MANAGEMENT OF *MELOIDOGYNE INCOGNITA* ON COTTON BY USE OF BOTANICAL AROMATIC COMPOUNDS

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ABSTRACT

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The efficacy of benzaldehyde (benzoic aldehyde), citral (3,7-dimethyl-2,6-octadienal), furfural (2-furaldehyde), menthol (5-methyl-2-(1-methylethyl)cyclohexanol), and α–terpineol (α, α, 4-trimethyl-3-cyclohexene-1-methanol) for management of *Meloidogyne incognita* was tested on 'Deltapine 50' cotton (*Gossipium hirsutum*) in a greenhouse experiment. Mixing these compounds into a sandy loam soil-sand mixture at rates ranging from 0.1 to 0.5 ml/kg reduced the number of *M. incognita* juveniles in cotton roots and soil. The number of galls/g root tissue was reduced, and the compounds had no adverse effects on cotton growth. The percentage of root colonized by vesicular-arbuscular mycorrhizae (VAM) was decreased with increasing concentrations of compounds. Citral and menthol significantly increased plant height at all rates of application. Benzaldehyde, citral, and furfural were tested in field microplots at rates ranging from 0.18 to 2.14 ml/kg soil. In all experiments, the compounds were incorporated into the soil approximately 2 weeks prior to planting. Benzaldehyde, citral, and furfural reduced populations of *M. incognita* at all rates without adversely affecting plant height or yield. Results indicate that these compounds could have practical application in the management of *M. incognita* on cotton and their further evaluation as components in integrated pest management is warranted.

Key words: chemical control, cotton, fumigants, *Meloidogyne incognita*, natural products, nematicides, pest management, root-knot nematode, terpene, volatile compounds.

RESUMEN

Bauske, E. M., R. Rodríguez-Kábana, V. Estaún, J. W. Kloepper, D. G. Robertson, C. F. Weaver and P. S. King. 1994. Manejo de *Meloidogyne incognita* usando compuestos fitoaromáticos en algodón. Nematrópica 24:143-150.

La eficacia de benzaldehido (benzoic aldehyde), citral (3,7-dimethyl-2,6-octadienal), furfural (2-furaldehyde), mentol (5-methyl-2-(1-methylethyl)cyclohexanol), y α-terpineol (α, α, 4-trimethyl-3-cyclohexene-1-methanol) para controlar *M. incognita* en algodón (*Gossypium hirsutum* var. Deltapine 50) fue evaluada a nivel de invernadero. El mezclar estos compuestos con suelo infestado con el nematodo a dosis de 0.1 a 0.5 ml/kg redujo el número de larvas juveniles en el suelo y raíces. Plantas tratadas mostraron menor número de agallas/gramo de raíz. El porcentaje de raíces colonizadas por micorrizas arbusculares disminuyó a medida que se aumentó la concentración de estos compuestos. La aplicación con citral y mentol aumentó significativamente la altura de las plantas. Dosis de benzaldehido, citral, y furfural (0.18 a 2.14 ml/kg suelo) fueron evaluadas en microparecelas de campo. Estos compuestos fueron incorporados al suelo dos semanas antes de la siembra. Las dosis evaluadas disminuyeron poblaciones de *M. incognita* efectivamente sin afectar la altura o el rendimiento de las plantas de algodón. El uso de estos compuestos en un programa de manejo integrado de plagas podría resultar como una medida de control efectiva.

Palabras clave: control químico, algodón, fumigantes, *Meloidogyne incognita*, productos naturales, nematicidas, manejo de plagas, nematodo nodulador, terpeno, compuestos volátiles.

INTRODUCTION

The root-knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood, is considered one of the most destructive pests of cotton (*Gossipium hirsutum*), causing damage alone and in combination with fungi in several disease complexes (15). Current management strategies for *M. incognita* in cotton are based on use of rotations, low levels of genetic resistance, and nematicides (1).

The widespread use of nematicides has resulted in contamination of groundwater and deleterious effects on animal and human health (7,8). As a result of these problems, several of the most efficacious nematicides (aldicarb, ethylene dibromide, and dibromochloropropane) have been particularly or totally removed from use. A recent report that methyl bromide, a broadspectrum soil biocide, is destructive to the earth's ozone layer (14) underscores the need for alternative nematode management methods.

