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Nematicidal Activity of Fatty Acid Esters on Soybean Cyst and Root-knot Nematodes[1]

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Abstract: Researchers have indicated that the C_9 fatty acid, pelargonic acid (nonanoic acid), has considerable nematicidal activity that could be increased by derivitization and improved emulsification. Microemulsions of methyl and ethylene glycol esters of pelargonic acid developed by Mycogen Corporation (San Diego, CA) were tested for nematicidal activity against root-knot and soybean cyst nematodes. All treatments were compared to a deionized water control and a microemulsion "blank" (minus active ingredient). Methyl pelargonate reduced gall numbers at concentrations ≥0.8 µl a.i./liter, and ethylene glycol pelargonate reduced gall numbers at ≥6.4 µl a.i./liter in a laboratory bioassay of *Meloidogyne javanica* on roots of tomato seedlings. Microscopic observation of treated *M. javanica* second-stage juveniles suggested that methyl pelargonate was toxic to nematodes at concentrations as low as 0.2 µl a.i./liter. Cysts of *Heterodera glycines* per gram of root were significantly reduced by weekly soil drenches of methyl pelargonate at 6.4, 3.2, and 1.6 µl a.i./liter compared to controls in one greenhouse experiment. Weekly soil drenches of methyl pelargonate at 4.8 or 3.2 µl a.i./liter also significantly reduced the number of eggs produced by *M. incognita* on soybean in a greenhouse test. In both greenhouse tests with soybean, rates of methyl pelargonate ≥4.8 µl a.i./liter had considerable phytotoxicity. No significant interaction of chemical treatment and different soil mixtures affected the nematode numbers produced or plant vigor observed. Soil drenches with microemulsions of methyl pelargonate at 3.2 µl a.i./liter applied weekly, or as two initial applications, were effective as a nematicide for root-knot and soybean cyst nematodes with negligible effects on plant vigor.

Key words: fatty acid, *Glycine max, Heterodera glycines, Meloidogyne incognita, Meloidogyne javanica,* nematicide, nonanoic acid, pelargonic acid methyl ester, pelargonic acid ethylene glycol ester, phytotoxicity, root-knot nematode, soybean cyst nematode.

Nematicides are an effective means to reduce field populations of plant-parasitic nematodes below economic crop damage thresholds (Heald, 1987; Johnson and Feldmesser, 1987). The common types of nematicides include fumigants that are halogenated aliphatic hydrocarbons or methyl isothiocyanate generators, and nonfumigants that are organophosphates and carbamates (Heald, 1987; Johnson and Feldmesser, 1987). The inherent toxicity of these compounds to humans and wildlife, and nematicide detection in groundwater, have led to suspension or severe restriction in use of many of the most effective nematicides, especially the soil fumigants (Thomason, 1987). The effective multi-purpose soil fumigant, methyl bromide, is scheduled to be removed from U.S. production and importation by 2001 (Thomas, 1996). The net result of these actions is that fewer nematicide options are now available to growers and many are often at an increased cost.

Renewed emphasis has been placed on the development and integration of nematode management tactics including modifications of cultural practices, crop rotations, resistant cultivars, and sustainable biological strategies. Recent research on nematicides includes the design of alternative formulations or application methods for existing nematicides, as well as the development of nematicides with alternative modes of action (Noling and Becker, 1994; Thomason, 1987). Some fatty acids and their derivatives have been reported to have nematicidal activity (Djian et al., 1994; Sayre et al., 1965; Tarjan and Cheo, 1956). Tarjan and Cheo (1956) reported that straight-chain fatty acid radicals of intermediate carbon-chain length (C_8–C_{10}) had the highest nematicidal activity of the fatty acids tested, and that better emulsions would improve nema-

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ticidal activity. Djian et al. (1994) have indicated that the nematicidal activity of dicarboxylic fatty acids could be improved by esterification of these compounds. Improved microemulsions of derivatives of the C_9 fatty acid, pelargonic acid (nonanoic acid), have recently been developed (Kim et al., 1997). The objective of this study was to test the nematicidal activity of microemulsions of the methyl and ethylene glycol esters of pelargonic acid (Fig. 1) on root-knot and soybean cyst nematodes.

