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Nematode suppression with brassicaceous amendments: application based upon glucosinolate profiles

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Abstract

Glucosinolate profiles differ among plant species and their isothiocyanate (ITC) derivatives differ in toxicity to nematodes. Successful management of plant–parasitic nematodes by ITCs requires the incorporation of appropriate amounts of glucosinolate-containing biomass. Plant materials, containing glucosinolate-precursors of the ITCs most toxic to nematodes, were selected and applied to soil based upon ITC lethal concentration (LC) values. This provided a reliable and repeatable basis for application rates for suppression of *Meloidogyne javanica* and *Tylenchulus semipenetrans* by *Brassica hirta* and *M. javanica* by *B. juncea*. Sufficient biomass of *B. hirta* to potentially yield 0.03–0.12 μmol ml^{-1} of glucotropeolin reduced nematode survival compared to similar amounts of broccoli (*Brassica oleraceae* var. *botrytis*). At biomass levels providing >0.37 μmol ml^{-1} of glucotropeolin, mortality of *M. javanica* was 100% with *B. hirta*. Biomass of *B. juncea* potentially yielding 2.82 μmol ml^{-1} of sinigrin reduced *M. javanica* survival 65% below that obtained by a similar amount of broccoli. Rates of *B. juncea* to yield lethal levels of allyl ITC to reduce *T. semipenetrans* survival underestimated the glucosinolate application rates for this amendment. Application of plant biomass to soil >2.9% w/w reduced *M. javanica* survival regardless of the glucosinolate concentration of the amendment material. Application of brassicaceous amendments to soil initiates complex and dynamic biological and chemical processes. Despite the inherent complexity, we find that brassicaceous amendments can be applied to achieve consistent and repeatable nematode suppression when based upon the chemistry of the incorporated material.

Keywords: Glucosinolate; Isothiocyanate; *Brassica juncea*; *Brassica hirta*; *Tylenchulus semipenetrans*; *Meloidogyne javanica*

1. Introduction

The application of organic amendments for reducing plant–parasitic nematode populations has met with both success and failure (Halbrendt, 1996). The underlying biology and chemistry of the suppressiveness of organic amendments must be understood for them to become a reliable nematode management option. Necessary data include: chemical composition of amendment material, lethal concentration values of plant-derived chemicals for specific organisms, the influence of biotic and abiotic factors upon decomposition and release of plant-derived chemicals, fate of amendments in the soil after application, and the potential for combining organic amendments with other pest management strategies. In this study we explore the plant-derived chemical component of amendments for nematode suppression, using the Brassicaceae as a model.

There are numerous reports of nematicidal chemicals for plants (Chitwood, 2002; Ferris and Zheng, 1999). Members of the Brassicaceae provide a rich array of variants for evaluation of specific chemical components for nematode suppression. The importance of the Brassicaceae and their breakdown products as food, feed and medicine has been recognized for centuries and there is an extensive knowledge base on their constituent chemistry (Fahey et al., 2001).

Brassicaceae produce glucosinolates which are β-D-thioglucosides, distinguished from one another by differences in their organic side chains (R groups). Glucosinolates, classified as aliphatic, aromatic or indole forms, occur in all parts of the plant and degrade via enzymatic hydrolysis. As a result of tissue damage, the relatively non-reactive glucosinolates react with myrosinase (thioglucoside glucohydrolase, EC 3.2.3.1), which is stored

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separately in the cell, to yield nitriles, epithionitriles, thiocyanates and isothiocyanates (ITCs) (Brown and Morra, 1997; Fahey et al., 2001). ITCs are highly toxic compounds of varying volatility. They are general biocides whose activity results from irreversible interactions with proteins (Brown and Morra, 1997). The toxicity of several ITCs to certain nematode species is known (Buskov et al., 2002; Lazzeri et al., 1993; Zasada and Ferris, 2003).

Lack of nematode suppression or inconsistencies among studies are often attributed to differences in glucosinolate concentrations of incorporated brassicaceous materials (Johnson et al., 1992; Mojtahedi et al., 1991). Incorporation of 1.3–2.7% w/w of fresh Brassica napus did not affect the population densities of several plant–parasitic nematodes (Johnson et al., 1992) however, 2% w/w of several dried Brassoca spp. was highly nematicidal (Potter et al., 1998). Depending on the variety, 7.8% w/w of fresh B.