This study was conducted as part of an on-going evaluation of the nematicidal potential of low-molecular weight volatile compounds. Benzaldehyde, citral, furfural, menthol, and α-terpineol are naturally occurring botanical compounds and are used commercially in the production of flavors and perfumes. Benzaldehyde (benzoic aldehyde) occurs in kernels of bitter almonds (*Prunus dulcis*) and is made synthetically from benzal chloride and lime or by oxidation of toluene (2). Citral (3,7-dimenthyl-2,6-octadienal) is a major constituent of oil of lemon grass (*Cymbopogon* spp.) and is used in the synthesis of vitamin A and in perfumery. Furfural (2-furaldehyde) is prepared industrially from cereal straws and brans. It has many industrial uses and has demonstrated effective control of plant-parasitic nematodes on 'Summer Crookneck' squash (*Cucurbita pepo*), soybean (*Glycine max*), and okra (*Hibiscus esculentum*) (11). Menthol (5-methyl-2-(1-methylethyl) cyclohexanol) is obtained from peppermint oil (*Litsea cubeba*) and is used in the production of liqueurs, confectionery, perfumery, and cigarettes. The compound, α-terpineol (α, α, 4-trimethyl-3-cyclohexene-1-methanol), is isolated from oil of long-leaf pine (*Pinus plaustris*) and is used in perfumes and to denature fats for soap manufacture. The objective of this study was to assess the efficacy of benzaldehyde, citral, furfural, menthol, and α-terpineol for of management of *M. incognita* on cotton.

MATERIALS AND METHODS

Greenhouse Experiment: A sandy loam field soil with pH 6.1, organic matter content < 1.0% (w/w), and a cation exchange capacity of < 10 meq/100 g was used in the greenhouse study. The soil was naturally infested with *Meloidogyne incognita* (Kofoid & White) Chitwood, and very low numbers of *Hoplolaimus galeatus* (Cobb) Thorn, *Paratrichodorus minor* Siddiqui, *Pratylenchus brachyurus* (Godfrey) Filipjev & Schuurmans Stekhoven, *P. zeae* Graham, and *Tylenchorhynchus claytoni* Steiner. Soil was screened to < 1 mm diam to remove large particles and debris and was then amended 1:1 (v:v) with fine siliceous sand (diam < 0.1 mm). Technical grade benzaldehyde, citral, furfural, menthol, and α-terpineol were obtained from Aldrige Chemical Company (Milwaukee, WI). Each compound was added to the soil at rates of 0.10, 0.25, and 0.50 ml/kg soil. The appropriate dosage was applied directly to 1 kg soil in 3-L polyethylene bags. After thorough mixing, the soil was transferred immediately to 1-L cylindrical plastic pots. Because menthol is a solid at room temperature, it was gently heated to the melting point (43°C) prior to application. A no-compound control was included in the experiment. The 16 treatments in the experiment were arranged on a greenhouse bench in a randomized complete block design with 8 replications. The soil was maintained moist, and after 11 days, four seeds of 'Deltapine 50' cotton were planted into each pot.

Nine weeks after planting, cotton plants were removed and soil nematode populations were enumerated using the 'salad bowl' method (12). Plant height and the number of galls/g fresh root tissue were determined. The roots from each pot were chopped to approximately 1 cm lengths, and nematodes were extracted and enumerated using the 'salad bowl' method. After extraction, the extent of root colonization by vesicular-arbuscular mycorrhizae (VAM) was determined. Roots were cleared and stained using procedures developed by Phillips and Hayman (9). The percentage of total root length colonized by VAM was estimated using the gridline intersect method (6).

Microplot Experiments: Three microplot experiments were established on the Old Agronomy Farm at the Auburn University campus at Auburn, AL, U.S.A. in May, 1993. Plots in the experiment were 30.5 cm × 30.5 cm and delimited by square terra-cotta chimney flutes embedded in the soil to a depth of 50 cm with 10 cm above ground level as previously described (11). Each microplot had a 15-cm-diam × 30 cm long PVC cylinder placed centrally. In each experiment, treatments consisted of an untreated control and one of the three compounds benzaldehyde, citral, and furfural applied at the following rates: 0.18, 0.35, 0.71, 1.07, 1.43, 1.78, and 2.14 ml/kg soil. As in greenhouse studies, the same sandy loam soil was screened and mixed 1:1 (v:v) with sand prior to application of the compounds. The compounds were applied to the soil and thoroughly incorporated using a cement mixer. Immediately after mixing, the PVC cylinder of the appropriate microplot was filled with treated soil. The cylinder was then removed. Microplots were equipped with a drip irrigation system, and water was applied as needed. After 10 to 14 days, soil samples were collected from each microplot by taking three 2.5-cm-diam soil cores to a depth of 25 cm. Cores were taken approximately 5 cm from each side of the microplot and from the center. The cores from a microplot were composited and a 100 cm^3 subsample was used for nematode analysis by means of the 'salad bowl' method (12). After sampling, 7 'Deltapine 50' cotton seeds were planted into each microplot. Approximately 2 weeks after planting, seedlings were thinned to 5 plants/plot. At harvest (190-200 days after planting), plant height and seed cotton (seed plus lint) yield were measured, and soil samples were collected as previously described.

