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Effect of Broccoli (*Brassica oleracea*) Tissue, Incorporated at Different Depths in a Soil Column, on *Meloidogyne incognita*

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Abstract: Brassicas have been used frequently for biofumigation, a pest-management strategy based on the release of biocidal volatiles during decomposition of soil-incorporated tissue. However, the role of such volatiles in control of plant-parasitic nematodes is unclear. The goal of this study was to determine the direct localized and indirect volatile effects of amending soil with broccoli tissue on root-knot nematode populations. *Meloidogyne incognita*-infested soil in 50-cm-long tubes was amended with broccoli tissue, which was mixed throughout the tube or concentrated in a 10-cm layer. After three weeks at 28°C, *M. incognita* populations in the amended tubes were 57 to 80% smaller than in non-amended tubes. Mixing broccoli throughout the tubes reduced *M. incognita* more than concentrating broccoli in a 10-cm layer. Amending a 10-cm layer reduced *M. incognita* in the non-amended layers of those tubes by 31 to 71%, probably due to a nematicidal effect of released volatiles. However, the localized direct effect was much stronger than the indirect effect of volatiles. The strong direct effect may have resulted from the release of non-volatile nematicidal compounds. Therefore, when using biofumigation with broccoli to control *M. incognita*, the tissue should be thoroughly and evenly mixed through the soil layer(s) where the target nematodes occur. Effects on saprophytic nematodes were the reverse. Amended soil layers had much greater numbers of saprophytic nematodes than non-amended layers, and there was no indirect effect of amendments on saprophytic nematodes in adjacent non-amended layers.

Key words: amendment, biofumigation, broccoli, *Brassica oleracea*, management, *Meloidogyne incognita*, root-knot nematode, soil.

Root-knot nematodes (*Meloidogyne* spp.) are economically the most damaging nematode pests on a range of crops in subtropical climates (Koenning et al., 1999; Stirling and Stirling, 2003). Methyl bromide, until recently widely used to control nematodes and other soil-borne problems in high value crops, is no longer available because of its negative impact on the stratospheric ozone layer. Other chemicals are less effective, too expensive, and often also have a negative impact on the environment and general public health (Braun and Supkoff, 1994). Consequently, there is an increased interest in non-chemical nematode-management strategies.

Biofumigation is a sustainable pest-management strategy that is lately receiving a lot of interest. Because of its potential efficacy against multiple soil-borne problems, its ease of incorporation into existing cropping regimes and the ability to combine it with other approaches to manage diseases and pests, such as soil solarization or the use of resistant crop cultivars, it could become an important component of an integrated pest-management program. In addition, like other techniques based on amending soil with organic matter, it has important soil building properties, improving the soil nutrient status and water-holding capacity and increasing the presence and activity of beneficial soil organisms, including those that are antagonistic to plant-parasitic nematodes (Stirling, 1991; Bridge, 1996; Lazarovits et al., 2001).

Biofumigation was initially defined as a process involving the biocidal action of volatile compounds released during the decomposition of brassica crops (Angus et al., 1994; Kirkegaard and Sarwar, 1998). Upon mechanical or biochemical disruption of brassica tissue, glucosinolates and myrosinase located in different parts of the cell come into contact (Poulton and Moller, 1993). The enzymatic hydrolysis of glucosinolates then leads to the formation of bioactive volatile isothiocyanates (Cole, 1976; Fenwick et al., 1983). Isothiocyanates are thought to be the main component of the pest-, pathogen- and weed-suppressive effect observed after soil incorporation of brassica tissue (Kirkegaard and Sarwar, 1998), and their inhibitory effect on fungi, bacteria, insects, nematodes and weeds has been amply demonstrated in in vitro experiments (Brown and Morra, 1997; Harvey et al., 2002; Bello et al., 2004). However, results from bio-assay experiments on the efficacy of adding brassica tissue to soil indicated that the actual conversion efficacy of glucosinolates to isothiocyanates may be very low and that the glucosinolate content of the tissue does not necessarily predict its pest suppressive activity (Angus et al., 1994; Bending and Lincoln, 1999; Charron and Sams, 1999; McLeod and Steel, 1999; Lazzeri and Manici, 2001; Harvey et al., 2002; Morra and Kirkegaard, 2002). This was particularly true in studies involving nematodes (Kirkegaard and Matthiessen, 2004), and the level of nematode control achieved through biofumigation that can be attributed to isothiocyanate gases remains unclear.