Napus did or did not reduce Meloidogyne chitwoodi populations compared to a wheat amendment (Mojtahedi et al., 1991).

The present study is part of a series of experiments to improve efficacy of nematode management by brassicaceous soil amendments. In an earlier paper, we identified the lethal concentrations (LC) of several purified ITCs to Tylenchulus semipenetrans and *M. javanica* (Zasada and Ferris, 2003). In the current paper we used these LC values to select plant material containing the glucosinolate-precursors with the most nematode-toxic ITCs. Biomass application rates were based on glucosinolate profiles of plant sources and LC values determined in a previous study (Zasada and Ferris, 2003) for target nematode species. Our objectives were to develop a chemistry-based application protocol and an experimental basis for reliable and repeatable nematode suppression.

2. Materials and methods

2.1. Plant material collection

Brassica hirta 'Martegena' leaves and stems were harvested from greenhouse-grown plants immediately prior to flower initiation. Broccoli (*B. oleracea* var. *botrytis*) and wheat (*Triticum aestivum*) leaves and stems were harvested from mature, field-grown plants and dried at 140 °C for 1 week. *Brassica juncea* 'Pacific Gold' meal was used. All material was ground and homogenized in a Wiley mill through a 850-μm-pore (20 mesh) sieve and stored at room temperature until use.

2.2. Nematode soil assay

Glucosinolate sources were assayed against *T. semipenetrans* and *M. javanica*. Mixed life-stages of *T. semipenetrans* were extracted from soil from an infested olive orchard in Orland, CA by decanting and sieving soil. Extracted material was placed on a Baermann funnel. Nematodes were collected after 24 h and used immediately (Ingham, 1994). Two-d-old second-stage juvenile *M. javanica* were obtained from hydroponic tomato cultures (Lambert et al., 1992), and used immediately.

A Yolo sandy loan (40% sand, 43% silt, 18% clay, 1.85% organic matter, and pH 7.3 (Sustainable Agriculture Farming Systems Project, Agronomy Farm, University of California, Davis, CA) was used in the experiments. The soil was spread 2.5 cm deep in a tray and defaunated at 60 °C for 3 h.

Based upon glucosinolate profiles biomass levels of *B. hirta* and *B. juncea* were applied to achieve target ITC LC_{50} and LC_{90} values determined in a previous study (Zasada and Ferris, 2003). A 10% conversion of glucosinolates to ITCs (Morra and Kirkegaard, 2002) was assumed. *B. hirta* application was based upon the concentration of glucotropeolin (Precursor to benzyl ITC) in the plant material, and *B. juncea* application based upon the concentration of sinigrin (precursor to allyl ITC) in the meal (Table 1). Other treatments included: broccoli a low-glucosinolate plant, and wheat a non-glucosinolate plant. Both were applied at the same biomass levels as the high-glucosinolate *Brassica* spp. In addition, there was an untreated control.

Twelve cubic centimeters of soil, with or without amendment, was placed into a 20-ml scintillation vial in three aliquots; the soil packed after each addition. The vials were hydrated to stimulate the production of ITCs. Assay nematodes (250–300) were inoculated in 1 ml water into a depression in the center of the soil in each vial. Water was added to the vials to achieve a soil moisture level approximately 60% of field capacity (Mitchell, 1997). The vials were closed to prevent loss of volatile ITCs and incubated for 72 h at 25 °C. All treatments were replicated five times and arranged in a completely randomized design in the incubator. Each trial was repeated at least twice. After incubation, the soil was removed from the vials and placed on Baermann funnels for 72 h (Ingham, 1994). Extracted nematodes were counted under a dissecting microscope.

