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Effect of Three Plant Residues and Chicken Manure used as Biofumigants at Three Temperatures on *Meloidogyne incognita* Infestation of Tomato in Greenhouse Experiments

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Abstract: Plant residues of broccoli, melon, and tomato with or without addition of chicken manure were used as biofumigants in two pot experiments with *Meloidogyne incognita*-infested soils. The efficacy of these biofumigants in controlling *M. incognita* infestation in susceptible tomato bio-assay plants was studied at soil temperatures of 20°, 25°, and 30 °C. None of the plant residues was effective at 20 °C, and broccoli was more effective than tomato or melon at 25 °C. At 30 °C all three plant residues reduced *M. incognita* infestation of tomato to very low levels. Chicken manure was effective in one of two experiments at 20 °C, and at 25 °C enhanced the efficacy of tomato and melon residue in one of two experiments. At 30 °C chicken manure was equally effective as the three plant residues but did not further decrease infestation levels in plant residue amended soils. It is concluded that biofumigation to control *M. incognita* is unlikely to be effective under cool conditions, that at soil temperatures around 25 °C broccoli is more effective than melon and tomato, and that the addition of chicken manure at this soil temperature may enhance the efficacy. At high soil temperatures, of approximately 30 °C, the biofumigant source seems of minor importance as strong reductions in tomato infestation by *M. incognita* were achieved by addition of each of the three plant residues as well as by addition of chicken manure.

Key words: biofumigation, broccoli, chicken manure, control, melon, root-knot nematode, temperature, tomato.

Root-knot nematodes (*Meloidogyne* spp.) are economically the most damaging nematodes in vegetable crops in California (Koenning et al., 1999). For control, chemical nematicides can be used but the range of available products is limited, they are expensive, and their use has negative impacts on the environment and on the general public health. As a result, there is growing interest in methods for nematode management that are economically viable and not polluting. Root-knot nematode-resistant varieties are available only for a limited number of vegetable crops (e.g., tomato, sweet pepper), and frequent cultivation could lead to selection of virulent nematode populations (Tzortzakakis and Gowen, 1996; Williamson et al., 1992). Crop rotation is difficult due to the wide host range of *Meloidogyne*.

An alternative management strategy that is increasingly receiving interest is biofumigation. Biofumigation was included as an alternative to methyl bromide by the Methyl Bromide Technical Options Committee (MBTOC) under the Montreal Protocol (MBTOC, 1997). The concept of biofumigation was described by Kirkegaard et al. (1993), and the first journal article on biofumigation was published in 1994 (Angus et al., 1994). Biofumigation was defined by several researchers (Bello et al., 2000a, 2000b; Halbrendt, 1996; Kirkegaard and Sarwar, 1998) as a process that occurs when volatile compounds with pesticidal properties are released into the soil during decomposition of plant material or animal by-products.

Brassica spp. contain glucosinolate compounds, known to release a number of toxic products (e.g., thiocyanate, isothiocyanate) during decomposition (Brown et al., 1991, Chew, 1988). Their efficacy in suppressing nematodes, weeds, and soil-borne diseases has been demonstrated (Angus et al., 1994; Boydston and Hang, 1995; Boydston and Vaughn, 2002; Brown et al., 1991; McFadden et al., 1992; Mojtahedi et al., 1993; Ploeg and Stapleton, 2001; Spak et al., 1993). However, it is not feasible to incorporate brassica crops in each cropping system, and transport of brassica residues to sites where they can be incorporated may be unpractical or too expensive. Therefore, Bello et al. (2000a, 2000b, 2004) looked at the efficacy of other sources of organic material and concluded that all can be used effectively in biofumigation to control *Meloidogyne,* depending on the method of application, dosage, and biochemical characteristics. As a general rule, they recommend incorporation of material or mixtures with a C/N ratio between 8 and 20, a general dosage of 50 t/ha, irrigating soil to near saturation, covering the soil with plastic for at least 2 weeks, and using the technique at soil temperatures above 20 °C (Bello et al., 2004).