The goal of this study was to determine the direct localized and indirect non-localized effects of broccoli tissue when used as a biofumigant to control *M. incognita* in soil columns.

Materials and Methods

An *M. incognita* race 3 population originally isolated from cotton in the San Joaquin Valley, CA, USA, was maintained and multiplied in a greenhouse on tomato var. 'UC82', grown in steam-sterilized sandy soil in 5-liter pots. Species and race identification had previously

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been confirmed by isozyme electrophoresis and by reproduction on differential hosts (Eisenback and Triantaphyllou, 1991). To prepare inoculum, heavily infested roots, approximately 3 mon after inoculation of the plants with 50,000 *M. incognita* eggs, were carefully removed from the pots, and the soil from the pots was collected after sieving over a 5-mm-pore sieve to remove remaining root pieces. Eggs of *M. incognita* were extracted by shaking the roots in a 1% NaOCl solution (Radewald et al., 2003) and collected on a 25-µm-pore sieve. They were added back to the sieved soil from the pots. This nematode-infested soil (1 part) was thoroughly mixed with steam-sterilized sandy soil (9 parts) originally obtained from the same location. Soil moisture levels were adjusted to 12% with tap water, and this nematode-infested soil was used in the experiments. To determine initial infestation levels, nematodes were extracted from three 100-g samples using a modified Baermann funnel technique (Rodriguez-Kabana and Pope, 1981) and counted.

An experimental unit consisted of five stacked sections of PVC tubing (10-cm long, 4-cm inner diam.), connected with plastic connector sleeves, resulting in a 50-cm-long tube. The bottom end of the tube was closed with a plastic cap. Leaves and stems of broccoli var. 'Liberty' plants were collected at time of broccoli harvest from an experimental field. Leaves and stems were stored on ice and transported to the lab. The next day, the leaves and stems were chopped into approximately 0.5 cm pieces using a food processor and immediately mixed thoroughly with the previously prepared nematode-infested soil.

Five treatments were applied: 1) broccoli-amended nematode-infested soil (20 g fresh broccoli tissue in 200 g soil = 10%) in the bottom layer (40–50 cm) of the tube; 2) broccoli-amended nematode-infested soil (10%) in the middle layer (20–30 cm) of the tube; 3) broccoli-amended nematode-infested soil (10%) in the top layer (0–10 cm) of the tube; 4) broccoli-amended nematode-infested soil (20 g fresh broccoli in 1 kg soil = 2%) throughout the tube; 5) non-amended nematode-infested soil throughout the tube (= control). Layers without broccoli were filled with 200 g nematode-infested soil. Thus, all the broccoli-containing tubes received the same amount (20 g) of broccoli tissue. Each treatment had five replicates. The tubes were then closed with a PVC cap and placed upright at 28°C in the dark.

Three weeks later, the individual layers of the tubes were carefully separated starting from the top, and nematodes were extracted by wet sieving over four sieves (sieve pore sizes 100, 75 and 2 x 45 µm). For final separation, nematodes collected on the sieves were allowed to migrate for 48 hr through a double layer of tissue paper (Kleenex, Kimberly-Clark, Neenah, WI) into a shallow dish with tap water, and *Meloidogyne* J2 and non-plant-parasitic nematodes were counted. The complete experiment was repeated once.

In a third experiment, with the same treatments, only 75 g of the soil from each tube layer was used for nematode extraction after 3 wk, and the remaining 125 g soil from each layer was used to fill a 200-ml plastic vial. The vials were placed on a greenhouse bench, and 2 d later two seeds of melon var. 'Durango' were added to each vial. After 1 wk, melon seedlings were thinned to leave 1 seedling/vial, and plants were grown for another 4 wk. The melon plants were then carefully removed from the vials, and the roots were indexed for severity of root galling on a 0 to 10 scale (0 = no galling; 10 = 100% of roots galled, plant dead or dying [Bridge and Page, 1980]). Egg masses on the root systems were stained overnight in 75 µg/ml eriyoglaucine (Sigma, MO) (Ehlers et al., 2000) and counted.

The statistical analysis of treatment effects was done with the GLM-procedure using SAS software (SAS Institute, Cary, NC). Least significant differences between individual treatments were determined at the 95% confidence level. The significance of differences between treatment combinations (e.g., amended vs. not-amended) was analyzed using the "Contrast" procedure in SAS. Nematode counts were transformed by $x^1 = \log_{10}(x + 1)$ before analysis. As results on non-plant-parasitic nematodes were very similar between the three experiments (treatment x experiment interaction not significant at $P = 0.05$), results are presented and discussed as averages for the three experiments.

