shoot fresh weights.

Lettuce seed viability. In the control, germination varied from 72% to 90%, with no significant differences among the treatment times (Table 2). At week 0, 0.5% *S. alba* seed meal was not phytotoxic and resulted in germination rates similar to that of the control. However, all mustard seed meal treatments containing *B. juncea* prevented or significantly reduced lettuce seed germination compared to the control and to 0.5% *S. alba* seed meal at week 0. The seed meal mixtures with the two highest rates of *B. juncea* (0.25 and 0.375%) resulted in 0% germination at week 0, and amendment with 0.2% *B. juncea* resulted in only 5% lettuce seed germination. During weeks 1 through 5, seed germination was not affected by any treatment; mustard seed meal treatments had germination rates of 65% - 86%, all being no different from the control.

Lettuce hypocotyl and root lengths. In general, lettuce seed hypocotyl lengths were more affected by the seed meals than were root lengths (Figs. 2A, 2B). At week 0, all of the seed meals that did not prevent germination resulted in hypocotyl lengths shorter than in the control. The only treatment that resulted in hypocotyl lengths similar to the control was 0.2% *B. juncea* at weeks 1, 4, and 5. Although 0.5% *S. alba* did not reduce germination, it inhibited hypocotyl growth at all time periods. Effects of seed meals on root lengths were similar to those observed for germination (Fig. 2B). Of the seeds that germinated, root lengths at week 0 were lowest with 0.2% *B. juncea* seed meal and with 0.375% *S. alba* + 0.125% *B. juncea*. After week 0, there was little

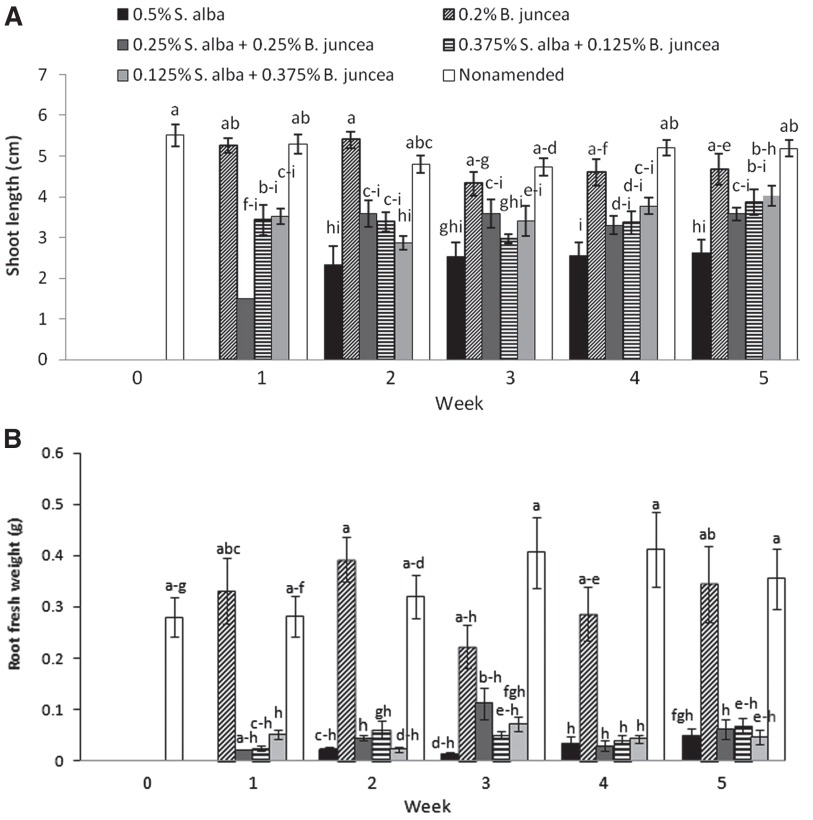

FIG. 1. Mean shoot lengths (A) and root fresh weights (B) of pepper seedlings transplanted into soil amended with seed meals of *Sinapis alba*, *Brassica juncea*, and combinations of these meals. Amended and nonamended soils were placed into pots in the greenhouse 0 to 5 weeks prior to pepper transplanting. Application rates are percentage dry weight seed meal/dry weight soil. Shown are the means of two trials with five replicates of each treatment per trial (n = 10) ± standard error. Values followed by the same letter are not significantly different ($P < 0.05$) according to Tukey's adjustment for multiple comparisons. Shoot lengths and root fresh weights were $\log_{10}(x+1)$-transformed prior to analysis; nontransformed data is presented.