The goal of this study was to compare the efficacy of biofumigation using three plant residues (broccoli, melon, tomato) with or without addition of chicken manure as an "activator" (Bello et al., 2004) at three soil temperatures to control *M. incognita* infestation in subsequent susceptible tomato.

Material and Methods

Experiment 1: Nematodes: A race 3 *M. incognita* population, originally isolated from cotton in the San Joaquin Valley, California, was maintained and multiplied in a greenhouse on tomato var. UC82. Species and race identification were confirmed by iso-zyme electrophoresis and by reproduction on differential hosts (Eisen-

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back and Triantaphyllou, 1991). To prepare inoculum, *M. incognita* eggs were extracted from tomato roots by shaking in a 1% NaOCl (Radewald et al., 2003) and the concentration was adjusted to contain 10^3 eggs/40 ml for inoculation.

The biofumigant action of three plant residues was compared: leaves and stems of broccoli var. Liberty, leaves and stems of tomato var. UC82, and leaves and stems of melon var. Durango. All leaves and stems were chopped into ca. 0.5-cm pieces using a food processor. Dry-weight percentages of broccoli, tomato, and melon were 16, 15, and 13%, respectively. The effect of adding dried chicken manure (87% dry weight), obtained from a poultry farm and ground and sieved over a 0.5-cm sieve, was also tested.

Carbon and nitrogen percentages of dried material were 40.1 and 3.3 for broccoli, 31.9 and 2.7 for tomato, 29.1 and 2.1 for melon, and 28.3 and 4.8 for chicken manure. The C/N ratios of the different soil amendments were broccoli 12.3, melon 14.0, tomato 11.7, chicken manure 5.9, broccoli plus chicken manure 7.7, melon plus chicken manure 7.3, and tomato plus chicken manure 7.3.

Two hundred and forty 500-g portions of steam-sterilized sand were prepared in plastic bags. To 120 portions, 40-ml egg suspension containing 10^3 *M. incognita* eggs was added, resulting in a soil moisture content of 12%. To the other 120 portions, 40 ml tap water was added. To each of 60 portions, 10 g freshly chopped broccoli, melon, or tomato were added (2% w/w, equivalent to 50 ton/ha). The remaining 60 portions did not receive any plant material. Each of the 60 portions was then divided in two lots of 30. Thirty received 3.3 g (fresh weight) chicken manure, and 30 remained without chicken manure. The soil, nematode inoculum, plant material, and chicken manure were thoroughly mixed and transferred to glass mason jars (Kerr mason jars, 473 ml), wrapped with aluminum foil, and closed with a lid. Of each treatment combination, five jars were placed in water baths at 20, 25, and 30° C (± 1° C) in a randomized block design with 1 jar/treatment combination in each block (5 blocks in each water bath). After 20 days, the jars were removed from the water baths and the soil was transferred to 500-ml plastic pots randomized on a greenhouse bench. Each of the pots was planted with a 3-week-old tomato var. UC82 plant 48 hours later. Plants were grown for 6 weeks and then carefully washed from the pots. Fresh shoot weights and root gall rating (scale 0 to 10, 0 = no galls, 10 = 100% galled) (Bridge and Page, 1980) were determined. The eggs were extracted from each root system by shaking with 1% NaOCl (Radewald et al., 2003) and counted. Data were analyzed separately for each temperature using ANOVA procedures, and the significance of differences between means was determined with Duncan's multiple-range tests at the 95% confidence level using SAS statistical software (SAS Institute, Cary, NC).

Experiment 2: The complete experiment was repeated, but with soil collected from an experimental field after a tomato crop that showed a high incidence of root galling. The field with sandy-loamy soil was located on the South Coast Research and Extension Center, Irvine, California, and had been inoculated the previous year with *M. incognita* from the same origin as was used in the first experiment. Half of the soil was spread out in a thin layer and air-dried for 3 weeks to eliminate root-knot nematodes. Water was then added and thoroughly mixed through the soil to achieve the same moisture percentage (11%) as the non-dried soil. Prior to setting up the experiment, both the previously dried and non-dried soils were thoroughly mixed and sieved over a 1-cm sieve, and nematodes were extracted from three 100-g samples from both soils using a modified Baermann funnel technique (Rodríguez-Kábana and Pope, 1981).