Results

Average (n = 3) initial *M. incognita* densities were 1,207, 670 and 226 J2/100 g soil, and initial non-plant-parasitic nematode numbers were 19, 33 and 22/100 g soil for replicated experiments 1, 2 and 3, respectively.

Meloidogyne J2: The total number of *Meloidogyne* J2 per tube was significantly smaller in the tubes that had been amended with broccoli tissue than in the non-amended tubes in all three replicated experiments (Table 1). Amending the nematode-infested soil in the tubes with broccoli tissue reduced *Meloidogyne* J2 numbers by 80, 79 and 57% compared to the non-amended control tubes in the first, second and third replicated experiments, respectively (Table 1). Mixing 20 g broccoli tissue with nematode-infested soil throughout the tube resulted in many fewer *Meloidogyne* J2 per tube than concentrating the same amount of broccoli tissue in one layer (Table 1). The vertical position of the broccoli-amended soil layer (top, middle or bottom) significantly affected the total number of *Meloidogyne* J2 per tube in the first and second replicated experiments, but not in the third. In the first experiment, amending the top or middle layer with broccoli tissue reduced *Meloidogyne* J2 compared to amending the bottom layer,

TABLE 1. Effect of different broccoli tissue-amendment treatments on the number of second-stage *Meloidogyne* juveniles (J2) per tube (1 kg soil/tube; average of 5 replicates) in 3 replicated experiments. Analysis on $\log_{10}(x \div 1)$-transformed data, non-transformed data (J2 per 200 g soil) shown.

Treatment	1st experiment	2nd experiment	3rd experiment
Non-amended	2,952	1,220	478
Amended throughout	223	97	17
Amended in top	459	181	311
Amended in middle	583	364	236
Amended in bottom	1,078	379	255
Amended in one layer	706	308	267
Amended	586	255	205
Contrasts (*F*-value):			
Amended vs. non-amended	132.34**[a]	110.22**	7.41*
Amended throughout vs. one layer	41.73**	36.71**	44.22**
Top vs. middle vs. bottom	10.25**	8.93**	0.06 ns

[a]** significant at $P = 0.01$; *significant at $P = 0.05$; ns $P > 0.05$.

but in the second replicated experiment amending the top layer reduced *Meloidogyne* J2 compared to amending the middle or bottom layer (Table 1).

Comparison of the number of *Meloidogyne* J2 from individual layers of the broccoli-amended tubes (treatments 1–4) showed that in two of the three replicated experiments fewer J2 were recovered from layers that had been amended with 20 g broccoli than from layers that had been amended with 4 g broccoli. Significantly more J2 were recovered from layers that had not been amended with broccoli tissue, but were from tubes that had received broccoli in another layer (Table 2).

To determine the indirect effect of broccoli tissue on *Meloidogyne* J2, the data from the tubes with broccoli tissue concentrated in one layer (treatments 1–3) were compared to the non-amended control (treatment 5). Amending a 200 g soil layer with 20 g broccoli tissue reduced the average number of J2 in these layers by 97, 98 and 96% compared to the average number of J2 per soil layer in the non-amended control in the first, second and third replicated experiments, respectively (Table 2). However, adding broccoli to one layer of a tube also reduced the number of J2 in the four non-amended layers of the tube compared to the number of J2 in layers of the non-amended control tubes (by 71, 69 and 31% for the first, second, and third replicated experiments, respectively; Table 2).

Data from the one-layer-amended tubes (treatments 1–3) did not show that the effect of incorporated broccoli tissue decreased as soil layers were further away from the broccoli-amended layer. Only in the third replicated experiment were fewer J2 recovered from layers adjacent to the broccoli tissue-amended layer than from layers further away from the broccoli-amended layer (Table 3).

Root-galling and egg-masses: Data on galling and egg masses on the roots of the melon plants from the bioassay in the third replicated experiment reflected the results obtained on the number of J2. Average galling and number of egg masses on melon roots were significantly reduced in the tubes that had been amended with broccoli tissue (Table 4). Mixing broccoli throughout the tube resulted in smaller galling indices and egg mass numbers than concentrating broccoli in one soil layer (Table 4). Placement of broccoli tissue in the top, middle or bottom layer of the tubes did not affect the average root-galling or egg mass numbers on roots of melon bio-assay plants (Table 4).

