

# Identification of alternatives for the management of foliar nematodes in floriculture

Ganpati B Jagdale\* and Parwinder S Grewal

Department of Entomology, Ohio State University, Ohio Agricultural Research and Development Center, Wooster, OH 44691, USA

**Abstract:** The foliar nematodes, *Aphelenchoides* spp, have emerged as important pests of ornamentals in North America during the last decade. Due to the ban on the use of potentially toxic pesticides, there are currently no nematicides registered to manage foliar nematodes on ornamentals. Therefore, we have evaluated a biological [*Burkholderia cepacia* (syn *Pseudomonas cepacia*)], two plant products [clove (*Syzygium aromaticum*) extract and Nimbecidine (azadirachtin)] and twelve chemical pesticides registered for the management of insects, mites, slugs or diseases of ornamentals, against *Aphelenchoides fragariae* on the most popular ornamental, hosta (*Hosta* spp), for two consecutive years. We found ZeroTol (270 g liter<sup>-1</sup> peroxyacetic acid), currently labeled as a broad-spectrum fungicide/algicide, to be a very potent nematicide that killed 100% of the nematodes in water suspension. It also caused over 70% reduction in *A fragariae* population in soil and in the leaves without any phytotoxicity. *B cepacia* caused 67–85% reduction in *A fragariae* population in leaves and 50% reduction in the soil whereas insecticidal soap caused over 72% reduction in leaves and 61% reduction in the soil. Clove extract and Nimbecidine did not show any potential for the control of *A fragariae* on hosta. Although all twelve chemical pesticides were effective in reducing the population of *A fragariae* in the soil 45 days after treatment (DAT), only diazinon 475 g liter<sup>-1</sup> EC, trichlorfon 800 g kg<sup>-1</sup> SP, ethoprophos 100 g kg<sup>-1</sup> GR, oxamyl 100 g kg<sup>-1</sup> GR and ZeroTol caused over 70% reduction in nematode population compared with the control. In the leaves, only diazinon EC, trichlorfon SP, insecticidal soap, oxamyl GR and ZeroTol consistently caused over 70% nematode population reduction compared with the control at 45 DAT in both years. Thus, only diazinon EC, trichlorfon SP, oxamyl GR and ZeroTol consistently caused over 70% reduction in nematode population both in soil and leaves. Due to the recent ban by the US Environmental Protection Agency on the use of the first three of these formulations, only ZeroTol would serve as an effective tool to manage foliar nematodes in ornamentals. Although not as effective as ZeroTol in the soil, insecticidal soap is the only other alternative for foliar nematode management.

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**Keywords:** *Aphelenchoides fragariae*; azadirachtin; biological control; *Burkholderia cepacia*; clove; *Hosta* spp, pesticides; plant-parasitic nematode; peroxyacetic acid

## 1 INTRODUCTION

Foliar nematodes, *Aphelenchoides fragariae* (Ritzema-Bos) Christie (Aphelenchida:Aphelenchinae), cause serious damage to alfalfa, strawberries and many ornamentals in nursery and landscape settings throughout the USA, Canada and Europe.<sup>1,2</sup> These nematodes have a wide host range, which includes many common ornamentals such as *Achimenes*, *Anemone*, *Angigozanthos*, *Begonia*, *Bergenia*, *Bouvardia*, *Chrysanthemum*, *Coleus*, *Columnnea*, *Cyclamen*, *Dahlia*, ferns, *Ficus*, *Fuchsia*, *Gloxinia*, *Hibiscus*, *Irish*, *Phloxy*, *Salvia*, *Saintpaulia*, *Sinningia*, *Streptocarpus*, *Viola* and *Zinnia*.<sup>3</sup> Foliar nematodes enter through stomata (natural openings) or directly through the tender leaf tissues. Once inside, they feed on the mesophyll cells, causing large sections of the leaf to

turn chlorotic. The chlorotic sections subsequently turn necrotic. These necrotic lesions are usually bounded by large veins<sup>4</sup> and, in severe cases, the entire leaf can dry and fall off the plant. Recent outbreaks of foliar nematodes on many ornamentals appear to coincide with the withdrawal of Vydate (oxamyl 100 g kg<sup>-1</sup> GR), the most effective nematicide to date,<sup>5,6</sup> from the market in 1995.

In the USA, cultivation and production of hosta (*Hosta* spp), one of the most popular perennial plants grown in urban landscapes, is a multimillion-dollar industry. Today, nurseries grow and sell selections from about 10 different hosta species and their hybrids. There are over 500 cultivars in the trade and the number is growing rapidly. While many gardeners grow them because of their shade tolerance,

\* Correspondence to: Ganpati B Jagdale, Department of Entomology, The Ohio State University, Ohio Agricultural Research and Development Center, Wooster, OH 44691, USA

E-mail: jagdale.1@osu.edu

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some varieties thrive in sunny sites as well. As hosta foliage offers various leaf shapes, textures and colors, there is a growing concern among the growers and nursery managers about the leaf damage caused by foliar nematodes. There is also great concern over the possible movement of nematode-infected plants across state and country boundaries, due to quarantine regulations and fear of nematode dissemination to uninfected areas.