Results

Experiment 1: The type of plant residue and the addition of chicken manure affected the tomato shoot weights at each of the three temperatures, but the nematodes did not affect tomato shoot weight ($P \leq 0.05$). Adding chicken manure resulted in an increase in shoot weight at all three temperatures, but the effects of plant residue on shoot weight were not consistent over the three temperatures. However, compared to the non-amended control, adding broccoli always resulted in a lower shoot weight (Table 1).

Gall ratings and number of eggs (root-knot nematode infection) were similarly affected by plant residues and chicken manure. Adding chicken manure reduced the overall root-knot nematode infection at each of the three temperatures. At 20 °C adding plant residue did not reduce nematode infection, but at 25 °C it did when compared to the non-amended control. At 25 °C, broccoli was more effective than melon or tomato. At 30 °C, chicken manure was effective only when compared to the absolute control (nothing added), and all three plant residues reduced nematode infection to

Table 1. Effect of biofumigant material on tomato fresh shoot weights at three temperatures in Experiment 1.

Biofumigant material	Temperature		
	20 °C	25 °C	30 °C
None	28.0 ab[a]	26.4 a	27.5 a
Broccoli	20.4 c	19.6 b	22.2 b
Melon	26.7 b	28.0 a	21.4 b
Tomato	33.2 a	24.2 ab	21.4 b

[a] Means that have a letter in common in the same column do not differ significantly according to Duncan's multiple-range test at $P \leq 0.05$

very low levels even without adding chicken manure (Figs. 1A–C;2A–C).

Experiment 2: Initial root-knot nematode densities in the three non-dried samples were 280, 380, and 330 (average 330) J2/100 g soil. No root-knot nematodes were detected in the previously dried soil. Chicken manure increased tomato shoot weight at all three temperatures. Plant residue affected the shoot weights at 25