2.3. Glucosinolate analysis

Four, 200 mg samples from each ground *Brassica* sp. Were processed for glucosinolate analysis by high pressure liquid chromatography (HPLC). Extraction was by established procedures in an 80 °C water bath (Matthaus and Luftmann, 2000; Kraling et al., 1990). In the first extraction step, the homogenized tissue was heated for 10 min in 2 ml of 70% aqueous ethanol to inhibit myrosinase activity. Internal standards were added, 500 μ1 of 1 mM of either glucotropeolin (Merk, Germany) or sinigrin (Sigma Chemical Co., St. Louis, MO), depending upon the material being extracted. After centrifugation (15 min, 8500*g*), the pellet was extracted a second time for 10 min using 2 ml of 70% ethanol and centrifuged again. One mililitre of

Table 1
Isothiocyanate-producing glucosinolate concentrations of incorporated brassicaceous material

Glucosinolate[b]	Corresponding Isothiocyanate	μmol g^{-1} dry material ± standard deviation[a]		
		Brassica hirta 'Martegena'	*Brassica juncea* 'Pacific Gold'	*B. oleraceae* var. *botrytis*[c]
Sinigrin	2-propenyl	–[d]	109.9 ± 3.0	Trace[e]
Glucotropeolin	Benzyl	5.0 ± 0.1	–	–
Sinalbin	*p*-Hydroxybenzyl	68.1 ± 7.7	–	–
Gluconasturtiin	2-phenyethyl	–	–	0.1 ± 0.1
Glucoraphanin	4-methylsulphinylbutyl	–	–	2.3 ± 0.4
Total		73.1	109.9	2.4

[a] Concentrations are the mean of at least four samples.
[b] Only ITC-producing glucosionlates are listed.
[c] Variety unknown.
[d] Not detected.
[e] Concentration <0.1 umol g^{-1}.

the combined extracts was applied to a DEAE-Sephadex A-25 (40 mg) column (pyridine acetate form). The column was washed three times with 20 mM pyridine–acetate and twice with water. The glucosinolates were converted to their desulfo analogs by overnight treatment with 100 μl of 0.25% (w/v) purified sulfatase (Type H1, Sigma Chemical Co., St Louis, MO). The desulfoglucosinolates were eluted with 1.5 ml water.

HPLC analysis was conducted using a Shimadzu VP liquid chromatograph with a dual length spectrophotometer. Samples (30 μl) were separated at 30 °C on a Phenomenex C18 column (150 by 4.6 mm, 3μ) (Phenomenex, Torrance, CA) using acetonitrile and water at a flow rate of 1 ml min^{-1}. The Procedure used 1.5% acetonitrile for the first 5 min, a linear gradient to20% acetonitrile over the next 15 min followed by 10 min at 20% acetonitrile. Absorbance was measured at 226 and 280 nm (Grubb et al., 2002). Individual glucosinolates were identified in relation to internal standards and concentrations were calculated using previously determined response factors (Dan Kliebenstein, personal communication).

2.4. Soil ITC analysis

One hour after biomass incorporation a 1 ml sample of each amended soil was added to 1.4 ml acetonitrile The mixture was incubated for 1 h, vortexed, centrifuged (10 min, 4000*g*) and the acetonitrile supernatant removed. HPLC analysis was conducted using a liquid chromatograph with a dual length spectrophotometer. Samples (20 μl) were separated at 25 °C on a reverse phase C18 column (5 μm) (Agilent Technology, Palo Alto, CA) using acetonitrile and water at a flow rate of 1 ml min^{-1}. The elution procedure started with a 0–5% linear gradient of acetonitrile over 5 min then decreased the gradient from 5 to 20% acetonitrile over the next 30 min. Absorbance was measured at 229, 280 and 210 nm. Identification of ITC was determined by elution standards and spectrophotometric graphs.

2.5. Statistical analysis

The number of nematodes retrieved from the untreated control for each experiment was used as the baseline for determining nematode mortality Percent reduction for each nematode-amendment combination is expressed as the average of at least two trials. Interactions of treatments with experimental trials were not significant ($P < 0.05$) and data were pooled across trials. Differences among treatments for each nematode were determined by analysis of variance and means were compared by Tukey's adjustment for multiple comparisons (SAS, Cary, NC). Percent nematode reductions for broccoli and wheat biomass levels were arcsin transformed and subjected to regression analysis. Differences between nematode-amendment regression lines were determined using *t*-tests ($P < 0.05$). The percentage of plant material applied is expressed as w/w of dry amendment to oven dried soil (% w/w).