Statistical Analysis: All data were analyzed following standard procedures for analysis of variance (13). Fisher's least significant differences were calculated when F values were significant. Unless otherwise stated all differences referred to in the text were significant ($P \leq 0.05$).

RESULTS

Greenhouse Experiment: None of the 5 compounds reduced plant height when compared with the nontreated control, and all rates of citral and menthol increased plant height (Table 1). Benzaldehyde at 0.25 ml/kg soil and furfural at 0.10 ml/kg soil increased plant height. VAM colonization (*Glomus* spp.) was reduced with increasing rates of all compounds. Root colonization was reduced by all rates of benzaldehyde and furfural, the two highest rates of citral and menthol, and the highest rate of α-terpineol. All compounds and rates, with the exception of benzaldehyde at 0.10 ml/kg soil, reduced the number of galls/g root.

The number of *M. incognita* juveniles in soil and roots was reduced by the compounds at all rates (Fig. 1). In all cases, application rates of 0.25 ml/kg soil were as effective as the 0.50 ml/kg dosage. The response of *M. incognita* juveniles was similar to that observed for the number of root galls/g root tissue (Table 1). Populations of nonparasitic nematodes were consistently higher in response to all rates of citral and menthol (Fig. 2). Populations of other genera of parasitic nematodes in all plots were negligible (< 20 nematodes/100 cm^3 soil).

Microplot Experiments: Preplant soil samples contained negligible numbers of parasitic nematodes (0-10 nematode/100 cm^3). At harvest, *M. incognita* populations in control treatments were 26.4, 242.6, and 71.6 juveniles/cm^3 soil in the furfural, citral, and benzaldehyde experiments, respectively (Tables 2, 3, and 4). Virtually no *M. incognita* juveniles were found in plots treated with all 3 rates of each the compounds. Populations of other genera of parasitic nematodes in all plots were negligible (< 20 nematodes/100 cm^3 soil).

Furfural had little effect on the number of nonparasitic nematodes in soil samples and had no adverse effects on plant height or seed cotton yield (Table 2). Application of the highest rate, 2.14 ml/kg soil, increased plant height compared to plants in nontreated control plots.

Application of citral at a rate of 0.18 ml/kg soil increased plant height from 64.7 cm to 71.9 cm (Table 3), and the highest rate (2.14 ml/kg soil) increased total yield from 82.7 g to 113.2 g seed cotton/microplot. With one exception of the 0.71 ml/kg soil rate, application of citral had no effect on the number of nonparasitic nematodes in the soil.

Seed cotton yield was unaffected by application of benzaldehyde (Table 4). Plant height increased in response to the highest level of application (2.14 ml/kg soil). However, when the LSD was calculated at $P \leq 0.10$ all rates greater than 1.07 ml/kg soil increased plant height. All rates but the 2.14 ml/kg soil rate of benzaldehyde significantly reduced populations of nonparasitic nematodes.

DISCUSSION

Benzaldehyde, citral, furfural, menthol, and α-terpineol provided effective control of *M. incognita* without adversely affecting cotton growth. Maximum control of *M. incognita* with all compounds under greenhouse conditions was achieved at 0.25 ml/kg soil, suggesting that effective rates may be between 0.10 and 0.25 ml/kg. Maximum control in microplot studies with benzaldehyde, citral, and furfural was achieved at a rate of 0.18 ml/kg soil. These results suggest that rates lower that 0.18 ml/kg soil should be tested in further field studies.

Two compounds tested in the greenhouse study were not included in microplot studies. Though α-terpineol

Table 1. Effects of aromatic compounds applied under greenhouse conditions on 'Deltapine 50' cotton height, root colonization by VAM (*Glomus* spp.), and the number of root galls caused by *M. incognita*.