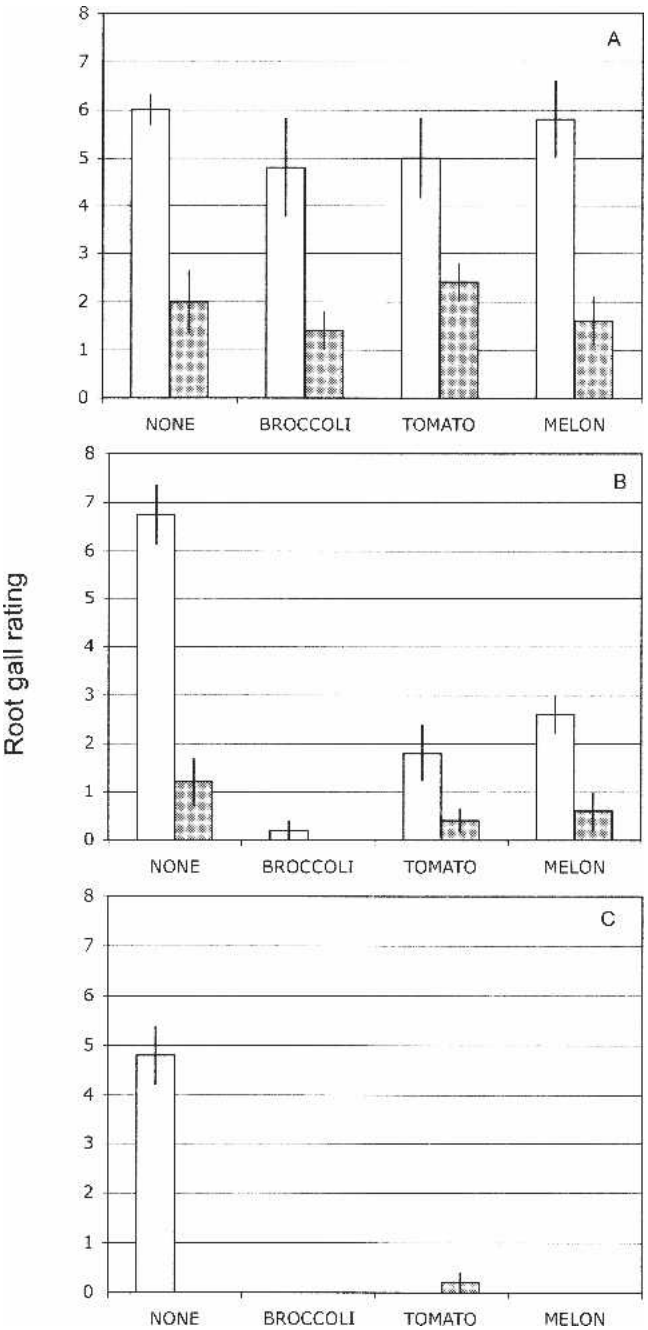

FIG. 1. Root gall rating of tomato grown in biofumigated, *Meloidogyne incognita*-infested soils in experiment 1. Biofumigants were fresh residue of broccoli, tomato, and melon. White bars represent soils without addition of chicken manure; dark bars represent soils with addition of chicken manure. Vertical bars represent 2× standard error. A) Biofumigation at 20 °C. B) Biofumigation at 25 °C; biofumigation at 30 °C.

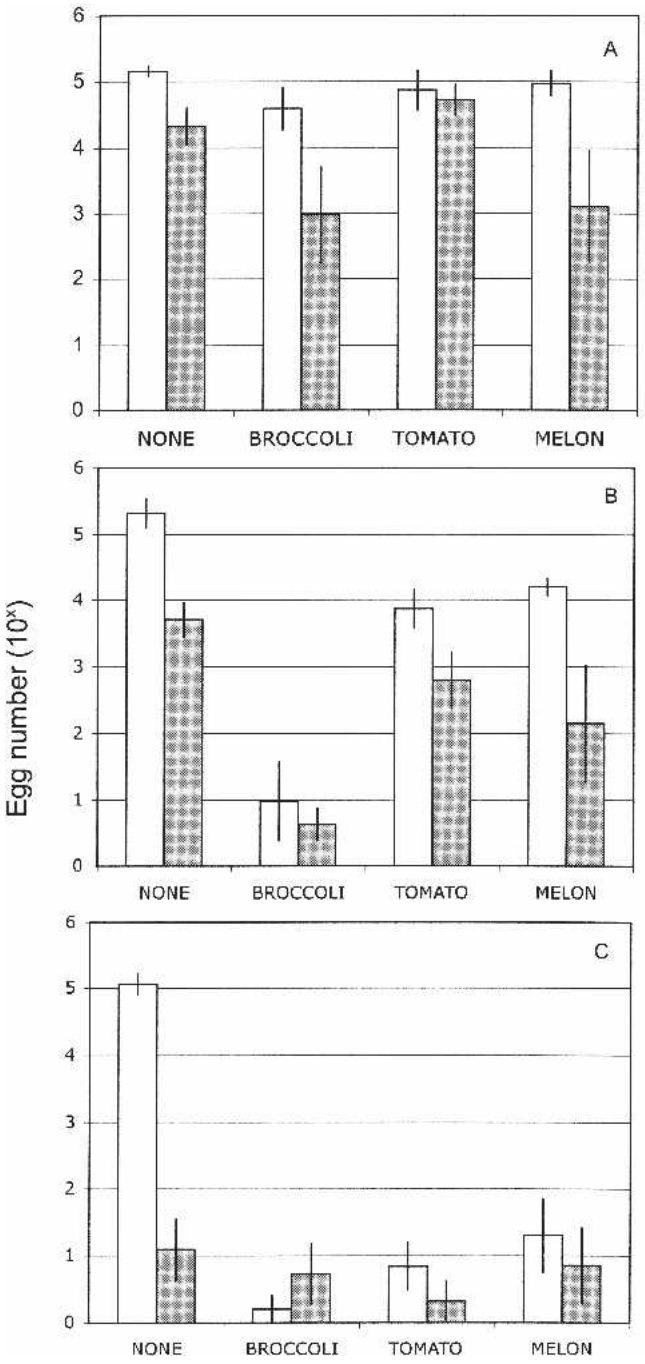

FIG. 2. Eggs extracted from tomato roots grown in biofumigated, *Meloidogyne incognita*-infested soils in experiment 1. Biofumigants were fresh residue of broccoli, tomato, and melon. White bars represent soils without addition of chicken manure; dark bars represent soils with addition of chicken manure. Vertical bars represent 2× standard error. A) Biofumigation at 20 °C. B) Biofumigation at 25 °C; biofumigation at 30 °C.