3. Results

3.1. Glucosinolate content of plant material

B. hirta 'Martegena' contained two ITC-producing forms, sinalbin and glucotropeolin, comprising 90 and 6%, respectively, of the glucosinolate profile (Table 1). The remainder of the profile contained unidentified or non-ITC producing glucosinolates. The majority of the glucosinolate profile of B. juncea 'Pacific Gold' was the ITC-producing glucosinolate, sinigrin. Broccoli contained small concentrations of the ITC-producing glucosinolates sinigrin, gluconasturtiin and glucoraphanin.

3.2. ITC production

ITC were produced by all brassicaceous amendments at all addition rates (data not shown) The production of ITCs in these systems was measured after 1 h incubation

and demonstrates only that ITCs were produced, not the concentration to which nematodes were exposed.

3.3. Nematode suppression related to glucosinolate addition

In experiments with *M. javanica*, *B. juncea* and corresponding broccoli and wheat treatments were applied at 0.7, 2.0 and 2.9% w/w biomass levels. For *B. juncea* these biomass levels were equivalent to sinigrin concentrations ranging from 0.99 to 4.23 μmol ml^{-1} (Fig.1A). At the lowest biomass level, corresponding to the LC_{50} of sinigrin, *M. javanica* reduction ranged from 6 to 24% and treatments were not different from the control. At a 2.0% w/w biomass level, *B. juncea* reduced *M. javanica* numbers almost 100% compared with approximately 34% for broccoli or wheat. Broccoli and wheat were different from the untreated control. At the highest level, all amendments reduced *M. javanica* populations by at least 72%. The percent reductions of *M. javanica* by *B. juncea* and broccoli were 100% and 94%, respectively.

The rates of *B. hirta*, broccoli and wheat applied for *M. javanica* suppression corresponded to *B. hirta* glucotropeolin concentrations ranging from 0.08 to 0.58 μmol ml^{-1} and biomass levels of 1.2, 5.4 and 8.5% w/w (Fig. 1B). At a 1.2% w/w biomass level, *B. hirta* reduced nematode populations by 70%. At this biomass level, nematode reduction by broccoli and wheat were not different from the untreated control. At the two higher levels, corresponding to the LC_{50} and LC_{90} values for benzyl ITC, the percent nematode reduction by wheat was less than the other two amendments. Both broccoli and *B. hirta* reduced *M. javanica* survival 100%.

Applications of *B. juncea*, broccoli and wheat for *T. semipenetrans* suppression at biomass levels of 0.4, 0.6 and 0.9% w/w were equivalent to *B. juncea* sinigrin concentrations of 0.62, 0.92 and 1.33 μmol ml^{-1} (Fig. 2A). Regardless of biomass level, *B. juncea* reduced *T. semipenetrans* populations significantly compared to the other amendments. There was no difference in nematode reduction between broc1coli and wheat at 0.4 and 0.6% w/w, with average reductions of 20 and 32%, respectively. At the highest biomass level, broccoli reduced the nematode population by 52%. Amendment with wheat did not reduce *T. semipenetrans* populations compared to the untreated control at any biomass level.

Biomass levels of 0.4, 1.2 and 1.8% w/w of *B. hirta*, broccoli and wheat for *T. semipenetrans* suppression were equivalent to glucotropeolin concentrations in *B. hirta* ranging from 0.03 to 0.12 μmol ml^{-1} (Fig. 2B). *B. hirta* reduced nematode populations from 61 to 98% across concentrations, and was different than broccoli and wheat, at all rates. Broccoli at 0.4% w/w and wheat at 0.4