Materials and Methods

Laboratory bioassay: A *Meloidogyne* spp. root-galling laboratory bioassay (Davis and Rich, 1987) was used to establish an effective range of concentrations of the active ingredients, ethylene glycol pelargonate and methyl pelargonate, that were received as microemulsions from Mycogen Corporation (San Diego, CA). Eggs of *Meloidogyne javanica* were extracted from roots of greenhouse cultures on tomato (*Lycopersicon esculentum*) using NaOCl (Hussey and Barker, 1973), and the eggs were suspended on Baermann pans to hatch at 27 °C. Second-stage juveniles (J2) were collected within 24 hours of hatching and incubated in either methyl pelargonate or ethylene glycol pelargonate at concentrations of 100, 50, 25, 12.5, 6.4, 3.2, 1.6, 0.8, 0.4, or 0.2 µl a.i./liter. Deionized water and a 6.4-µl/liter "blank" of the microemulsion (minus active ingredient) were included as treatment controls in all experiments. After 4 hours in treatment solution at room temperature, an aliquot containing approximately 500 J2 suspended in treatment solution was added as a soil drench to sand supporting a 10-cm-tall tomato seedling in a 14.8-ml (4-dram) glass vial. The remaining J2 in treatment solution were immediately observed under a dissecting microscope. Nematode movement and the development of vacuoles within nematodes were used as a measure of nematode viability, and treated nematodes were transferred to deionized water and observed after 24 hours to assess recovery from pelargonate treatment. Deionized water was added daily to the sand supporting each inoculated tomato seedling to approximate field capacity. At 10 days after inoculation with treated J2, the sand was washed from the tomato seedling roots and the numbers of galls induced by treated *M. javanica* were observed and recorded. The single experiment contained three replicates of each treatment.

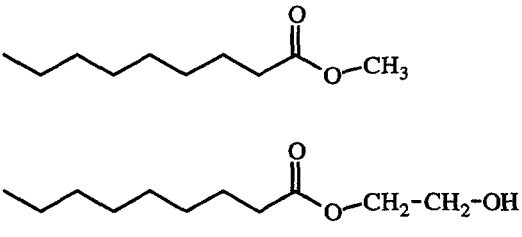

FIG. 1. Chemical structures of esters of the fatty acid, pelargonic acid (nonanoic acid). Above: Methyl pelargonate. Below: Ethylene glycol pelargonate.

Greenhouse assays: Two greenhouse assays were conducted to assess the effect of the methyl pelargonate microemulsion on nematodes infecting soybean (*Glycine max*). Several different soil mixtures and schedules of soil drench were included to assay rates of methyl pelargonate, which were selected based upon the results of the laboratory bioassay above. In the first greenhouse test, single plants of Lee 68 soybean were germinated and grown in 15-cm-diam. clay pots for 2 weeks before concomitant chemical treatment and infestation of soil at the base of each plant with about 10,000 eggs of race 1 of the soybean cyst nematode (SCN), *Heterodera glycines*. Eggs of SCN were obtained by gently crushing cysts that were extracted from greenhouse cultures (Davis et al., 1996). Different ratios of sand and field soil (loamy sand: 88.9% sand, 8.3% silt, 2.8% clay) were mixed, steam-pasteurized, and used as potting media to assess the effects of different soil textures on the activity of the test compound. For each concentration of methyl pelargonate, three sand:field soil mixtures (1:3, 1:1, 3:1) were tested, and four replicates of each treatment combination were included. The methyl pelargonate emulsion was added at concentrations of 12.5, 6.4, 3.2, 1.6, 0.8 µl a.i./liter as a soil

drench immediately before eggs of SCN were added to soil; identical soil drenches of the chemical were made at weekly intervals for the next 7 weeks. Weekly "water" and 6.4-µl/liter microemulsion blank treatments were included as controls. The 12.5-µl a.i./liter rate of methyl pelargonate also was evaluated with just two initial applications that were 1 week apart. Soybean plants were observed for vigor throughout the course of the experiment. At 8 weeks after inoculation with SCN and the first chemical treatment, the plants were harvested, cysts were extracted from soil and roots and counted, roots and shoots were weighed, and the percentage of necrosis of the root system was estimated.