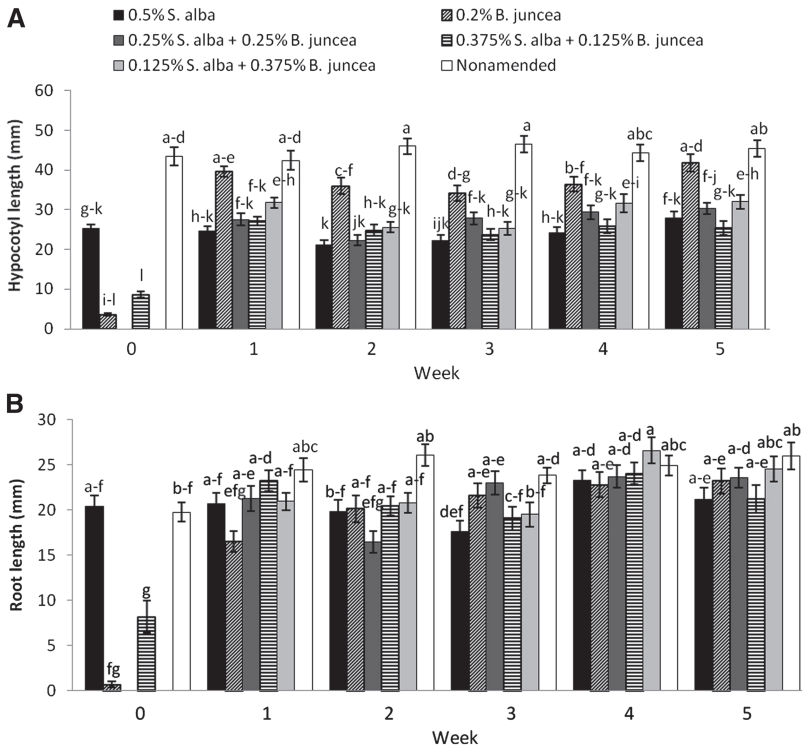

FIG. 2. Mean hypocotyl lengths (A) and root lengths (B) of germinating lettuce seeds exposed to soil amended with seed meals of *Sinapis alba*, *Brassica juncea*, and combinations of these meals. Amended and nonamended soils were placed into pots in the greenhouse 0 to 5 weeks prior to lettuce seed germination in Petri dishes. Application rates are percentage dry weight seed meal/dry weight soil. Shown are the means of two trials with thirty replicates of each treatment per trial (n = 60) ± standard error. Values followed by the same letter are not significantly different ($P < 0.05$) according to Tukey's adjustment for multiple comparisons.

significant reduction with any seed meal compared to the control, the exceptions being 0.2% *B. juncea* seed meal at week 1 and 0.25% *S. alba* + 0.25% *B. juncea* at week 2.

Suppression of M. incognita on pepper by seed meals: Shoot and root growth. Trials 1 and 2 could not be combined due to the significant interaction between trial × treatment ($P < 0.05$) for the recorded variables. In Trials 1 and 2, pepper shoots were consistently long with 0.15 and 0.2% *B. juncea* seed meal, and with 0.05% *S. alba* + 0.15% *B. juncea* (Table 3). The 0.2% *S. alba* and 0.15% *S. alba* + 0.05% *B. juncea* seed meals resulted in the

TABLE 3. The effects of *Sinapis alba* and *Brassica juncea* seed meal soil amendments on pepper shoot lengths and weights and on root galling indices.