Treatment (ml/kg soil)	Plant height (cm)	VAM root colonization[z]	Galls/g root
Control			
0	15.7	0.589	21.1
Benzaldehyde			
0.10	16.8	0.449*	18.0
0.25	17.2**	0.271**	9.3**
0.50	16.8	0.304**	0.3**
Citral			
0.10	18.2**	0.616	0.9**
0.25	17.2**	0.404**	0.0**
0.50	17.1*	0.245**	0.0**
Furfural			
0.10	17.0*	0.345**	8.8**
0.25	15.7	0.309**	1.2**
0.50	16.3	0.216**	0.2**
Menthol			
0.10	17.7**	0.479	4.1**
0.25	18.4**	0.292**	0.0**
0.50	18.1**	0.141**	0.0**
α-terpineol			
0.10	16.5	0.626	12.2**
0.25	16.8	0.521	11.7**
0.50	16.8	0.466*	8.6**
$FLSD_{0.05}$	1.2	0.115	5.0
$FLSD_{0.01}$	1.5	0.155	6.6

[z]Arcsin transformed percentages of root colonized by vesicular-arbuscular mycorrhizae.
*, ** = Significantly different from the control at $P \leq 0.05$ and $P \leq 0.01$, respectively.

reduced *M. incognita* populations in greenhouse tests, it was the least effective compound at controlling soil populations (Fig. 1). Menthol has a relatively high melting point (43°C) and must be heated to apply in liquid form (2). This physical characteristic would make it difficult to apply with conventional spray equipment; therefore, it was not included in microplot studies.

All compounds tested in the greenhouse study adversely affected VAM colonization of cotton roots, and these compounds may affect other soil fungi as well. The fungicidal properties of furfural have been studied by Flor (5) and Raeder *et al.* (10), who reported limited control of *Rhizoctonia solani* in potato (*Solanum tubersum*). Canullo *et al.* (4) demonstrated con-

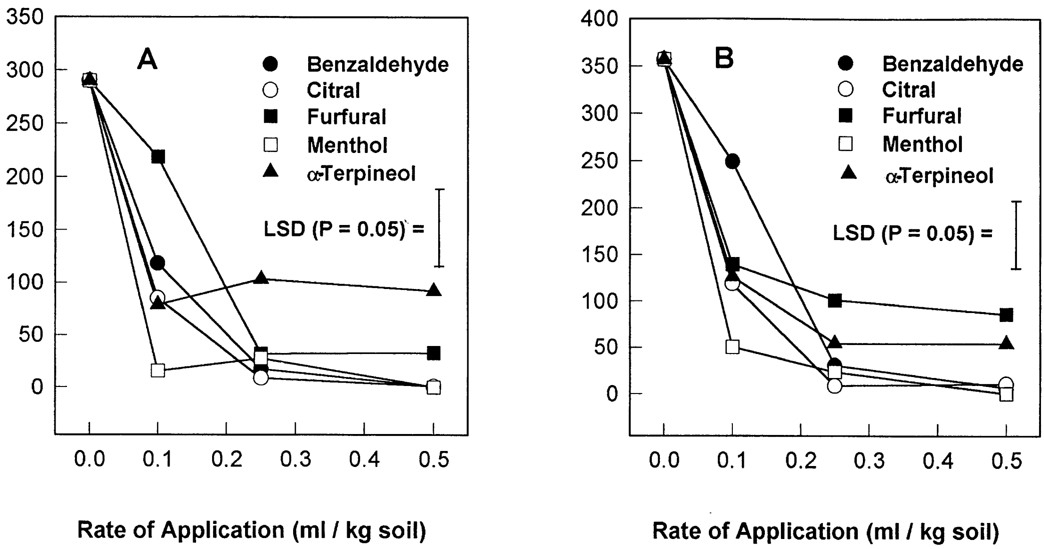

Figure 1. Effects of soil application of benzaldehyde, citral, furfural, menthol, and α-terpineol on populations of *M. incognita* juveniles extracted A) from 100 cm^3 soil and B) from the root mass of 'Deltapine 50' cotton nine weeks after planting in a greenhouse experiment.

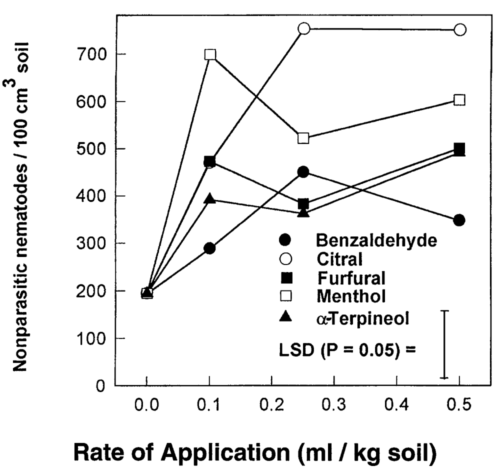

Figure 2. Effects of soil application of benzaldehyde, citral, furfural, menthol, and α-terpineol on populations of nonparasitic nematodes extracted from 100 cm^3 soil nine weeks after planting 'Deltapine 50' cotton in a greenhouse experiment.

trol of southern blight caused by *Sclerotium rolfsii* in lentil (*Lens culinaris*). Population densities of *Trichoderma* spp. increased in the same study (4). Possible effects of these compounds on common damping-off diseases of cotton remain to be determined.