and 30 °C, but the effects were inconsistent: At 25 °C amending with melon resulted in lower shoot weights, whereas at 30 °C the tomatoes in the no-plant amended soils had lower shoot weights (Table 2). Nematodes did not affect the shoot weights at 20 or 25 °C, but increased shoot weights at 30 °C (data not shown).

At 20 °C chicken manure or plant residue did not

TABLE 2. Effect of biofumigant material on tomato fresh shoot weights at three temperatures in Experiment 2.

	Temperature		
Biofumigant material	20 °C	25 °C	30 °C
None	40.2 a[a]	46.4 a	40.3 b
Broccoli	45.3 a	46.2 a	53.5 a
Melon	43.0 a	36.0 b	55.4 a
Tomato	42.1 a	44.9 a	53.1 a

[a] Means that have a letter in common in the same column do not differ significantly according to Duncan's multiple-range test at $P \leq 0.05$.

affect the gall rating. At 25 °C and at 30 °C both chicken manure and plant residue reduced galling. However, at these temperatures, chicken manure reduced galling only in the treatment without plant residue, and amending with plant residue reduced galling only compared to the absolute control. There were no differences between the three plant residues (Fig. 3A–C).

In general, the effects on the final egg numbers were similar to the effects on galling. At 20 °C, neither chicken manure nor plant residue affected egg numbers. At 25 °C, amending the soil with plant residue reduced the number of eggs compared to the nonamended soils and broccoli was more effective than melon or tomato. Chicken manure did not affect egg numbers at this temperature, but at 30 °C it reduced egg numbers where no plant residue had been added. The degree of reduction at this temperature resulting from adding only chicken manure was similar to the one resulting from adding only broccoli, melon, or tomato residue (Fig. 4A–C).