Rates (% w/w)[a]	Mean shoot length (cm)[b]		Mean shoot fresh weight (g)		Mean Root Galling index	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Nonamended, -*M. incognita*	15.3 abc	17.5 abcd	2.6 cd	3.2 c	0.0 d	0.0 e
0.2% *S. alba*	11.2 c	19.8 abc	2.2 d	4.4 ab	2.9 cd	5.8 bcd
0.2% *B. juncea*	18.2 a	21.3 a	4.1 ab	4.8 a	1.3 d	2.3 cde
0.15% *S. alba*	13.7 abc	20.8 ab	2.7 cd	4.8 a	5.9 bc	11.2 a
0.15% *B. juncea*	17.2 ab	20.1 ab	3.6 abc	4.7 a	1.8 d	5.1 bcd
0.1% *S. alba*	13.9 abc	15.6 d	3.1 abcd	3.6 bc	9.4 ab	7.0 abc
0.1% *B. juncea*	15.8 abc	18.8 abcd	3.1 abcd	3.6 bc	9.4 ab	4.0 bcde
0.05% *S. alba*	15.0 abc	17.1 bcd	2.8 bcd	2.9 c	7.5 ab	6.9 abc
0.05% *B. juncea*	15.5 abc	17.6 abcd	2.9 bcd	3.4 c	6.9 ab	6.9 abc
0.1% *S. alba* +0.1% *B. juncea*	16.1 abc	20.3 abc	3.4 abcd	4.6 a	1.6 d	1.9 de
0.15% *S. alba* +0.05% *B. juncea*	12.5 bc	19.0 abcd	2.4 cd	4.4 ab	1.1 d	5.1 bcd
0.05% *S. alba* +0.15% *B. juncea*	18.6 a	20.4 abc	4.4 a	4.5 a	1.0 d	1.9 de
Nonamended, +*M. incognita*	15.8 abc	16.4 cd	2.8 bcd	2.8 c	10.0 a	7.5 ab

[a]Amended and nonamended soils were inoculated with 5,000 *Meloidogyne incognita* eggs and placed into pots in the greenhouse 2 weeks prior to seedling transplant.

[b]Values are the mean of eight replicates of each treatment per trial (n = 8); the two trials were not combined for analysis. Within a column, values followed by the same letter are not significantly different ($P < 0.05$) according to Tukey's adjustment for multiple comparisons. Significance letters are not comparable among trials or among columns.

shortest shoots in Trial 1, but had longer shoots in Trial 2. In Trial 2, 0.1% S. *alba* seed meal resulted in short shoots, along with *M. incognita*-inoculated plants that were not amended with any meal. Shoot fresh weights also showed differences between the two trials, but 0.2% *B. juncea* and 0.05% *S. alba* + 0.15% *B. juncea* had consistently high shoot fresh weights in both trials, while 0.05% *S. alba* and nonamended *M. incognita*-inoculated plants had consistently low shoot fresh weights. The two seed meals that consistently resulted in high shoot lengths and shoot fresh weights in both trials were 0.2% *B. juncea* and 0.05% *S. alba* + 0.15% *B. juncea* (Table 3). The three seed meals with consistently high root fresh weights in both trials were 0.15% and 0.2% *B. juncea*, and 0.05% *S. alba* + 0.15% *B. juncea* (data not shown). However, there was no treatment that resulted in a consistent, significant increase or decrease in root weight in both trials.

Root gall index and egg numbers. Trials 1 and 2 could not be combined due to the significant interaction between trial × treatment ($P < 0.05$) for the recorded variables. In Trial 1, the root galling index was highest in the control + *M. incognita*, and also high with individual low seed meal rates of 0.1% and 0.05% (Table 3). The higher rates of *B. juncea* (0.2 and 0.15%) and the mustard seed meal combinations resulted in the lowest root gall indices in Trial 1. Along with high gall indices, the control + *M. incognita* also had the highest number of eggs/g root in Trial 1 (Fig. 3). The number of eggs/g root was lowest in the treatments that had low gall indices; i.e. the two highest rates of *B. juncea* (0.2 and 0.15%), and all three seed meal combination amendments.