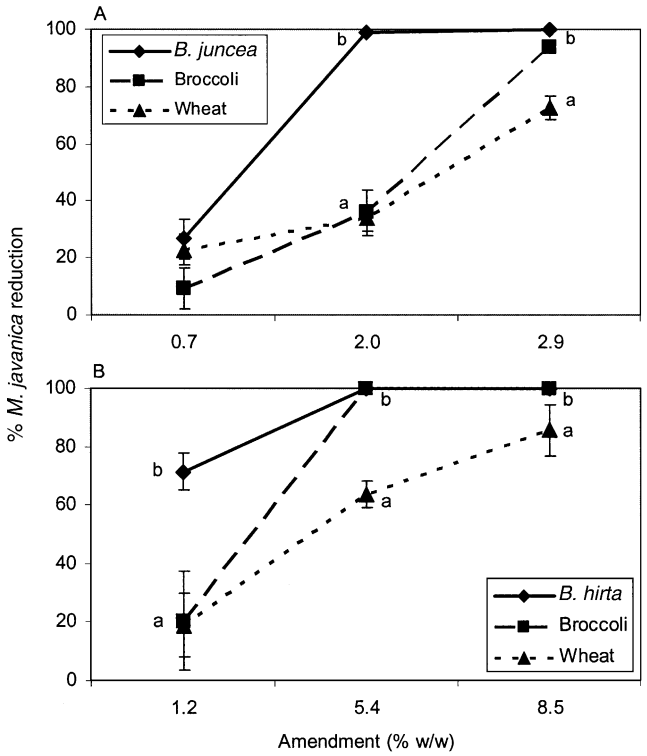

Fig. 1. Percent *Meloidogyne javanica* reduction by *Brassica juncea* (A) and *Brassica hirta* (B) at different glucosinolate application rates (% w/w). The data are the means of 10 replications. The vertical bars are the standard errors of the means. Points followed by the same letter within a concentration are not significantly different at $P < 0.01$ according to Tukey's adjustment for multiple comparisons.

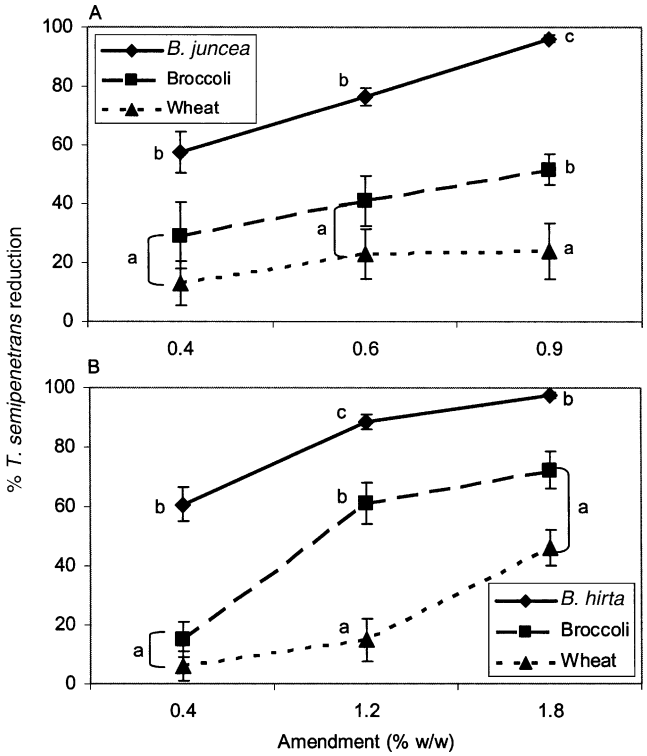

Fig. 2. Percent *Tylenchulus semipenetrans* reduction by *Brassica juncea* (A) and *Brassica hirta* (B) at different glucosinolate application rates (% w/w). The data are the means of 10 replications. The vertical bars are the standard errors of the means. Points followed by the same letter within a concentration are not significantly different at $P < 0.301$ according to Tukey's adjustment for multiple comparisons.

and 1.2% w/w did not reduce *T. semipenetrans* numbers compared to the control. Nematode reduction was 61% with broccoli at 1.2% w/w but at 1.8% w/w there was no difference between broccoli and wheat.

3.4. Nematode suppression related to amendment biomass

The amendment biomass levels (maximum 1.8% w/w) used in *T. semipenetrans* experiments were lower than for *M. javanica* (Fig. 3). There was no reduction in *T. semipenetrans* survival compared to the untreated control below 0.4% w/w for broccoli and wheat (Fig. 3A). There was a significant difference in nematode reduction between the amendments, with increasing percent w/w of broccoli resulting in greater *T. semipenetrans* reduction than wheat.

Up to biomass levels of 1.2% w/w, *M. javanica* reduction (Fig. 3). There was no reduction in *T. semipenetrans* survival compared to the untreated control below 0.4% w/w for broccoli and wheat (Fig. 3A). There was a significant difference in nematode reduction between the amendments, with increasing percent w/w of broccoli in greater *T. semipenetrans* reduction than wheat.