Root-galling of melons grown in layers amended with 20 g broccoli was less than of those grown in layers amended with 4 g broccoli (Table 5). Roots of plants grown in layers that had not been amended with broccoli tissue but were from tubes that had received broccoli in another layer had significantly greater galling indices, but still less galling than plants grown in soil from the non-amended controls (Table 5). Effects on the number of egg masses per root system were less pronounced; only differences between the non-amended and broccoli-amended layers were significant (Table 5).

There was no evidence that the effect of incorporated broccoli tissue on root-galling or number of egg masses decreased as soil layers in which melon plants were grown were further away from the broccoli-amended layer (Table 6).

Non-plant-parasitic nematodes: Non-plant-parasitic nematodes were almost exclusively bacterivores (Rhab-

TABLE 2. Average number of second-stage *Meloidogyne* juveniles in broccoli tissue-amended or non-amended soil layers (200 g) in 3 replicated experiments.[a]

Treatment	1st experiment	2nd experiment	3rd experiment
Broccoli-tissue amended tubes			
g fresh broccoli tissue added per 200 g soil layer			
0	858 (29)[b] b[c]	378 (31) b	330 (69) b
4	223 (8) c	97 (8) c	18 (4) c
20	100 (3) d	27 (2) d	17 (4) c
Non-amended controls	2,952 (100) a	1,220 (100) a	478 (100) a

[a] Estimated initial density per 200 g: 2,414, 1,340 and 452 for 1st, 2nd and 3rd experiment.
[b] In brackets: percentage relative to non-amended control.
[c] Different letters within a column indicate significant differences at 95% level of confidence [lsd-test, statistics on $\log_{10}(x + 1)$-transformed data; non-transformed data shown].

TABLE 3. Number of second-stage *Meloidogyne* juveniles in soil layers (200 g soil per layer) in relation to their distance from the broccoli tissue-amended layer (20 g/200 g soil), in 3 replicated experiments.[a]

Distance of layer to broccoli-amended layer	1st experiment	2nd experiment	3rd experiment
0 cm (= broccoli-amended layer)	100 b[b]	27 b	18 c
>0–10 cm	670 a	300 a	203 b
>10–20 cm	924 a	429 a	341 a
>20–30 cm	1,002 a	421 a	389 a
>30–40 cm	958 a	389 a	501 a

[a] Estimated initial density per 200 g: 2,414, 1,340 and 452 for 1st, 2nd and 3rd experiment.
[b] Different letters within a column indicate significant differences at 95% level of confidence [lsd-test, statistics on log(x + 1)-transformed data; non-transformed data shown].

ditids). The effects of treatments on non-plant parasites were very similar for all three experiments and were the reverse of effects on *Meloidogyne* J2. Tubes with broccoli had more non-plant parasites than did non-amended tubes, and mixing 20 g broccoli tissue with nematode-infested soil throughout the tube resulted in a much larger number of non-plant parasites per tube than concentrating the same amount of broccoli tissue in one layer (Table 7). Adding broccoli tissue to the top, middle or bottom layer of the tubes did not result in significantly different numbers of non-plant-parasitic nematodes in the tubes (Table 7).

There was no significant difference ($P \leq 0.05$) in the number of non-plant-parasitic nematodes recovered from 200 g soil layers amended with 4 or 20 g broccoli tissue (1,623 and 1,749 nematodes/200 g, respectively). The average number of non-plant parasites from soil layers of non-amended tubes (119/200 g) was very similar to numbers from non-amended layers of broccoli-amended tubes (167/200 g), indicating that the increase in non-plant-parasitic nematodes in broccoli-amended tubes occurred almost exclusively in the broccoli-amended layers. This was further demonstrated by analysis of the number of non-plant-parasitic nematodes in soil layers in relation to the distance of the soil layer to the broccoli-amended layer. Non-plant-parasite numbers were significantly ($P \leq 0.05$) greater in the broccoli-amended layers (1,749/200 g) than in layers up to 10 cm (240/200 g), 20 cm (254/200 g), 30 cm (265/200 g) or 40 cm (148/200 g) from the amended layer.

TABLE 4. Effect of different broccoli tissue-amendment treatments on average galling[a] and egg masses on roots of melon bio-assay plants after 5 wk in the third experiment.