At present, there are no registered nematicides available for the control of foliar nematodes in the USA. Although some insecticides have been effective against *A. fragariae* on some ornamentals,<sup>7</sup> they are not yet registered as nematicides and therefore cannot be used by nursery managers. There is thus a need to identify alternative biological and/or chemical products that can be used safely by homeowners and nursery managers to control foliar nematodes. Since several plant species and their by-products are known to have nematicidal activity,<sup>8,9</sup> we have evaluated clove (*Syzygium aromaticum* (L) Merr & Perry) extract and Nimbecidine (azadirachtin) against *A. fragariae*. We also evaluated Deny, a formulation containing *Burkholderia cepacia* (Burkholder) Yabuuchi *et al* (= *Pseudomonas cepacia* (Burkholder) Palleroni & Holmes), a root-colonizing non-parasitic rhizobacterium which has been used for controlling fungal diseases<sup>10,11</sup> and a plant-parasitic nematode, *Meloidogyne incognita* (Kof & White) Chitwood.<sup>12</sup> We included an environment-friendly broad-spectrum fungicide/algicide, ZeroTol (aqueous peroxyacetic acid, claimed to act through the formation of hydrogen dioxide radicals) that has been recently registered for use on ornamentals. In addition, several chemical pesticides registered for the management of other pests and diseases on ornamentals were included in this investigation along with two nematicides, oxamyl 100 g kg<sup>-1</sup> GR (Vydate 10G) and ethoprophos 100 g kg<sup>-1</sup> GR (Mocap 10G), for comparison. We evaluated the efficacy of all the products

against *A. fragariae* in laboratory bioassay and in the greenhouse. We also made observations on the phytotoxicity of ZeroTol.

## 2 MATERIALS AND METHODS

### 2.1 Source of nematodes

Hosta plants (variety 'Patriot') infected with *A. fragariae* were obtained from a nursery in Lake Country, Ohio. These plants were used in all greenhouse and field experiments. Nematodes were extracted from infested leaf tissues by incubating in water for >48 h in 5-cm Petri dishes at 25 (±2) °C. The freshly extracted nematodes were collected in a beaker and used within 2–3 days for laboratory experiments.

### 2.2 Source of pesticides

A complete list of biological and chemical pesticides that were used in the field, greenhouse and laboratory studies conducted on hosta is given in Table 1.

### 2.3 Laboratory bioassays

A preliminary experiment was conducted in 24-well plates to test the effects of chemical and biological products (see Table 2) on *A. fragariae* at 25 (±2) °C. There were 15 treatments, and four wells from a 24-well plate were assigned for each treatment, each well being considered a separate replicate. Since no recommendations were available for the control of foliar nematodes on hosta, a double-strength concentration of each product was prepared based on the recommendation made by the pesticide companies or other researchers for the management of insects, mites, slugs, snails, other plant-parasitic nematode species or fungal diseases of ornamentals or other crops.<sup>12–16</sup> An aliquot (500 µl) of a solution of each concentration was transferred into each well and 500 µl of a suspension containing about 1500 nematodes was added to each well to achieve a desired

**Table 1.** Trade and chemical names, formulations and sources of pesticides/biologicals used in this study

Trade name	Chemical/scientific name and formulation	Source
Clove extract <sup>a</sup>	<i>Syzygium aromaticum</i>	Wal-mart, Wooster, OH, USA
Deny	<i>Burkholderia cepacia</i> 20g liter <sup>-1</sup> SC	CCT Corp, Carlsbad, CA, USA
Diazinon 4E	Diazinon 475g liter <sup>-1</sup> EC	Terra International Inc, Sioux City, IA, USA
Dylox 6.2G	Trichlorfon 62g kg <sup>-1</sup> GR	Bayer Corp, Kansas City, MO, USA
Dylox 80S	Trichlorfon 800g kg <sup>-1</sup> SP	Bayer Corp, Kansas City, MO, USA
Insecticidal soap	Potassium salts of fatty acids 250g kg <sup>-1</sup> AI	The Murphy-Phoenix Co, Beachwood, OH, USA
Merit 0.5G	Imidacloprid 5.0g kg <sup>-1</sup> GR	Bayer Corp, Kansas City, MO, USA
Merit 75WP	Imidacloprid 750g kg <sup>-1</sup> WP	Bayer Corp, Kansas City, MO, USA
Mesorul 75WP	Methiocarb 750g kg <sup>-1</sup> WP	Gown Co, Yuma, AR, USA
Nimbecidine	Azadirachtin 0.3g liter <sup>-1</sup> TC	PBT International, North Potomac, MD, USA
Mocap 10G	Ethoprophos 100g kg <sup>-1</sup> GR	Rhone-Poulenc AG Co, Research Triangle Park, NC, USA
Oftanol 2-S	Isofenphos 220g liter <sup>-1</sup> EC	Bayer Corp, Kansas City, MO, USA
Orthene 75S	Acephate 750g kg <sup>-1</sup> SP	Whitmire Micro-Gen Res Lab Inc, St Louis, MO, USA
Vydate 10G	Oxamyl 100g kg <sup>-1</sup> GR	Miller Chem & Fertilizer Corp, Hanover, PA, USA
ZeroTol	Peroxyacetic acid 270g liter <sup>-1</sup> aqueous solution	BioSafe Systems, Glastonbury, CT, USA