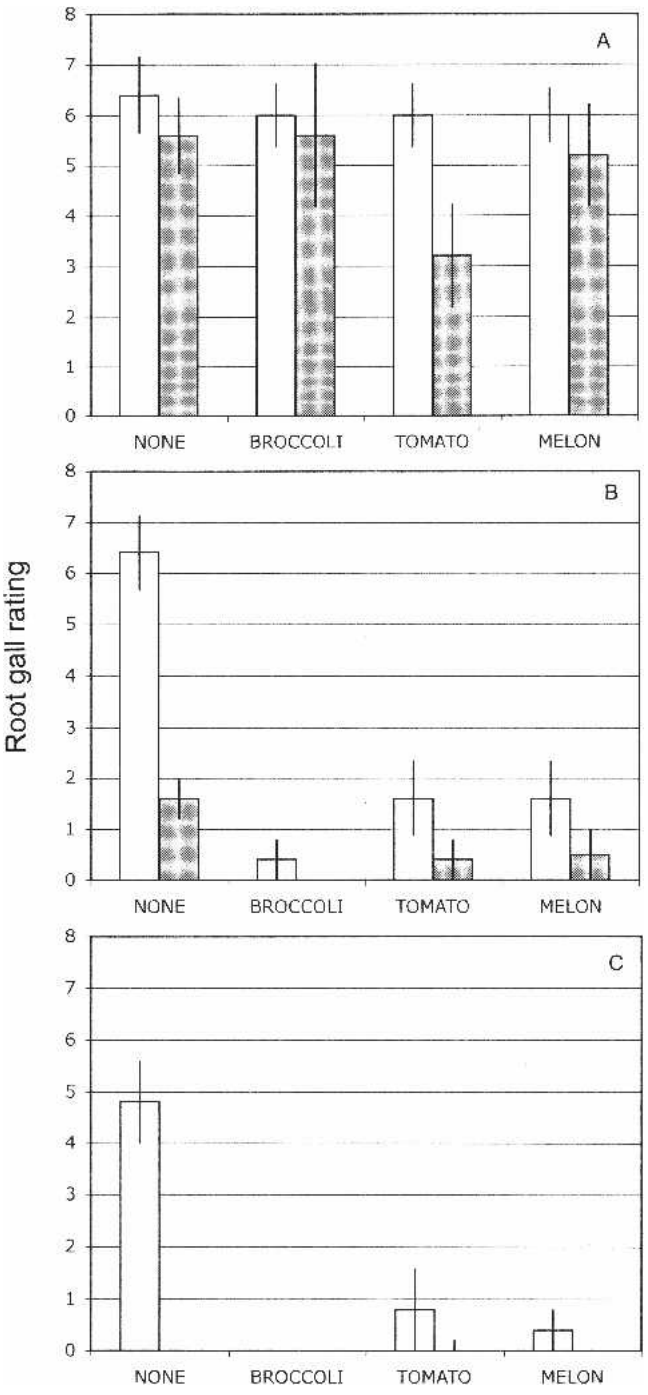

FIG. 3. Root gall rating of tomato grown in biofumigated, *Meloidogyne incognita*-infested soils in experiment 2. Biofumigants were fresh residue of broccoli, tomato, and melon. White bars represent soils without addition of chicken manure; dark bars represent soils with addition of chicken manure. Vertical bars represent 2× standard error. A) Biofumigation at 20 °C. B) Biofumigation at 25 °C; biofumigation at 30 °C.

DISCUSSION

Adding chicken manure to the soil increased the tomato shoot weights at all three temperatures in both experiments. Most likely this can be attributed to the increased availability of nitrogen from the chicken manure. The effects of amending soils with the different plant residues on the shoot weights were generally inconsistent although the broccoli amendment in the first experiment was slightly phytotoxic. Phytotoxic effects of the breakdown products of glucosinolates, compounds present in many brassica crops, were earlier reported by Bialy et al. (1990) and Brown and Morra (1996).

Amending *M. incognita*-infested soils with residue of broccoli, tomato, or melon plants reduced galling and *M. incognita* infestation of subsequently grown tomato at soil temperatures of 25 and 30, but not at 20 °C. This corresponds with earlier results by Ploeg and Stapleton (2001) and with recommendations by Bello et al. (2004). Of the three plant residues tested, amending with broccoli resulted in a lower *M. incognita* infestation than amending with melon or tomato at 25 °C. At 30 °C the three plant residues were equally effective. Adding chicken manure to *M. incognita*-infested soils without plant residues at 30 °C also reduced galling and *M. incognita* infestation of subsequently grown tomato, and at this temperature was as effective as using plant residues. When soil temperatures were lower, chicken manure was effective only in the first experiment. Where