Treatment	Galling	Egg masses
Non-amended	7.8	66.8
Amended throughout	3.3	13.7
Amended in top	5.9	64.1
Amended in middle	5.9	64.0
Amended in bottom	5.3	44.1
Amended in one layer	5.7	57.4
Amended	5.1	46.5
Contrasts (*F*-value):		
Amended vs. non-amended	47.22**[b]	5.47*
Amended throughout vs. one layer	35.76**	23.72**
Top vs. middle vs. bottom	1.11 ns	2.20 ns

[a] Galling index: 0 = no galling; 10 = 100% of root galled.
[b]** Significant at $P = 0.01$; *significant at $P = 0.05$; ns $P > 0.05$.

TABLE 5. Average galling index (0 - no galling; 10 = 100% of root galled) and number of *Meloidogyne* egg masses on roots of melon bioassay plants grown for 5 wk on broccoli tissue-amended or non-amended soil layers (125 g).

Treatment	Galling	Egg masses
Broccoli tissue-amended treatments		
g Fresh broccoli tissue added per 200 g soil layer		
0	6.7 b[a]	71.2 a
4	3.3 c	13.7 b
20	1.9 d	2.0 b
Non-amended controls	7.8 a	66.8 a

[a] Different letters within a column indicate significant differences at 95% level of confidence (lsd-test).

DISCUSSION

Amending *M. incognita*-infested soil (1 kg) in 50-cm-long tubes with 20 g fresh broccoli tissue reduced the number of *Meloidogyne* J2 that were recovered from the tubes three weeks later by 57 to 80%, compared to non-amended tubes in three replicated experiments. By mixing broccoli tissue throughout the length of the tube rather than concentrating it in a 10-cm (200 g soil) layer, a much greater reduction (up to 96%) could be achieved. Although the greatest reductions (up to 98%) occurred within the 10-cm layers that had been amended with 20 g broccoli tissue, a significant portion of nematodes survived in the non-amended layers of these tubes. Because of this, mixing broccoli throughout the tube was more effective in reducing *Meloidogyne* J2 than concentrating the broccoli in one layer of the tube. In tubes where one layer was amended with broccoli, J2 numbers in the four remaining non-amended

TABLE 6. Average galling index (0 = no galling; 10 = 100% of root galled) and number of *Meloidogyne* egg masses on roots of melon bioassay plants grown for 5 wk on broccoli tissue-amended (20 g/200 g soil) or non-amended soil layers (125 g).

Distance of layer to broccoli-amended layer	Galling	Egg masses
0 cm (= broccoli-amended layer)	1.9 b[a]	2.0 b
>0–10 cm	5.9 a	44.3 b
>10–20 cm	6.8 a	87.5 a
>20–30 cm	7.1 a	91.8 a
>30–40 cm	7.6 a	71.9 ab

[a] Different letters within a column indicate significant differences at 95% level of confidence (lsd-test).

TABLE 7. Effect of different broccoli tissue-amendment treatments on the number of non-plant-parasitic nematodes per tube (1 kg soil/tube; average of 5 replicates and 3 replicated experiments). Analysis on $\log_{10}(x + 1)$-transformed data, non-transformed data (nematodes per 200 g soil) shown.

Treatment	Average of three experiments
Non-amended	119
Amended throughout	1,623
Amended in top	435
Amended in middle	486
Amended in bottom	529
Amended in one layer	483
Amended	768
Contrasts (*F*-value):	
Amended vs. non-amended	60.81**[a]
Amended throughout vs. one layer	32.78**
Top vs. middle vs. bottom	0.27ns

[a]** significant at $P = 0.01$; *significant at $P = 0.05$; ns $P > 0.05$.

layers were still 31 to 71% lower than in layers from the non-amended control tubes. This indirect effect of broccoli tissue amendment on nematodes in soil layers up to 30 to 40 cm separated from the amended layer may be the result of volatiles with a nematostatic or nematicidal action that are released during tissue decomposition. Studies on fungal plant pathogens and weeds showed that volatiles released during brassica tissue decomposition strongly inhibited fungal growth and weed germination (Fenwick et al., 1983; Brown and Morra, 1997; Rosa et al., 1997; Sarwar et al., 1998; Smolinska and Horbowicz, 1999). However, the role of volatiles in reducing nematode populations remains unclear. To increase the pest-suppressive effect of biofumigant brassica-type crops, a lot of effort has been directed towards characterization of glucosinolates, the precursors of the biocidal volatile isothiocyanates, and towards selecting and breeding crop varieties with increased glucosinolate levels (Potter et al., 1998; Kirkegaard and Sarwar, 1999; Potter et al., 2000; Morra and Kirkegaard, 2002). However, in several studies (Mojtahedi et al., 1993; Potter et al., 1998; Charron and Sams, 1999; McLeod and Steel, 1999; Kirkegaard et al., 2000) aimed at correlating the pest- or pathogen-suppressive activity of plant tissue with its glucosinolate level, such a correlation was not found. This was particularly true with studies involving nematodes (Kirkegaard and Matthiessen, 2004). For example, Potter et al. (1998) and McLeod and Steel (1999) strongly reduced *Pratylenchus neglectus* and *M. javanica* with various brassica amendments, but did not find a correlation between the suppressive effect and the glucosinolate profiles of the amendments. Results from our study suggest that volatile effects from decomposing broccoli tissue on *M. incognita* are much weaker than the localized effects. Mojtahedi et al. (1993), using open-ended tubes with *M. chitwoodi*-infested soil, reported an even stronger difference between localized and non-localized effects. They achieved high levels of control of *M. chitwoodi* in rapeseed-amended soil, but not in soil only 5 cm from the amended soil. The difference in experimental set-up between our study (closed tubes) and theirs (open-ended tubes) may explain the difference in results on non-localized effects.