In Trial 2, the root galling index was greatest with 0.15% *S. alba* seed meal, followed by the control + *M. incognita* (Table 3). In both trials, gall indices were lowest with 0.2% *B. juncea*, 0.1% *S. alba* + 0.1% *B. juncea*, and 0.05% *S. alba* + 0.15% *B. juncea* seed meals (Table 3). Control + *M. incognita*, 0.1% *S. alba*, 0.05% *S. alba*, and 0.05% *B. juncea* consistently resulted in the highest gall indices.

In Trial 2, the numbers of eggs/g root showed overall similar treatment effects to Trial 1 (Fig. 3). The primary exception was that in Trial 2, 0.1% *S. alba* seed meal had more eggs/g root than all treatments except 0.15% *S. alba* or the control. Lowest egg numbers/g root again occurred in most treatments containing *B. juncea* seed meal.

Discussion

In our phytotoxicity study with pepper seedlings and lettuce seeds, *S. alba* seed meal was applied at a higher rate than *B. juncea* seed meal, because the latter had demonstrated greater nematotoxicity (Zasada et al., 2009), and our ultimate goal was to suppress nematode

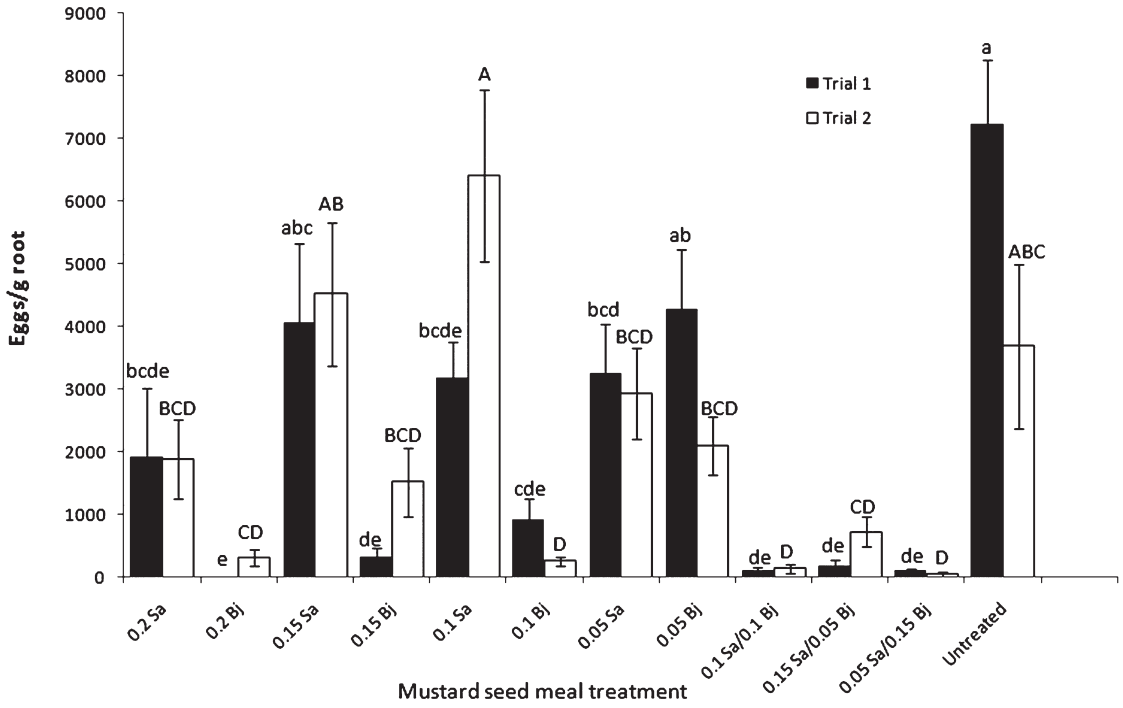

Fig. 3. Effects of *Sinapis alba* (Sa) and *Brassica juncea* (Bj) seed meals applied alone and in combinations on numbers of *Meloidogyne incognita* (root-knot nematode) eggs/g root on greenhouse-grown pepper plants. Amended and nonamended soils were inoculated with *M. incognita* eggs (5,000 per pot) and placed into pots 2 weeks prior to pepper seedling transplant. Application rates are percentage dry weight seed meal:dry weight soil. Shown are the means of eight replicates of each treatment (n = 8) per trial; the two trials were not combined for analysis. Values followed by the same letter are not significantly different ($P < 0.05$) within a trial according to Tukey's adjustment for multiple comparisons; significance values are not comparable between trials.