Up to biomass levels of 1.2% w/w, *M. javanica* reduction was not different from the untreated control for either amendment (Fig. 3B). The relationship between percent *M. javanica* reduction and biomass was linear for both amendments. Across biomass levels, broccoli resulted in significantly greater nematode reductions than wheat.

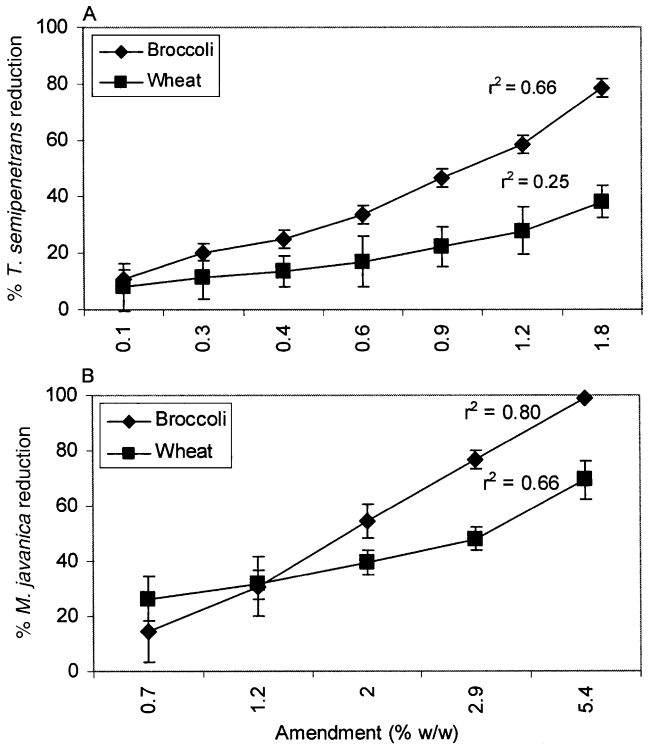

Fig. 3. Percent *Tylenchulus semipenetrans* (A) and *Meloidogyne javanica* (B) reduction as influenced by increasing amounts of biomass (% w/w) of broccoli and wheat. The data are the means of at least 10 replications. The vertical bars are the standard errors of the means.

4. Discussion

Glucosinolate profiles of plant sources, guided by ITC LC values, provided a reliable repeatable basis for determining biomass levels of *B. hirta* effective against both *M. javanica* and *T. semipenetrans*. For these amendment-nematode combinations, the amount of glucosinolate (at a 10% conversion rate) necessary to produce ITC LC_{50} and LC_{90} concentrations resulted in nematode suppression >50 and 90%, respectively. The benefit of applying brassicaceous amendments based upon glucosinolate profiles was demonstrated by the application of *B. hirta* for *T. semipenetrans* suppression. At all biomass levels, the addition of *B. hirta* suppressed the nematode more than a broccoli amendment. Glucosinolate content of broccoli was insufficient for reducing nematode populations at the same biomass levels.

Glucotropeolin comprised only 7% of the glucosinolate profile of *B. hirta*, with sinalbin predominating. Enzymatic hydrolysis of sinalbin yields *p*-hydroxybenzyl ITC which resulted in only 8% mortality of Heterodera schachtii (Lazzeri et al., 1993) and 13% of Globodera rostochiensis juveniles (Buskov et al., 2000). In those experiments, exposure to the same concentrations of glucotropeolin resulted in 100% nematode mortality. Some of the nematode suppression by *B. hirta* in this study was probably due to *p*-hydroxybenzyl ITC but the major effect was likely due to the more toxic benzyl ITC hydrolyzed from glucotropeolin.