<sup>a</sup> Clove extract was prepared by boiling of 1.25g of cloves in 5ml of tap water for 1 min in a microwave and the strength of a resulting solution was considered as 100% AI.

percentage active ingredient (AI) level for each chemical and biological product (see Table 2). The percentage nematode mortality was recorded 24, 48 and 72 h after exposure. At each observation, a thoroughly mixed 200- $\mu$ l sub-sample from each well was transferred into a 5-cm diameter dish containing 5 ml of water and held at room temperature for 72 h for the recovery of nematodes. Numbers of live and dead nematodes were counted after concentrating the suspension to 1 ml. Non-motile nematodes were considered alive if they responded to prodding with a fine probe.

## 2.4 Greenhouse experiments

### 2.4.1 Spray application

Greenhouse trials were conducted in 1999 and in 2000 to evaluate the efficacy of the same biological and chemical products that were tested in the laboratory bioassays against *A. fragariae* (see Table 3). These trials were initiated on October 1, 1999 and October 18, 2000 in plastic pots (0.53 cm<sup>2</sup> surface area in the pot) containing nematode-infected plants. All the treatments were arranged in a randomized block design with four replications. Based on the label instructions, both liquid and wettable powder formulations were dispersed in water (1 litre) and sprayed onto each plant using a hand-operated carbon dioxide pressurized-sprayer until run-off into the pot. Pesticides that were available in granular formulations were directly mixed in the soil around the plant. Although Deny, a liquid formulation, is recommended only for soil application for control of plant parasitic nematodes,<sup>12</sup> the required dosages of AI were prepared in water and sprayed onto the hosta plants as described above. As a control treatment, water (1 litre) was sprayed onto each of four plants (replicates).

Nematode populations in the soil (in year 2000 only) and in the infected leaves (in 1999 and 2000) were recorded before (initial population  $p_i$ ) and 15, 30 and 45 (final population,  $p_f$ ) days after treatment (DAT). For assessment of the population of *A. fragariae* in the leaf tissues, one infected leaf from each plant of each treatment was collected at every observation and the area showing nematode infection was quantified using a NIH 1.62 image analysis system with a flexcam. The infected leaves were then cut into 1-cm<sup>2</sup> pieces and incubated in 14-cm diameter Petri dishes containing water (40 ml) for 72 h for the nematodes to emerge. The numbers of nematodes that emerged from each leaf were counted and expressed as nematodes per cm<sup>2</sup> of infected leaf area. To assess the population of *A. fragariae* in the soil, about 30 g of soil was collected from each pot at every observation. The Baermann funnel technique<sup>17</sup> was used to extract nematodes from thoroughly mixed 10-g subsamples of soil, and nematode numbers were expressed per 10 g of soil.

### 2.4.2 Soil drench application

A greenhouse experiment was conducted to study the

effect of soil drenching with Nimbecidine and ZeroTol on the population of *A. fragariae* in pots containing infected hosta plants. Both Nimbecidine and ZeroTol were drench-applied at the rate of 0.01 and 5.4 g AI liter<sup>-1</sup> per pot, respectively, for three consecutive days. Drenching was performed until all the soil in the pot was fully saturated with the chemicals or with water as a control treatment. Treated pots were arranged in a randomized block design with four replicates. Nematode populations in the soil and in the infected hosta leaves were recorded before and 15, 30 and 45 days after drenching of products to the pot. The nematode populations both in the soil and in the leaves were assessed as described above.

## 2.5 Field experiments

### 2.5.1 Effects of ZeroTol concentration on foliar nematodes

The efficacy of different concentrations of ZeroTol on *A. fragariae* in the leaves of hosta was studied in the field. The pots with the infected plants were arranged in a randomized block design with four replicates. ZeroTol concentrations of 0, 0.675, 1.35 and 2.7 g AI liter<sup>-1</sup> were prepared in water and sprayed onto each plant for three consecutive days as described in Section 2.4.1. In addition, based on label instructions, the area around the experiment was disinfected with ZeroTol (5.4 g AI liter<sup>-1</sup>) before the initiation of the experiments. Nematode population in the infected leaves was assessed at 5 DAT as described in Section 2.4.1.