It is known that, apart from isothiocyanates, a large variety of other potentially toxic compounds are formed during brassica tissue decomposition (Bending and Lincoln, 1999). This could explain the strong localized suppressive effect of broccoli tissue amendment.

Amending soil with broccoli tissue also reduced galling and the number of egg masses on roots of melon bio-assay plants. Of the three replicated experiments, the volatile effect of broccoli amendment on J2 was weakest in the third experiment. The reduction in J2 from 478 in the non-amended layers of the control tubes to 330 in the non-amended layers of broccoli-amended tubes corresponded with a slight reduction in melon root-galling (from 7.8 to 6.7), but was not reflected in a reduction in the number of egg masses on the melon roots (67 and 71). This further demonstrated that the localized effect of broccoli amendment on *Meloidogyne* population densities and subsequent infestation of a susceptible host was much stronger than the indirect volatile effect.

Amending nematode-infested soil with broccoli tissue resulted in a strong increase in levels of non-plant-parasitic nematodes in the amended layers but had very little effect on levels of non-plant parasites in adjacent non-amended layers. Thus, the moderately strong non-localized effect of the amendment on *Meloidogyne* J2 was largely absent with non-plant-parasitic nematodes. The non-plant-parasitic nematodes, mostly bacterivores, were probably introduced together with the *M. incognita* inoculum from the tomato cultures. An increase in levels of saprophytic nematodes after amending soil with organic material is common (Bello et al., 2004). Such a response was also reported by Stirling and Stirling (2003) after amending soil with brassica tissue. It is known that the biocidal activity of brassica-produced isothiocyanates is short-lived and has its peak within the first few hours after soil incorporation (Morra and Kirkegaard, 2002). Halbrendt and Jing (1996) reported that non-plant-parasitic nematodes were less sensitive to brassica-induced toxins than plant parasites. Therefore, although numbers of non-plant parasites in our experiments may have declined shortly after the broccoli amendment, the generally short generation time of saprophytic nematodes and ample food supply resulted in their quick recovery and in large populations in the broccoli-amended layers at harvest.

Our results suggest that the fumigant nematicidal activity of biofumigation is limited and that for biofumigation to be effective a thorough and even distribution

of the biofumigant material through the soil profile where the target nematodes occur is required. This corresponds with application protocols for isothiocyanate-releasing synthetic nematicides such as metam-sodium and dazomet, also known to have limited fumigant action (Smelt and Leistra, 1974; Anonymous, 1998).

Other factors shown to greatly enhance the pest-suppressive activity of biofumigation include a very thorough disruption of the plant tissue prior to soil incorporation and sufficient soil moisture at the time of tissue incorporation (Morra and Kirkegaard, 2002; Matthiessen et al., 2004). Under field conditions, numerous other variables may influence the efficacy of biofumigation. For example, soil temperature at time of tissue incorporation appears to determine to a large extent the level of control and the time needed to achieve control (Ploeg and Stapleton, 2001). Soil characteristics also affected biofumigant efficacy, and particularly soil fluoride levels were positively correlated with the biocidal activity of the tissue used to amend the soil (Matthiessen and Kirkegaard, 2003).

Combined with on-going breeding efforts aimed at developing varieties that release high levels of effective biocidal compounds and identifying nematode-resistant varieties, biofumigation may become an important tool to manage nematodes and soil-borne pests in general, while at the same time maintaining and improving soil structure and fertility.

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