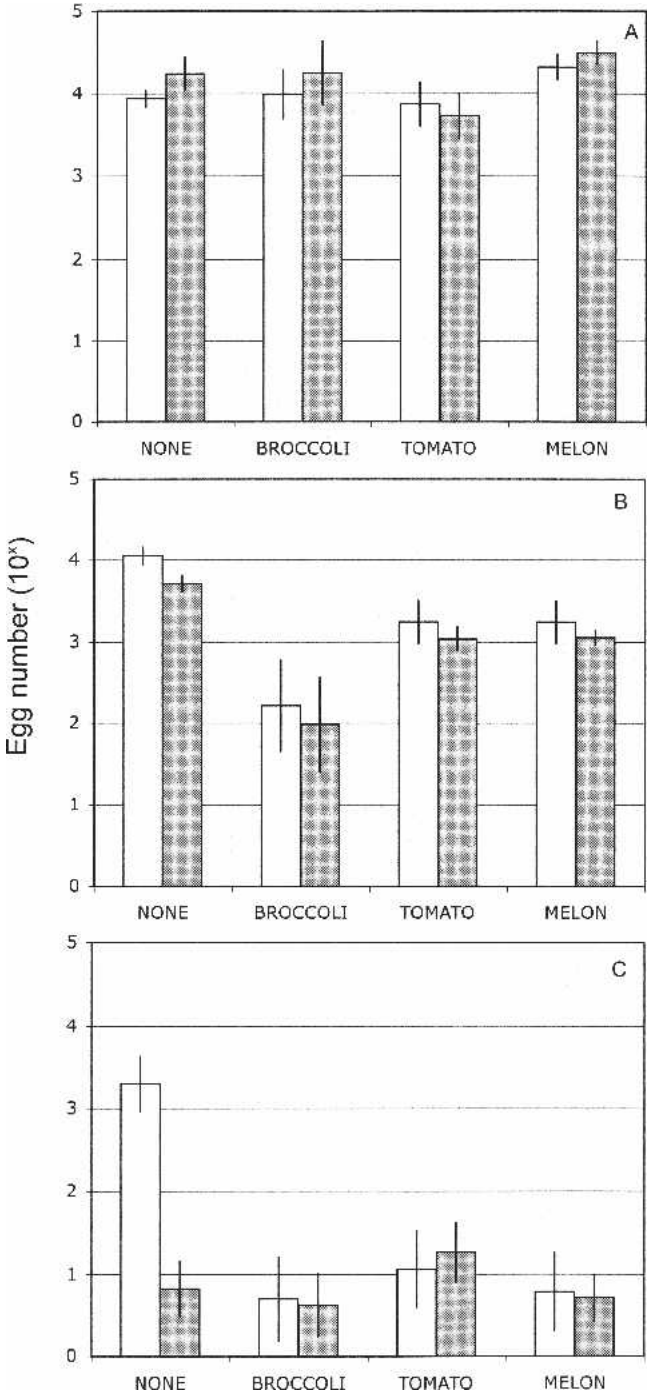

FIG. 4. Eggs extracted from tomato roots grown in biofumigated, *Meloidogyne incognita*-infested soils in experiment 2. Biofumigants were fresh residue of broccoli, tomato, and melon. White bars represent soils without addition of chicken manure; dark bars represent soils with addition of chicken manure. Vertical bars represent 2× standard error. A) Biofumigation at 20 °C. B) biofumigation at 25 °C; biofumigation at 30 °C.

the effect of amending soils with plant residue was significant, chicken manure rarely resulted in an additional reduction in galling and *M. incognita* infestation (only in melon and tomato-amended soils, 25 °C, experiment 1).

It can be concluded that under cool conditions (soil temperature approximately 20 °C) biofumigation to control *M. incognita* is unlikely to be effective. When soil temperatures are around 25 °C, amending with broccoli is likely to be more effective than amending with tomato or melon. This suggests that, at this temperature, breakdown of glucosinolates, present in broccoli but not in melon or tomato, is important in nematode suppression. At this temperature, applying a mix of plant residue and chicken manure may increase efficacy. When soil temperatures of 30 °C can be achieved, biofumigation can reduce *M. incognita* infestation to low levels and its efficacy is less likely to depend on the type of biofumigant material. At this temperature, chicken manure as well as residues of broccoli, tomato, and melon reduced *M. incognita* infestation of subsequently grown tomato to very low levels. This suggests that mechanisms other than the breakdown of glucosinolates, reported to be the main mode of action of brassica crops (Angus et al., 1994; Kirkegaard and Sarwar, 1998), also may be important. The successful control of root-knot nematodes by amending soil with chicken manure has been well documented (Chindo and Khan, 1990; Kaplan and Noe, 1993; Riegel et al., 1996). The mode of action of chicken manure is thought to be based on the release of toxic levels of ammonium, although alterations in soil structure, the stimulation of antagonistic organisms, and improved plant tolerance also may play a role (Lazarovits et al., 2001). The creation of an anaerobic environment underneath plastic-covered soil during decomposition of organic matter also was found to be an important event in the control of soil-borne fungal pathogens (Blok et al., 2000).

In conclusion, although biofumigation often results in satisfactory levels of nematode, fungi, insect, or weed control, the underlying mechanisms responsible for control are still largely unknown but appear to be temperature dependent. In spite of this, biofumigation appears to be a very promising technique that could easily be integrated with other weed, pest, or pathogen management strategies such as crop rotation, cover cropping, and use of resistant varieties. In addition, it may offer alternative uses for some agricultural byproducts.

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