### 2.5.2 Evaluation of ZeroTol phytotoxicity

The phytotoxicity of different concentrations of ZeroTol was studied on healthy hosta plants (variety 'Patriot') in the field. The plants were arranged in a randomized block design with four replicates. ZeroTol concentrations (0, 0.675, 1.35 and 2.7 g AI liter<sup>-1</sup>) were prepared in water and sprayed onto each plant for three consecutive days as described in Section 2.4.1. Phytotoxic symptoms, if any, were recorded 5 days after the application of ZeroTol, and the plants were examined daily for another 20 days for the initiation of symptoms.

## 2.6 Statistical analyses

Data on the soil and leaf nematode population from greenhouse and field experiments and arcsine-transformed values of corrected percentage mean mortalities from laboratory bioassays were subjected to analysis of variance (ANOVA) using a General Linear Models Procedure (SAS Institute, 1988). Significant differences between treatments were determined using Tukey's multiple range test at  $P < 0.05$ .

## 3 RESULTS

### 3.1 Laboratory bioassays

In water suspension, all compounds, except Deny and insecticidal soap, caused significant ( $P < 0.05$ ) nematode mortality within 24 h of exposure compared with

Product	Concentration <sup>a</sup> (g AI liter <sup>-1</sup> )	Mortality at hours after exposure <sup>b</sup> (%)		
		24	48	72
Clove extract <sup>c</sup>	100.0	7.9 ef	8.2 g	6.0 gh
<i>Burkholderia cepacia</i>	0.25	5.6 fg	37.8 ef	33.9 ef
Diazinon 475g liter <sup>-1</sup> EC	2.90	96.5 a	100.0 a	100.0 a
Trichlorfon 62g kg <sup>-1</sup> GR	0.07	41.4 bcd	40.6 ef	76.6 bcd
Trichlorfon 800g kg <sup>-1</sup> SP	4.00	49.0 bc	78.3 bc	91.4 ab
Insecticidal soap	15.00	1.3 fg	6.3 g	5.4 gh
Imidacloprid 5.0g kg <sup>-1</sup> GR	0.002	35.6 cd	50.6 cde	73.6 cd
Imidacloprid 750g kg <sup>-1</sup> WP	0.52	7.9 ef	8.9 g	11.7 fg
Methiocarb 750g kg <sup>-1</sup> WP	2.30	66.1 b	85.1 b	89.0 bc
Ethoprophos 100g kg <sup>-1</sup> GR	1.20	32.6 cd	42.2 ef	74.8 bcd
Isofenphos 220g liter <sup>-1</sup> EC	2.40	55.7 bc	65.2 cd	67.7 d
Acephate 750g kg <sup>-1</sup> SP	1.50	30.9 cd	48.8 def	70.1 cd
Oxamyl 100g kg <sup>-1</sup> GR	1.00	24.5 de	38.1 ef	39.2 e
ZeroTol	6.70	98.9 a	100.0 a	100.0 a
Control (water)		0.0 g	0.0 g	0.0 h

<sup>a</sup> See Section 2.3.

<sup>b</sup> Data are corrected percentage mortality means of four replicates, and values in the same column followed by the same letter are not significantly different (Tukey's multiple range test,  $P < 0.05$ ).

<sup>c</sup> 100 µl of boiled clove extract (see Table 1) mixed with 400 µl of tap water was transferred into each well containing 500 µl of nematode suspension, thus resulting in 100g liter<sup>-1</sup> nominal AI.

**Table 2.** Effect of biological and chemical pesticides on *Aphelenchoides fragariae* in water

the untreated controls (Table 2). Highest mortality was observed when nematodes were exposed to ZeroTol (98.9%) or diazinon 475g liter<sup>-1</sup> EC (96.5%) during the first 24h, whereas lowest mortality (7.9%) was recorded with clove extract and imidacloprid 750g kg<sup>-1</sup> WP. Nematode mortality increased with increased exposure period in all treatments except clove extract, insecticidal soap and imidacloprid WP (Table 2).