populations. For example, an individual application rate of 0.5% *B. juncea* and *S. alba* seed meals resulted in *M. incognita* suppression of 100% and ca. 80%, respectively (Zasada et al., 2009). We also chose to apply meals with a reduced flake size, because a flake application of *S. alba* seed meal, particularly smaller flakes, improved activity against *P. penetrans* (Zasada et al., 2009). The improved efficacy against pathogens with decreased particle size is most likely a result of higher isothiocyanate generation with small particles (Mazzola and Zhao, 2010). With these results in mind, the nematotoxic rates of 0.5% *S. alba* and 0.2% *B. juncea*, applied as ground seed meals, were selected for individual meal applications in our phytotoxicity tests. The 0.5% *S. alba* amendment was more phytotoxic to pepper seedlings than the 0.2% *B. juncea* seed meal amendment, resulting in more pepper plant death and in smaller shoots and roots than the *B. juncea* seed meal. The seed meal combinations tended to be intermediate in effect on pepper seedlings, although roots were small in all seed meals except 0.2% *B. juncea*. This study indicated that, based on the recorded parameters, only the 0.2% *B. juncea* amendment could be used without some phytotoxic effects within weeks of pepper seedling transplant, with the earliest application time being 2 weeks prior to transplant.

The response of lettuce seeds to seed meals was different from that of pepper seedlings. Immediately after application (week 0), 0.5% *S. alba* did not inhibit lettuce seed germination, but all treatments containing *B. juncea* did. This toxic effect of *B. juncea* was gone by week 1. *Sinapis alba* seed meal did decrease hypocotyl lengths compared to the control at all times, although root length was unaffected. *Brassica juncea* seed meal decreased hypocotyl length to some extent the first few weeks, and root lengths weeks 0 and 1.

The differential effects of the seed meals in these phytotoxicity tests may have been due to several factors, including seed meal species, application rates, plant species, and seedling growth vs. seed germination. Mustard plant species differ in types and amounts of glucosinolates, and therefore in breakdown products such as isothiocyanates (ITC), ionic thiocyanate (SCN^-), nitriles, and oxazolidinethiones (Borek and Morra, 2005; Brown and Morra, 2005; Hansson et al., 2008). Both types of seed meals tested are known to have high glucosinolate contents; *B. juncea* 'Pacific Gold' mainly contains sinigrin (2-propenyl glucosinolate), while *S. alba* "IdaGold' contains a large concentration of sinalbin (4-hydroxybenzyl glucosinolate) (Borek and Morra, 2005; Rice et al., 2007; Hansson et al., 2008). Typical concentrations of sinigrin in 'Pacific Gold' seed meal range from 108-134 μmol/g, and typical sinalbin concentrations in 'IdaGold' meal range from 125-160 μmol/g (Morra, unpublished). The glucosinolates in *S. alba* form SCN^- in soil, which is known to act as an herbicide (Borek and Morra, 2005); glucosinolates in *B. juncea* seed meal produce ITCs that are also phytotoxic (Rice et al., 2007). Also, while *B. juncea* produces compounds that are toxic on contact, so the plant is either tolerant or dies quickly, SCN^- is translocated and accumulates in plant tissues (Stiehl and Bible, 1989; Brown and Morra, 2005). An example of disparate activity of these two seed meals on the same crop plant species was shown with carrot; *S. alba* amendment inhibited emergence compared with *B. juncea* (Hansson et al., 2008; Snyder et al., 2009). In addition, the seed meal application rates used in our study were selected for nematotoxicity, so *S. alba* was applied at a higher rate, which could also cause some differences in phytotoxicity between seed meals.