The predicted allyl ITC LC values of *B. juncea* for *T. semipenetrans* overestimated the observed effect. When *B. juncea* sinigrin was applied at levels assuming a 10% conversion rate to allyl ITC, there was no nematode reduction at the LC_{50} value, and 39% reduction at the LC_{90} value. These sinigrin addition rates corresponded to 0.1 and 0.3% w/w biomass application levels. To achieve approximately 50 and 90% reductions in *T. semipenetrans* populations. Biomass application levels had to be increased 73 and 67% over the calculated LC_{50} and LC_{90} values, respectively. The application of *B. juncea* for *M. javanica* suppression based upon the allyl ITC LC_{50} value also underestimated the required biomass. Lack of nematode suppression at lower *B. juncea* biomass application levels may be explained by the difficulty in achieving uniform distribution of the amendment in the soil and the high volatility of allyl ITC. The high sinigrin concentration of *B. juncea* meal dictated a very small biomass application which may have been insufficient for adequate distribution through the soil. Further, volatility of ITCs is thought to increase with decreasing molecular weight (Lewis and Papavizas, 1971), and allyl ITC is one of the move volatile ITCs. At 20 °C, allyl ITC has a vapor pressure of 4 mm Hg. At higher temperatures ITC volatility would increased so that the chemical would move rapidly through the soil profile and limit exposure time for target organisms. With the expectation that the observed nematode suppression

would be due to the production of ITCs or other toxic chemicals, not elevated temperature, we used an incubation temperature of 25 °C during our experiments. Ploeg and Stapleton (2001) demonstrated that 2% w/w broccoli, incubated at 25 °C for 72 h, did not compromise the ability of *M. javanica* to parasitize a plant. The practical inference of these conclusions is that when *Brassica* spp. with high glucosinolate content are used as amendments, sufficient biomass must be applied to allow uniform distribution through the soil profile for subsequent volatilization. This requirement may be even more critical in cooler climates.

In previous studies, *B. juncea* meal was highly effective against *T. semipenetrans*, and the nematode was undetectable in soil amended with meal at levels $\geq 1\%$ w/w (Walker, 1997). At *B. juncea* meal levels of 0.9% w/w in our study, *T. semipenetrans* survival was reduced 98%. The toxicity of allyl ITC against a variety of plant–parasitic nematodes is well documented (Buskov et al., 2000; Lazzeri et al., 1993; Zasada and Ferris, 2003). Since sinigrin is the predominant glucosinolate in *B. juncea*, we assume that the nematode suppression was primarily due to allyl ITC.

It is likely that other degradation products of glucosinolates occurred in these systems and may have played a role in nematode suppression. In soil amended with 2% w/w dried cabbage, methanethiol, ethanol and occasionally acetic acid and methanol were detected (Gamliel and Stapleton, 1993). Carbon-disulphide, dimethyl-disulphide, dimethyl-sulphide and methanethiol were the dominant headspace component after a 0.6% w/w dry *B. juncea* amendment was incubated at 20 °C. All of these compounds are toxic to a broad range of organisms and probably contribute to the toxicity of brassicaceous tissue (Bending and Lincoln, 1999).

Environmental and chemical factors have profound influences on amendment-based management systems. In our *Brassicaceae* experiments, we strove to create an environment that would optimize conversion of glucosinolate to ITC. Actual conversion of glucosinolates to ITCs can range from 1 to 25%. The total conversion of rapeseed meal glucosinolates to ITCs was 15% of the potential amount (Borek et al., 1997). Only 1% or less of the predicted ITC from tissue glucosinolate concentrations was measured in soil amended with repeseed or mustard varieties (Morra and Kirkegaard, 2002). In the same study, more thorough tissue disruption at a cellular level, afforded by freezing the tissues, increased release efficiencies by 13 and 25% over coarsely-chopped incorporated material. We chose to apply *B. juncea* and *B. hirta* glucosinolates with expectation of a 10% conversion rate to their respective ITCs. The plant material used in this study was finely ground and homogenized, allowing for event distribution in the soil profile and facilitating exposure to soil moisture. The smaller size of the amendment material, compared to coarsely chopped material, would increase cell disruption and permit greater conversion efficiency of glucosinolates to ITCs.

Collectively, available data indicate that most ITCs will be released within the first 4 days after tissue incorporation into soil (Morra and Kirkegaard, 2002). ITCs extracted from soils amended with *B. napus* were at maximum concentration after 30 h and decreased by 75% after 72 h (Gardiner et al., 1999). The half-life for allyl ITC ranged from 20 to 60 h (Borek et al., 1995). Our experiments were incubated for 72 h, a sufficient amount of time of ITC production to occur. Soil is a complex system in which many factors, including soil water, pH, and microbial activity have direct impact on the fate of glucosinolates and ITCs (Brown et al., 1991). Under neutral conditions ITCs are the predominant degradation products of ITC-producing glucosinolates (Fahey et al., 2001). The pH of the soil used in this study was 7.3, optimal for enzymatic hydrolysis of glucosinolates to ITCs.