### 3.2 Greenhouse experiments

#### 3.2.1 Spray application

Overall, the population of *A. fragariae* in the leaves of hosta was higher in 1999 than in 2000. The initial population (before the application of pesticides) in 1999 ranged from 321 to 750 (mean 449) whereas in 2000 it ranged from 126 to 346 (mean 199) nematodes per cm<sup>2</sup> of infected leaf area (Table 3). Nematode population increased over tenfold in the control

**Table 3.** Effect of biological and chemical pesticides on the population of *Aphelenchoides fragariae* in infected hosta leaves

Product	AI (g) <sup>a</sup>	Nematodes per cm <sup>2</sup> infected leaf area (1999) <sup>b</sup>					Nematodes per cm <sup>2</sup> infected leaf area (2000) <sup>b</sup>				
		Days after treatment				ROC <sup>d</sup> (%)	Days after treatment				ROC <sup>d</sup> (%)
		15	30	45 (pf)	RF <sup>c</sup>		15	30	45 (pf)	RF <sup>c</sup>	
Clove extract <sup>e</sup>	5.0	763 a	1019 a	946 c	2.1 c	78.1	62.7 a	117 ab	394 ab	2.0 ab	29.6
<i>Burkholderia cepacia</i>	0.25	659 a	1208 a	631 c	1.4 c	85.4	60.3 a	134 ab	185 bcd	0.9 bcd	67.0
Diazinon 475g liter <sup>-1</sup> EC	2.90	1033 a	1713 a	1155 c	2.6 c	73.2	380 a	79.7 ab	79.9 cd	0.4 cd	85.7
Trichlorfon 62g kg <sup>-1</sup> GR	0.07	588 a	1547 a	5746 a	12.8 a	-33.1	74.5 a	143 ab	189 bcd	0.9 bcd	66.3
Trichlorfon 800g kg <sup>-1</sup> SP	4.0	349 a	2290 a	600 c	1.3 c	86.1	104 a	59.8 b	84.3 cd	0.4 cd	84.9
Insecticidal soap	15.0	793 a	1051 a	1151 c	2.6 c	73.3	124 a	136 ab	157 bcd	0.8 bcd	71.9
Imidacloprid 5.0g kg <sup>-1</sup> GR	0.002	805 a	1072 a	1754 bc	3.9 bc	59.3	328 a	185 ab	346 abc	1.7 abc	38.2
Imidacloprid 750g kg <sup>-1</sup> WP	0.52	598 a	997 a	986 c	2.2 c	77.2	182 a	258 ab	242 bcd	1.2 bcd	56.7
Methiocarb 750g kg <sup>-1</sup> WP	2.30	606 a	1107 a	1983 bc	4.4 bc	54.1	53.5 a	69.1 b	404 ab	2.0 ab	27.8
Ethoprophos 100g kg <sup>-1</sup> GR	1.20	334 a	2250 a	1620 bc	3.6 bc	62.7	96.6 a	101 ab	65.8 d	0.3 d	88.3
Isofenphos 220g liter <sup>-1</sup> EC	2.40	225 a	1761 a	2386 bc	5.3 bc	44.7	157 a	204 ab	209 bcd	1.0 bcd	62.6
Acephate 750g kg <sup>-1</sup> SP	1.50	579 a	825 a	1139 c	2.5 c	73.6	93.4 a	97.7 ab	254 bcd	1.3 bcd	54.7
Oxamyl 100g kg <sup>-1</sup> GR	1.00	— <sup>f</sup>	—	598 c	1.3 c	86.1	73.3 a	73.9 b	156 bcd	0.8 bcd	72.2
ZeroTol	6.70	452 a	1267 a	680 c	1.5 c	84.2	73.3 a	123 ab	152 bcd	0.8 bcd	72.9
Control (water)		474 <sup>a</sup>	2939 <sup>a</sup>	4316 <sup>a,b</sup>	9.6 <sup>a,b</sup>		232 <sup>a</sup>	388 <sup>a</sup>	560 <sup>a</sup>	2.8 <sup>a</sup>	

<sup>a</sup> Applied in 1 liter of water for dispersible formulations; for granules, equivalent amount applied directly to soil.

<sup>b</sup> Data are means of four replicates and values in the same column followed by the same letter are not significantly different (Tukey's multiple range test,  $P < 0.05$ ).

<sup>c</sup> RF (reproduction factor) =  $p_f$  (final population) ÷  $p_i$  (mean 449 and 199, initial populations for 1999 and 2000 respectively).

<sup>d</sup> ROC = reduction of population over control.

<sup>e</sup> 5ml of boiled clove extract (see Table 1) was mixed in 1 liter of water.

<sup>f</sup> Dash indicates no data.