The second greenhouse test was conducted like the first, except that the effect of methyl pelargonate on the root-knot nematode, *Meloidogyne incognita* race 4 (Mi4), was evaluated on Lee 68 soybean. The same sand:field soil mixtures were used, and three replicates of each treatment were included. Methyl pelargonate treatments were added as soil drenches to each pot containing a 2-week-old soybean seedling. Eggs of Mi4 were extracted from greenhouse cultures as described above, and about 10,000 eggs were added to the soil in each pot immediately after the first methyl pelargonate drench. The methyl pelargonate microemulsions, 4.8, 3.2, and 1.6 µl a.i./liter, and the water control were added as weekly soil drenches until 2 weeks before the termination of the experiment. In addition, separate treatments of the methyl pelargonate microemulsions, 6.4, 4.8, and 3.2 µl a.i./liter, and the 6.4-µl/liter microemulsion blank were added as soil drenches only at inoculation and 1 week later. The soybean plants were observed for vigor throughout the course of the experiment. At 12 weeks after inoculation with Mi4 and the first chemical treatment, the plants were harvested, the roots and shoots were weighed, and the percentage of galling and necrosis of each root system were estimated. Eggs of Mi4 were extracted with NaOCl from a random 5-g sample from each root system and used to calculate eggs per gram of root and total Mi4 eggs per root system.

All tests were arranged in a randomized complete-block design with methyl pelargonate rate-schedule and soil mixture as the main treatments. Data from the greenhouse test with Mi4 were transformed with $\log_{10}(x + 1)$ before analysis to standardize the variance, but nontransformed data were used for statistical analyses of the laboratory bioassay and the greenhouse test with SCN. All data were subjected to analysis of variance with the SAS General Linear Models procedure (SAS Institute, Cary, NC), and interaction main effects were analyzed for all dependent variables presented above. Treatment means were compared with the Tukey-Kramer HSD ($P = 0.05$).

Results

Laboratory bioassay: Both ethylene glycol pelargonate and methyl pelargonate reduced galling of tomato roots induced by *M. javanica* when compared to the controls ($P = 0.05$). Methyl pelargonate was found to reduce gall numbers to concentrations as low as 0.8 µl a.i./liter, and ethylene glycol pelargonate strongly reduced gall numbers down to concentrations of 6.4 µl a.i./liter (Table 1). Microscopic observation of treated *M. javanica* J2 suggested that methyl pelargonate was toxic to J2 at concentrations as low as 0.2 µl a.i./liter. Methyl pelargonate-treated J2 were S-shaped and vacuolated, and the lack of spontaneous movement when placed in deionized water for 24 hours suggested that they were nonviable. Similar observations of J2 viability were made with ethylene glycol pelargonate at concentrations as low as 1.6 µl a.i./liter. At concentrations of ethylene glycol pelargonate of 0.8 µl a.i./liter and lower, the nematodes were viable, but the majority of J2 were vacuolated at the 0.8- and 0.4-µl a.i./liter concentrations. Tomato seedlings were killed at the highest three concentrations of either chemical treatment, so data on root galling were not available for those treatments. Greater than 50% of the tomato leaf area was chlorotic at con-

TABLE 1. Effect of microemulsions of ethylene glycol pelargonate and methyl pelargonate on *Meloidogyne javanica,* subsequent galling of tomato seedling roots, and phytotoxicity in a laboratory bioassay.