Broccoli was included in our studies as a low glucosinolate-containing plant material and because it is commonly used in California biofumigation pest management systems (Ramirez-Villapudua and Munnecke, 1988; Xiao et al., 1998). The broccoli we used contained 2.44 μmol g^{-1} of ITC-producing glucosinolates. The addition of broccoli, regardless of biomass level, did not suppress *T. semipenetrans* to the same extent as *B. hirta* and *B. juncea*. Based upon the concentration of gluconasturtiin in broccoli, and the nematode-specific toxicity of its glucosinolate-degradation product 2-phenylethyl ITC (Zasada and Ferris, 2003), 54% w/w of broccoli would be required to reduce T. semipenetrans survival to 10%. Based upon the concentration of glucoraphanin in broccoli, the precursor to 4-methylsulfinyl(butyl), this application level could be reduced to 14% w/w. Greater biomass levels would be needed to produce toxic concentrations of 2-phenylethyl and 4-methysulfiny(butyl) ITCs for a 90% reduction in *M. javanica* survival. The amount of wet broccoli residue left in a field after harvest is approximately 8.5% w/w (Shetty et al., 2000) or 0.9% w/w dry material (10:1 wet/dry ratio). Clearly, the amount of broccoli needed to provide glucosinolate concentrations at required rates would be prohibitive. Broccoli varieties containing higher concentrations of sinigrin, gluconasturtiin and glucoraphanin would reduce the amount of plant biomass needed to generate toxic concentrations of ITCs.

The nematode suppression observed with a broccoli amendment in these experiments would have increased at higher temperatures as discussed earlier. An application level of 2% w/w fresh broccoli, incubated at 25 °C, reduced *M. javanica* galling after 10 days compared to unamended soil. Soil had to be heated to 40 °C for 10 days to eliminate root-galling compared to three days with a broccoli amendment (Ploeg and Stapleton, 2001).

Differences in sensitivity of nematodes to organic amendment control practices have been reported (Halbrendt and Jing, 1994; Ploeg and Stapleton, 2001; Zasada and Ferris, 2003). At comparable levels of broccoli,

T. semipenetrans suppression was three times greater than that of *M. javanica*. This response is in concurrence with the lower LC values for 2-phenylethyl and 4-methylsulfiny (butyl) to *T. semipenetrans* (Zasada and Ferris, 2003).

This study allowedboth evaluation of nematode suppression associated with specific glucosinolates, and the influence of amendment biomass on nematode suppression. *B. hirta* contained 5 μmol g^{-1} of glucotropeolin and large applications of biomass were requiredto achieve LC of glucotropeolin. Less *B. juncea* meal was necessary to achieve requires sinigrin rates due to the high concentration (197 μmol g^{-1}) in the meal. The suppression of *M. javanica* continued to increase with increasing biomass. Blok et al. (2000) incorporated an equivalent of 0.3% w/w of dry broccoli and grass into soil. In plots amended and covered with plastic tarp, anaerobic soil conditions developed, resulting in a decrease in redox potential values. Pathogens were inactivated in covered plots as effectively with grass as with broccoli, suggesting that non-specific fermentation products rather than crop-specific toxins were involved (Blok et al., 2000). Although we did not observe nematode mortality at an amendment level of 0.3% w/w, anaerobic soil conditions may have occurred at higher biomass levels and carbon dioxide, ethylene, hydrogen, methane, organic acids, alcohols and aldehydes may have accumulated (Ponnamperuma, 1972).

The application of brassicaceous amendments to soil for plant–parasitic nematode management initiates dynamic and complex biological and chemical processes. Issues that require further investigation include: the rate of ITC conversion in soil, sensitivity of target species to specific ITCs, application technology and the development of varieties containing high-glucosinolate levels. Out work indicates that brassicaceous amendments can provide consistent and repeatable plant–parasitic nematode management if levels are based on the glucosinolate profile of incorporated material. The determination of ITC lethal concentration values for specific nematodes allows the selection and application of species of Brassicaceae containing glucosinolate precursors of the ITCs most toxic to target nematodes.

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