Product	AI (g) <sup>a</sup>	Mean numbers of nematodes per 10g soil <sup>b</sup>				
		Days after treatment			RF <sup>c</sup>	ROC <sup>d</sup> (%)
		15	30	45 (pf)		
Clove extract	5.0	975 ab	1137 ab	585 b	1.1 b	30.8
<i>Burkholderia cepacia</i>	0.25	650 abc	812 bc	422 bcd	0.8 bcd	50.0
Diazinon 475g liter <sup>-1</sup> EC	2.90	520 bc	487 c	162 f	0.3 f	80.8
Trichlorfon 6.2g kg <sup>-1</sup> GR	0.07	422 c	390 c	390 bcde	0.7 bcde	53.8
Trichlorfon 800g kg <sup>-1</sup> SP	4.00	845 abc	780 bc	195 ef	0.4 ef	76.9
Insecticidal soap	15.0	780 abc	812 bc	325 cdef	0.6 cdef	61.5
Imidacloprid 0.5g kg <sup>-1</sup> GR	0.002	520 bc	552 c	325 cdef	0.6 cdef	61.5
Imidacloprid 750g kg <sup>-1</sup> WP	0.52	975 ab	780 bc	487 bc	0.9 bc	42.3
Methiocarb 750g kg <sup>-1</sup> WP	2.30	747 abc	520 c	260 def	0.5 def	69.2
Ethoprophos 100g kg <sup>-1</sup> GR	1.20	585 bc	617 c	162 f	0.3 f	80.8
Isofenfos 220g liter <sup>-1</sup> EC	2.40	390 c	682 c	292 cdef	0.5 cdef	65.4
Acephate 750g kg <sup>-1</sup> SP	1.50	650 abc	715 bc	390 bcde	0.7 bcde	53.8
Oxamyl 100g kg <sup>-1</sup> GR	1.00	325 c	520 c	195 ef	0.4 ef	76.9
ZeroTol	6.70	357 c	390 c	195 ef	0.4 ef	76.9
Control		1170 a	1332 a	845 a	1.6 a	

<sup>a</sup> See Table 3.

<sup>b</sup> Data are means of four replicates and values in the same column followed by the same letter are not significantly different (Tukey's multiple range test,  $P < 0.05$ ).

<sup>c</sup> See Table 3.

<sup>d</sup> See Table 3.

**Table 4.** Effect of biological and chemical pesticides on the population of *Aphelenchoides fragariae* in soil around hosta plants (2000)

in 1999 whereas in 2000 it increased only threefold within 45 days of treatment. Almost all compounds tested during 1999 and 2000 had no significant ( $P < 0.05$ ) effect on the population of *A. fragariae* within the first 30 days of application, but the nematode population was significantly reduced in most of the treatments compared with the control at 45 DAT. Compared with the control, significant ( $P < 0.05$ ) reduction in *A. fragariae* population in the leaves was observed in treatments with *B. cepacia*, diazinon EC, trichlorfon SP, insecticidal soap, imidacloprid WP, acephate SP, oxamyl GR and ZeroTol at 45 DAT in both 1999 and 2000 (Table 3). Five of these formulations, diazinon EC, trichlorfon SP, insecticidal soap, oxamyl GR and ZeroTol, produced over 70% reduction in nematode population in leaves

compared with the control in both years, whereas the other three, *B. cepacia*, imidacloprid WP and acephate SP, produced reductions of 55–85%.

In general, pesticides had a much more rapid effect on *A. fragariae* populations in the soil than in the leaves. Seven of the 14 products caused significant reduction at 15 DAT compared with control (Table 4). All products had significantly ( $P < 0.05$ ) reduced nematode population in soil at 45 DAT. Five products, diazinon EC, trichlorfon SP, ethoprophos GR, oxamyl GR and ZeroTol, caused 77–81% reduction in nematode population compared with the control (Table 4).

### 3.2.2 Soil drench application

Selected pesticides applied exclusively as a soil drench

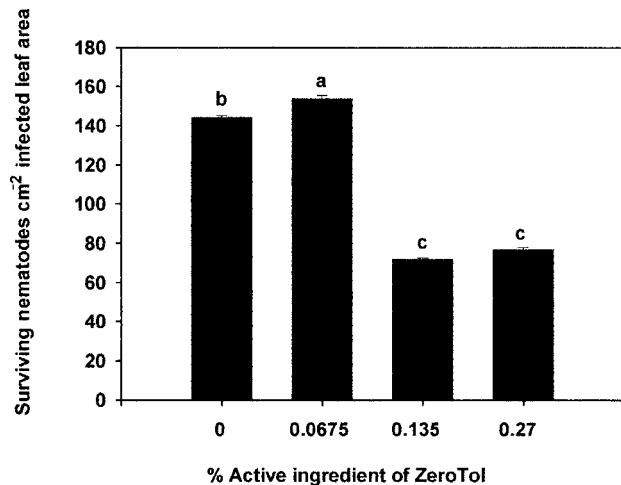
Product	AI (g liter <sup>-1</sup> )	Nematode population at days after the treatment <sup>a</sup>				RF <sup>b</sup>	ROC <sup>c</sup> (%)
		15	30	45 (pf)			
Per cm <sup>2</sup> of infected leaf area							
Nimbecidine	0.001	186.9 a	135.7 ab	158.6 ab	0.7 ab	45.7	
ZeroTol	0.54	146.8 a	115.8 ab	43.3 b	0.2 b	85.2	
Control (water)		200.4 a	174.6 a	291.9 a	1.3 a		
Per 10g of soil							
Nimbecidine	0.001	520.0 a	845.0 ab	585.0 ab	0.9 ab	21.7	
ZeroTol	0.54	520.0 a	585.0 b	422.5 b	0.6 b	43.5	
Control (water)		682.5 a	1007.5 a	747.5 a	1.1 a		

<sup>a</sup> Data are means of four replicates and values in the same column followed by the same letter are not significantly different (Tukey's multiple range test,  $P < 0.05$ ).