Concentration (µl a.i./liter)	Ethylene glycol pelargonate			Methyl pelargonate		
	Nematode[a]	Plant[b]	Galls[c]	Nematode	Plant	Galls
100	D	+++	—	D	+++	—
50	D	+++	—	D	+++	—
25	D	+++	—	D	+++	—
12.5	D	++	1.0 a	D	++	0.0 a
6.4	D	++	1.7 a	D	++	0.3 a
3.2	D	+	5.3 ab	D	+	0.0 a
1.6	D	ND	10.3 bcd	D	ND	1.0 a
0.8	LV	ND	10.3 bcd	D	ND	0.3 a
0.4	LV	ND	13.3 d	D	ND	6.3 ab
0.2	L	ND	6.0 abc	D	ND	10.0 b
Blank[d]	L	ND	12.0 cd	L	ND	12.0 b
Water	L	ND	14.6 d	L	ND	14.6 b

[a] Microscopic observation of second-stage juveniles (J2) of *M. javanica* after 4-hour incubation in test solution: D = dead; LV = living, but the majority of J2 vacuolated; L = J2 living and apparently unaffected by treatment.

[b] Relative phytotoxicity of treatment based upon observation of tomato seedlings: +++ = plants dead; ++ greater than 50% of leaf area chlorotic, plants stunted; + = less than 50% of leaf area chlorotic, no stunting. ND = no phytotoxicity detected.

[c] Mean number of galls per seedling root system from three replicates. Dashes indicate absence of values due to phytotoxicity. Column means followed by the same letter are not significantly different according to the Tukey-Kramer HSD ($P = 0.05$).

[d] The same microemulsion blank (minus active ingredient) treatment (at 6.4 µl/liter) and water control were used for comparisons in both treatments.

centrations of either compound at 12.5 and 6.4 µl a.i./liter, and phytotoxicity was minimal or not detectable at concentrations of either chemical at 3.2 µl a.i./liter and below.

Greenhouse assays: No interaction effects of the chemical treatments and sand:field soil mixtures on cyst numbers or plant vigor were obtained. Therefore, data were combined and analyzed by chemical treatment. The total number of SCN cysts per root system ranged from 1,933 to 18 cysts for the microemulsion blank and 12.5 µl a.i./liter methyl pelargonate treatments, respectively. Considerable differences in soybean root mass among the treatments were observed, however, so data on cyst numbers are presented as cysts per gram of root (Fig. 2). Weekly soil drenches of methyl pelargonate at 1.6 µl a.i./liter or greater reduced the number of cysts per gram of root when compared to controls ($P = 0.05$). Cyst numbers per gram of root were greatly reduced by two applications of methyl pelargonate at a concentration of 12.5 µl a.i./liter and weekly applications of this compound at 6.4 and 3.2 µl a.i./liter. Plant vigor (root and shoot weights) was reduced by methyl pelargonate

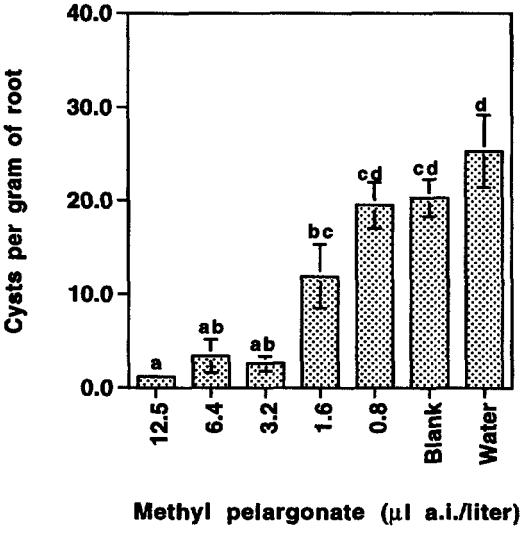

FIG. 2. Effect of 7 weekly soil drenches with a microemulsion of methyl pelargonate at concentrations of 6.4, 3.2, 1.6, and 0.8 µl a.i./liter, and a 12.5-µl a.i./liter rate applied twice, on cyst production by *Heterodera glycines* on Lee 68 soybean. The blank represents the microemulsion at 6.4 µl/liter minus active ingredient. Bars represent the mean and standard error of four replications of each treatment. Treatments designated with the same letter are not statistically different according to the Tukey-Kramer HSD ($P = 0.05$).