The dissimilar results observed on pepper compared with lettuce may also be due to differential sensitivity to glucosinolate breakdown products that have been observed among plant species (Vaughn et al., 2006). For example, when seeds and seedlings of 39 crop plant species were exposed to SCN^-, 44% of the crop plants tested did not exhibit adverse effects (Stiehl and Bible, 1989). Not all plant species are equally sensitive to the phytotoxic chemicals. Finally, another factor that could affect results with pepper seedlings vs. lettuce seeds is that even when percentage seed germination is not inhibited by SCN^-, subsequent plant growth can still be inhibited by this compound (Stiehl and Bible, 1989; Brown and Morra, 2005). Similarly, with *B. juncea* seed meal, phytotoxicity was observed with sweet corn, but germination was not inhibited (Yu et al., 2007).

In the *M. incognita* suppression studies with pepper, *S. alba* and *B. juncea* seed meal application rates were based on the phytotoxicity test results. The two seed meals were applied at equal rates, and as 1:1 and 1:3 combinations. The three seed meal application rates that resulted in the longest pepper shoots and the greatest shoot and root weights were 0.2% *B. juncea*, 0.15% *B. juncea* and the low *S. alba* + high *B. juncea* (0.05% + 0.15%) combination. Of these three seed meal amendments, 0.2% *B. juncea* and 0.05% *S. alba* + 0.15% *B. juncea* also were among the lowest in root galling indices; 0.15% *B. juncea* varied between trials. The 0.1% *S. alba* + 0.1% *B. juncea* combination also tended to have high shoot and root growth and low gall indices. In both trials, when similar rates of *S. alba* and *B. juncea* were applied as individual seed meals, *B. juncea* generally had a more suppressive effect on *M. incognita* populations than *S. alba*, except at the lowest rate (0.05%). These results agree with previous studies indicating that *B. juncea* seed meal is more active than *S. alba* against *P. penetrans* and *M. incognita* (Mazzola et al., 2007, 2009; Zasada et al., 2009). *Brassica juncea* seed meal also suppressed *M. javanica* populations in vineyards (Rahman and Somers, 2005), *Tylenchulus semipenetrans* in soil (Walker, 1997), and *P. penetrans* on sweet corn (Yu et al., 2007). This activity may be at least in part due to high levels of sinigrin. Seed meal made

from *Brassica carinata*, which is also high in this compound, was efficacious for suppressing *M. incognita* on zucchini in a commercial greenhouse (Lazzeri et al., 2009).

The seed meal combinations tested in our study also tended to be suppressive to *M. incognita*. The range of seed meals available provides for the possibility of producing combinations that are active against multiple plant pests or pathogens. Soil application of a 1:1 ratio of *B. juncea* to *B. napus* seed meal improved apple replant disease control, partly because of differential effects of each meal on plant-pathogenic fungi (Mazzola and Brown, 2010). Consequently, a combination of *S. alba* and *B. juncea* seed meals active against *M. incognita* might have additional benefits for suppression of weeds or of other pathogens.

The results of this study indicate that the lower phytotoxicity of *B. juncea* seed meal to pepper, combined with the greater nematotoxicity of this seed meal, make it a better candidate than *S. alba* when used alone for suppression of *M. incognita* on pepper. However, seed meal amendments containing a higher rate of *B. juncea* combined with a lower rate of *S. alba* could also be formulated for concurrent nematode and weed suppression. Further studies would indicate whether both of these pests can indeed be minimized with such a combination. Additional tests can also determine whether long-term growth of pepper in amended, unpasteurized soil could allow for enhanced microbial activity, possibly resulting in even greater suppressive activity.

Acknowledgments. Thanks are extended to Shannon Rupprecht for greenhouse and laboratory work and data analysis, to Ashlee Green and Emily Brinker for assistance in the greenhouse and laboratory, and to Vladimir Borek for providing mustard seed meal. Dr. Orisajo's visit to the Nematology Laboratory was supported by a Norman E. Borlaug International Agricultural Science and Technology Fellows Program grant from the World Cocoa Foundation and the USDA.

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