<sup>b</sup> RF (reproduction factor) =  $p_t$  (final population) ÷  $p_i$  (mean 223 and 666, initial populations from leaves and soil respectively).

<sup>c</sup> ROC = reduction of population over control.

**Table 5.** Effect of biological and chemical pesticides when applied as drench on the population of *Aphelenchoides fragariae* during 2000



**Figure 1.** Effect of different concentrations of ZeroTol on *Aphelenchoides fragariae* in infected hosta leaves in the field 5 DAT. Each bar represents a mean ( $\pm$  SE) of four replicates. Bars with the same lower case letter are not significantly different (Tukey's multiple range test,  $P < 0.05$ ).

influenced reproduction of *A. fragariae* in the soil as well as in the hosta leaves (Table 5). ZeroTol had significantly reduced *A. fragariae* population in the soil at 30 DAT and in the leaves at 45 DAT. The reduced population of *A. fragariae* both in the soil and in the leaves coincided with the reduced nematode reproduction (Table 5). Nimbecidine had no effect on either the soil or leaf populations of *A. fragariae* even at 45 DAT (Table 5).

### 3.3 Field experiments

#### 3.3.1 Effects of ZeroTol concentrations on foliar nematodes

Three consecutive daily applications of ZeroTol killed 30–50% of *A. fragariae* in hosta leaves (Fig 1). At 1.35 and 2.7 g AI liter<sup>-1</sup>, ZeroTol significantly ( $P < 0.05$ ) reduced *A. fragariae* populations in hosta leaves compared with the control and with 0.675 g AI liter<sup>-1</sup> treatment.

#### 3.3.2 Evaluation of ZeroTol phytotoxicity

No phytotoxicity was observed during the experimental period (5 days) on uninfected hosta plants with any of the concentrations of ZeroTol tested, and all the plants appeared healthy 20 days after the termination of experiment.

## 4 DISCUSSION

This study identified several new chemical alternatives for managing foliar nematodes. ZeroTol (270 g liter<sup>-1</sup> peroxyacetic acid), a broad-spectrum fungicide and algicide which is claimed to act through the production of hydrogen dioxide radicals and is currently registered for use on ornamental plants, showed exceptional potential as a nematicide. We found that ZeroTol was potent against *A. fragariae* with 100% mortality of the nematodes, even at the lowest concentration tested in

water (0.675 g AI liter<sup>-1</sup>) within 72 h. Furthermore, when ZeroTol was applied as a spray or a soil drench, it caused a significant reduction in nematode reproduction in the soil and leaves. These results were comparable with those obtained with chemical nematicides including oxamyl 100 g kg<sup>-1</sup> GR and ethoprophos 100 g kg<sup>-1</sup> GR. In addition, no phytotoxicity arising from ZeroTol was observed on healthy hosta plants even at the highest concentration of 5.4 g AI liter<sup>-1</sup>. Therefore, we suggest that ZeroTol should be evaluated further as a nematicide to control foliar nematodes in home gardens, nurseries and other urban landscapes.

The use of biocontrol agents to manage foliar nematodes may provide another environmentally safe alternative to chemical nematicides. We found that the *B. cepacia*, a non-parasitic rhizobacterium, was as effective as oxamyl in reducing the reproduction of nematodes in hosta leaves (see Table 3 for reproduction factor values). While the mode of action of *B. cepacia* against *A. fragariae* is unclear, it has been suggested that some species of the non-parasitic bacterium colonize the rhizosphere of the host plant aggressively, which enhances plant development and induces systemic resistance against pathogens including nematodes.<sup>18,19</sup> Although *B. cepacia* is recommended only for soil application, in this study we sprayed the formulated product directly onto the plants and also tested its toxicity in aqueous suspension against foliar nematodes. We observed that about 34% of the nematodes were killed when directly exposed to *B. cepacia* in water suspension. When sprayed on hosta foliage, it caused 50 and 67–85% reduction in the nematode population in the soil around the plants and in the leaves, respectively. Meyer *et al*<sup>12</sup> reported that the culture filtrates of *B. cepacia* reduced only the mobility of infective juveniles of *M. incognita*, and in the soil only non-viable *B. cepacia* was effective against *M. incognita*. This suggests that the suppressive effect of *B. cepacia* could be nematode species-specific. None of the plant-based products showed nematicidal activity against *A. fragariae* infesting hosta leaves.