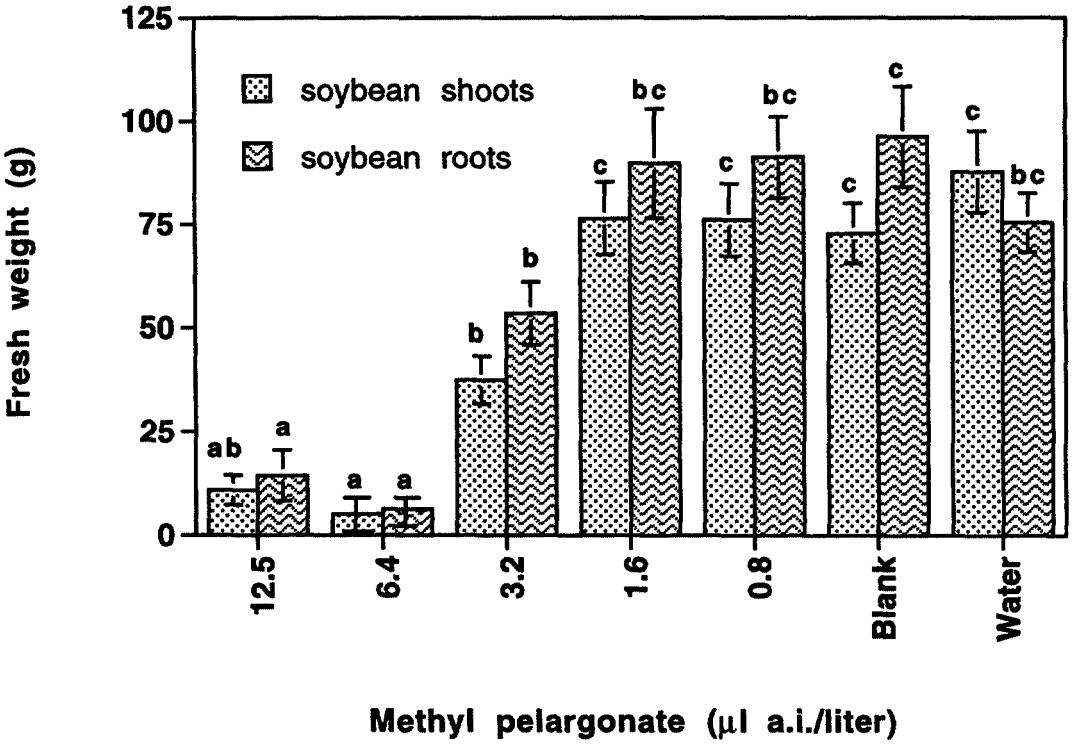

FIG. 3. Effect of 7 weekly soil drenches with a microemulsion of methyl pelargonate at concentrations of 6.4, 3.2, 1.6, and 0.8 µl a.i./liter, and a 12.5-µl a.i./liter rate applied twice, on root and shoot development of Lee 68 soybean plants inoculated with *Heterodera glycines*. The blank represents the microemulsion at 6.4 µl/liter minus active ingredient. Bars represent the mean and standard error of four replications of each treatment. Treatments designated with the same letter compared among shoot or root weights are not statistically different according to the Tukey-Kramer HSD ($P = 0.05$).

treatment ($P \leq 0.01$), and the microemulsion blank had minimal effects on plant vigor (Fig. 3). No significant treatment effects on root necrosis were obtained. Soybean plants were almost killed by the two soil applications of methyl pelargonate at 12.5 µl a.i./liter, and they were >75% stunted and chlorotic after weekly treatments of 6.4 µl a.i./liter. Plants were only about 10% stunted with minimal chlorosis after weekly soil drenches with methyl pelargonate at 3.2 µl a.i./liter. No phytotoxicity to soybean plants was observed in weekly methyl pelargonate treatments of 1.6 and 0.8 µl a.i./liter, or in the blank and water controls.