In the greenhouse test, the number of nonparasitic nematodes tended to increase in response to application of the compounds, whereas in microplot tests the number of nonparasitic nematodes was slightly reduced or remained the same with application of benzaldehyde, citral, and furfural. Greenhouse soil samples, taken 9 weeks after planting, represent short term effects of the compounds. Because nonparasitic nematodes have relatively short life cycles, they may be able to recover from treatment more rapidly than *M. incognita.* They also may be responding to the increased bacterial populations which accompany treatment with these compounds (3,4). Samples from microplot experiments, taken at harvest, indicate effects of the compounds long after application.

Citral, benzaldehyde, furfural, menthol, and α-terpineol were chosen for testing because they are volatile and aromatic.

Table 2. Effects of furfural application on nematode populations, plant height, and yield of 'Deltapine 50' cotton in field microplots.[z]

Rate of Application (ml/kg soil)	Number of *M. incognita*	Nonparasitic nematode spp.	Plant height (cm)	Yield in g seed cotton
0	26.4	48.3	67.2	118.6
0.18	0.0**	59.0	66.2	119.2
0.35	0.0**	46.6	68.5	106.9
0.71	0.0**	50.5	65.4	117.2
1.07	0.0**	45.8	72.8	130.2
1.43	0.0**	43.9	67.7	111.9
1.78	0.0**	14.4**	68.1	126.1
2.14	0.0**	49.6	76.3*	101.4
$FLSD_{0.05}$	6.89	23.32	7.78	37.25
$FLSD_{0.01}$	9.18	31.10	10.47	50.12

[z]Data are means of 8 replications and nematodes were extracted from 100 cm^3 soil.
*, ** = Significantly different from the control at $P \leq 0.05$ and $P \leq 0.01$, respectively.

It was postulated that these compounds may function as signal compounds for nematodes and may be active in blocking recognition of host plants. Future studies will focus on the mode of action of these compounds. In addition, these aromatics should be evaluated in field trials for utility as components in integrated management systems of soil-borne pathogenic fungi and plant- parasitic nematodes of cotton.

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Table 3. Effects of citral application on nematode populations, plant height, and yield of 'Deltapine 50' cotton in field microplots.[z]

Application (ml/kg soil)	Number of *M. incognita*	Nonparasitic nematode spp.	Plant height (cm)	Yield in g seed cotton
0	242.6	95.6	64.7	82.7
0.18	0.6**	47.0**	71.9*	94.7
0.35	0.1**	73.8	62.5	86.3
0.71	0.0**	28.3**	65.6	72.6
1.07	0.0**	89.4	65.8	73.5
1.43	0.0**	87.8	66.6	93.5
1.78	0.0**	67.1	68.5	100.4
2.14	0.0**	69.1	67.6	113.2*
$FLSD_{0.05}$	34.7	31.4	5.8	24.5
$FLSD_{0.01}$	46.2	41.9	7.8	33.0

[z]Data are means of 8 replications and nematodes were extracted from 100 cm^3 soil.
*, ** = Significantly different from the control at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Table 4. Effects of benzaldehyde application on nematode populations, plant height, and yield of 'Deltapine 50' cotton in field microplots.[z]

Application (ml/kg soil)	Number of *M. incognita*	Nonparasitic nematode spp.	Plant height (cm)	Yield in g seed cotton
0	71.6	128.1	36.5	48.4
0.18	1.0**	31.6**	37.2	43.4
0.35	0.6**	37.4**	45.9	61.7
0.71	0.0**	31.5**	34.2	33.6
1.07	0.0**	40.8**	44.8	61.9
1.43	0.0**	52.5*	46.1	59.7
1.78	0.0**	60.6*	46.3	57.7
2.14	0.0**	83.0	50.2*	63.4
$FLSD_{0.05}$	24.2	57.2	10.7	24.0
$FLSD_{0.01}$	28.2	76.3	14.3	32.3

[z]Data are means of 8 replications and nematodes were extracted from 100 cm^3 soil.
*, ** = Significantly different from the control at $P \leq 0.05$ and $P \leq 0.01$, respectively.

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