This study demonstrated that all the insecticides and nematicides except imidacloprid 5.0 g kg<sup>-1</sup> GR and methiocarb 750 g kg<sup>-1</sup> WP significantly reduced *A. fragariae* population in hosta leaves at 45 DAT, but the efficacies of only acephate 750 g kg<sup>-1</sup> SP, diazinon 475 g liter<sup>-1</sup> EC, imidacloprid 750 g kg<sup>-1</sup> WP, insecticidal soap, oxamyl 100 g kg<sup>-1</sup> GR and trichlorfon 800 g kg<sup>-1</sup> SP were consistent for 1999 and 2000. The reduction in nematode population (from 55 to 86%) may have been caused by the decreased nematode reproduction in the hosta leaves 45 days after application of the six most effective pesticides. The results obtained in the present study were in agreement with the findings of some studies on foliar nematodes but were not in agreement with the findings of other studies. For example, our results contradict the findings of Strider,<sup>20</sup> who observed the ineffec-

tiveness of diazinon 480 g liter<sup>-1</sup> EC against *A fragariae* on begonia, but support the findings of LaMondia,<sup>7</sup> who showed diazinon (unspecified formulation) was highly effective in reducing *A fragariae* populations in lamium (*Lamium maculatum* L) leaves. Furthermore, our results on oxamyl (Vydate 10G) applied as granules in the soil support the findings of Strider,<sup>20</sup> who showed that oxamyl 252 g liter<sup>-1</sup> SL as a soil drench reduced the number of nematodes in begonia leaves within 20 days. In addition, LaMondia<sup>7</sup> reported that methiocarb (unspecified formulation) also reduced the nematode population in lamium leaves; however, we found that methiocarb 750 g kg<sup>-1</sup> WP (Mesurul 75WP) significantly reduced the nematode population only in the soil and not in hosta leaves. Furthermore, we showed that imidacloprid 750 g kg<sup>-1</sup> WP (Merit 75WP) was effective in controlling *A fragariae* population both in the soil and in hosta leaves, but Walker *et al*<sup>6</sup> found no effect of imidacloprid 200 g liter<sup>-1</sup> EC on *A fragariae* infesting begonia. Yamada and Takakura<sup>21</sup> reported that trichlorfon 500 g liter<sup>-1</sup> SL reduced the population of *A fragariae* in lily bulbs (*Lilium* spp). We obtained similar results with trichlorfon 800 g kg<sup>-1</sup> SP (Dylox 80S) against *A fragariae* infecting hosta. These differences between studies may be due to differences in the concentrations and formulations used and/or differences in the host plants.

Of the two nematicides, oxamyl 100 g kg<sup>-1</sup> GR and ethoprophos 100 g kg<sup>-1</sup> GR, only oxamyl was effective in reducing *A fragariae* reproduction in hosta leaves for both years. However, in the soil, both nematicides were equally effective in reducing reproduction of *A fragariae*. These observations on the efficacy of oxamyl are in agreement with studies on other ornamentals<sup>5,6,22-24</sup> and suggest that oxamyl GR functions as a systemic nematicide. This study showed that all the chemical pesticides, including two nematicides, were effective in reducing both reproduction and overall *A fragariae* population (from 55 to 81%) in the soil. Based on the reduction in the population of nematodes in hosta leaves over control (ROC) during 1999 and 2000, all the biological and chemical pesticides could be grouped as highly ( $\geq 70\%$  ROC), moderately (51–69% ROC) and least ( $\leq 50\%$  ROC) effective products against *A fragariae*. The highly effective products against *A fragariae* were diazinon 475 g liter<sup>-1</sup> EC, ZeroTol, insecticidal soap, oxamyl 100 g kg<sup>-1</sup> GR and trichlorfon 800 g kg<sup>-1</sup> SP, while the moderately effective products included acephate 750 g kg<sup>-1</sup> SP, *B cepacia*, ethoprophos 100 g kg<sup>-1</sup> GR and imidacloprid 750 g kg<sup>-1</sup> WP. The least effective products against *A fragariae* were clove extract, imidacloprid 5.0 g kg<sup>-1</sup> GR, methiocarb 750 g kg<sup>-1</sup> WP, isofenphos 220 g liter<sup>-1</sup> EC and trichlorfon 62 g kg<sup>-1</sup> GR.

Due to the recent ban on the use of Diazinon 4E, Dylox 80S and Vydate 10G by the US Environmental Protection Agency<sup>25,26</sup> only ZeroTol and insecticidal soap could serve as good alternatives to standard nematicides for the management of foliar nematodes.

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