Weekly applications of methyl pelargonate at 4.8 and 3.2 µl a.i./liter also reduced the number of eggs produced by *M. incognita* on soybean ($P = 0.05$) (Fig. 4). No interaction effects of the chemical treatments and the sand:soil mixtures on egg numbers or plant vigor were obtained, so data were combined and analyzed by chemical treatment. Egg production by *M. incognita* on soybean was low in all treatments (including controls) and did not significantly correlate with root galling or root necrosis. Two soil applications of methyl pelargonate at either 6.4, 4.8, or 3.2 µl a.i./liter also reduced egg numbers below those of the controls, although not significantly. Soybean plants were about 30% stunted with about 50% of the leaf area chlorotic after weekly treatment with methyl pelargonate at 4.8 µl a.i./liter, but these symptoms were barely detectable on plants treated weekly with this compound at 3.2 µl a.i./liter. Soybean plants initially were stunted and chlorotic after two soil applications of methyl pelargonate at

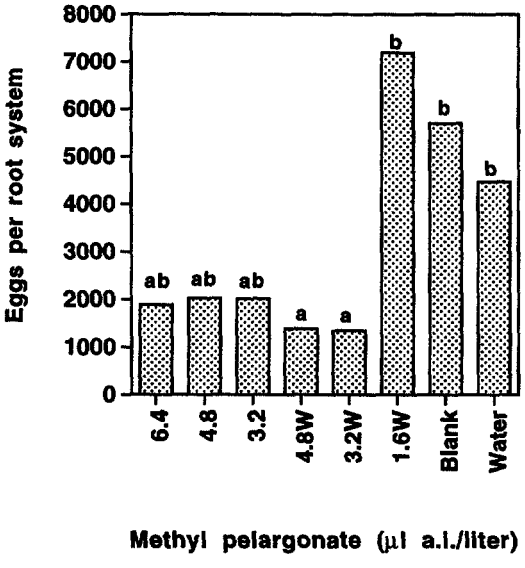

FIG. 4. Effect of two soil drenches with a microemulsion of methyl pelargonate at concentrations of 6.4, 4.8, and 3.2 µl a.i./liter, and 4.8-, 3.2-, and 1.6-µl a.i./liter rates applied as 7 weekly (W) soil drenches in separate treatments, on egg production by *Meloidogyne incognita* on Lee 68 soybean. The blank represents the microemulsion at 6.4 µl/liter minus active ingredient. Bars represent the mean of three replications from each treatment, and data were transformed with $\log_{10}(x+1)$ before analysis to standardize the variance. Treatments designated with the same letter are not statistically different according to the Tukey-Kramer HSD ($P = 0.05$).

6.4 and 4.8 µl a.i./liter, but these plants recovered after chemical treatments were terminated, resulting in only about 10% stunting of soybean plants by the end of the experiment. No significant effects of methyl pelargonate treatment on soybean shoot or root weights were observed in this test.

DISCUSSION

Microemulsions of the C_9 fatty acid esters methyl pelargonate and ethylene glycol pelargonate were inhibitory to both root-knot and soybean cyst nematodes in these experiments. This corroborates the findings of Tarjan and Cheo (1956), who reported that complete control of cyst nematodes could be achieved with a 4.0% formulation of pelargonic acid radical. In the bioassay reported here, concentrations of pelargonate as low as 0.8 µl a.i./liter were able to significantly reduce root galling when compared to controls. The improved microemulsion combined with esterification of pelargonic acid may explain the increased activity of pelargonic acid compared with previous reports. Methyl pelargonate was more effective in reducing tomato root galling by *M. javanica* in laboratory bioassays compared to ethylene glycol pelargonate. It has been suggested by Djian et al. (1994) that the physical properties of methyl esters of fatty acids as compared to ethyl esters promote increased permeation into nematodes with a resultant increase in toxicity. It is unusual that *M. javanica* J2 that were incubated in methyl pelargonate appeared S-shaped, rather than straight, when killed. This may be due to a potential rapid inactivation of the nematodes when immersed in pelargonic acid, as Tarjan and Cheo (1956) observed. Time-course observations using the test compounds presented here will be necessary to confirm this hypothesis. While the mode of action of methyl pelargonate is unknown, its physical properties might promote some type of detergent (solubilization) effect that may adversely interfere with the integrity and permeability of the nematode cuticle or hypodermis. Jalal and Read (1983) suggested that the inhibitory effects of fatty acids of intermediate chain length that have been observed in biological systems may involve a direct interaction between the fatty acids and lipophilic regions of the target plasma membranes. A few J2 in the lowest concentrations of methyl pelargonate were able to induce tomato root galls even though they appeared to be nonviable (as observed after a 24-hour recovery period in water). Perhaps methyl pelargonate is nematostatic rather than nematicidal, but more definitive tests of nematode viability should be conducted to determine concentrations that kill nematodes.

The effects of methyl pelargonate on nematodes and plants in the bioassay were corroborated in greenhouse assays. It was considered that soils of different textures may influence the activity of methyl pelargonate since the movement of this aliphatic compound in sandy soils might be limited, but no effects of different soil mixtures were observed. This does not preclude the possi-

bility, however, that soils different than those used here will not affect the activity of this test compound. It also must be considered that pasteurized soils were used in these greenhouse tests and, thus, the potential effects of soil microbes on the integrity and activity of this compound remain in question. Weekly soil drenches of methyl pelargonate at concentrations as low as 3.2 µl a.i./liter significantly reduced the number of cysts per gram of root compared to controls. This same treatment reduced egg production by *M. incognita* on soybean. Weekly applications above 3.2 µl a.i./liter did not have a much greater effect on reducing SCN or root-knot nematode populations in either greenhouse test. The same degree of reduction in nematode numbers also may be achieved, however, by more limited applications of methyl pelargonate. Two applications of methyl pelargonate at 3.2 µl a.i./liter at the time of inoculation and 1 week later were almost as effective in reducing the number of root-knot nematode eggs as the weekly soil drenches.

The efficacy of methyl pelargonate in reducing cyst and root-knot nematode numbers must be balanced with this compound's potential adverse effects on plant vigor. Concentrations above 12.5 µl a.i./liter were phytotoxic to tomato plants grown in sand in the bioassay. It has been reported that derivatives of fatty acids also have phytotoxic and fungitoxic effects, and that these effects may be manifested in disruption of plasma membrane activity (Jalal and Read, 1983). In another report, nonanoic acid was used to increase the activity of the herbicide glyphosate (Arnold et al., 1993). Phytotoxic effects may differ depending upon soil type, but this was not apparent from the phytotoxicity data observed in greenhouse tests with soybean plants grown in different soil mixtures. In both greenhouse tests with soybean, rates of methyl pelargonate of 4.8 µl a.i./liter or higher had significant adverse effects on plant vigor. Concentrations of methyl pelargonate at 3.2 µl a.i./liter had little adverse effect on tomato or soybean plant vigor, especially when only two applications of the compound were administered.

Pelargonic acid esters have potential as effective nematicides. These fatty acid derivatives are relatively inexpensive to produce, they have low mammalian toxicity, and their chemistry suggests that they may be degraded by soil microbes and pose minimal environmental threat. Care should be taken to assess the application method and schedule that maintain optimal nematicidal activity with minimal effects on plant vigor. In these studies, methyl pelargonate at 3.2 µl a.i./liter was effective as a nematicide for root-knot and soybean cyst nematodes with negligible effects on plant vigor.

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