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Effectiveness of a Hot Water Drench for the Control of Foliar Nematodes *Aphelenchoides fragariae* in Floriculture[1]

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Abstract: Effectiveness of a hot water drench for the control of *Aphelenchoides fragariae* infesting hosta (*Hosta* sp.) and ferns (*Matteuccia pensylvanica*) was studied. Drenching with hot water at 70 °C and 90 °C in October reduced ($P < 0.05$) *A. fragariae* in the soil but not in the leaves relative to the control (25 °C) 300 days after treatment (DAT). Plants drenched with 90 °C water had lower numbers of nematode-infected leaves per plant than those treated with 25 °C and 70 °C water ($P < 0.05$). Hot water treatments had no adverse effect on the growth parameters of hosta. Boiling water (100 °C) applied once a month for 3 consecutive months (April, May, June) consistently reduced the number of infected leaves and the severity of infection relative to the control 150 DAT in hosta but not in ferns ($P < 0.05$). Boiling water (100 °C) caused a 67% reduction in *A. fragariae* population in hosta leaves, 50% in fern fronds, and 61% to 98% in the soil over the control 150 DAT. A boiling water drench had no effect on the fern growth but caused 49% and 22% reduction in the number and size of hosta leaves, respectively, over the control in 2002. We conclude that 90 °C water soil drench in the autumn or early spring could prove effective in managing foliar nematodes on hosta in nurseries and landscapes.

Key words: Aphelenchoides fragariae, fern, foliar nematode, *Hosta* spp, hot water, *Matteuccia pensylvanica.*

Foliar nematodes *Aphelenchoides fragariae* (Ritzema Bos, 1890) Christie, 1932 (Aphelenchida: Aphelenchidae) cause serious damage to alfalfa, strawberries, and many ornamentals including hosta and ferns in nursery and landscape settings throughout the United States, Canada, and Europe (Grewal and Jagdale, 2001; Heinlein, 1982; Johnson and Gill, 1975; Richardson and Grewal, 1993; Southey, 1993). The nematodes infect young leaves, presumably through stomata, and feed on the mesophyll cells, causing large sections of the leaf to become chlorotic. The chlorotic sections subsequently turn necrotic. These necrotic lesions are usually bounded by large veins (Sanwal, 1959) and, in severe cases, death and abscissions of the leaf may occur. In the Midwest, typical symptoms of foliar nematodes on hosta can first be observed in July (Grewal and Jagdale, 2001). The nematodes overwinter in the soil, dormant crowns, and dry leaves and migrate to the leaves in the spring (Jagdale and Grewal, unpubl. data).

In the commercial nursery setting, thousands of ornamentals are traded each year and there is a growing concern among growers about the movement of nematode-infected plants and(or) cut foliage across state and country boundaries despite quarantine regulations. Nurseries lose millions of dollars in revenue because of returned shipments of nematode-infected plants. Although several nematicides, insecticides, and fungicides can provide effective suppression of nematode populations (Jagdale and Grewal, 2002; LaMondia, 1999), the U.S. Environmental Protection Agency (EPA) has imposed bans on the usage of most of these pesticides due to concerns about environmental pollution and human health risks (Nixon, 2001; Schulze, 2001). Thus, there is a need to develop alternative methods that can be safely used by nursery managers and homeowners for control of the foliar nematodes.

Hot water treatment has been used to disinfest plant materials of insect pests (Hara et al., 1993, 1994) and plant-parasitic nematodes (Birchfield, 1954; Birchfield and van Pelt, 1958; Tsang et al., 2001). In the United Kingdom, narcissus bulbs are routinely treated with hot water for the control of stem nematode *Ditylenchus dipsaci* prior to planting (Gratwick and Southey, 1986). Typically, bare-rooted plants, dormant crowns, bulbs, stocks, and runners of many economically important plants are removed from the soil and directly immersed in hot water for control of foliar nematodes (MacLachlan and Duggan, 1979; Powell and Riedel, 1973; Qiu et al., 1993; Yamada and Takakura, 1989). This practice is time consuming and labor intensive and requires controlled temperature equipment. This practice is also cumbersome for homeowners. Therefore, it is important to develop easy-to-use hot water treatments for the management of foliar nematodes.

The purpose of this study was to examine the potential of a hot water drench (as opposed to a hot water dip) as preventive treatment for intact plants to reduce overwintering *A. fragariae* populations prior to their migration to the leaves. Specifically, we examined the effects of hot (70 °C and 90 °C) and boiling water (100 °C) as a soil drench in the autumn and spring, respectively, on *A. fragariae* populations in the soil, hosta leaves, and fern fronds. We also evaluated the effects of hot and boiling water on growth parameters of hosta and ferns.

Materials and Methods

Sources of nematode-infested plants: Aphelenchoides fragariae infected hosta (*Hosta* sp.; variety Patriot) and Ostrich fern (*Matteuccia pensylvanica*) plants were obtained from commercial nurseries in Perry, Ohio.

Effectiveness of hot water drench: A greenhouse trial on dormant hosta Patriot crowns was initiated in autumn

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2001 to evaluate the effectiveness of different water temperatures applied as a soil drench on the development of symptoms of *A. fragariae* infection in the following summer. Water temperatures (treatments) included 25 °C (control treatment), 70 °C, and 90 °C. Temperature was maintained using a circulating hot-water bath, and 700 ml of hot water was drench-applied to each plastic pot (346 cm^2 surface area), saturating the soil. Prior to the application of all the treatments, the soil temperature in each pot was ca. 14 °C but increased to 20 °C, 35°C, and 57 °C within 2 to 3 minutes after drenching with water from all three treatments, 25 °C, 70 °C, and 90 °C, respectively. All treatments were applied on 6 December 2001, and pots were arranged in a randomized block design with four replications and maintained in a polyhouse throughout the winter. At the beginning of spring 2002, the experiment was moved into a greenhouse for the rest of the growing season. Nematode populations in the soil and in infected leaves were recorded 300 DAT. For assessment of the population of *A. fragariae* in the leaf tissues, all the leaves showing characteristic lesions from each treatment were collected and their total area quantified using a LI-3100 Area Meter (LI-COR, Inc., Lincoln, NE). Leaves were then cut into 1-cm^2 pieces and transferred to a misting chamber (Seinhorst, 1950) for 72 hours for the emergence of the nematodes. The numbers of nematodes were counted and expressed as nematodes per cm^2 of leaf. To assess the *A. fragariae* population in the soil, about 30 g of soil was collected from each pot and the nematodes were extracted from a 10-g subsample using the Baermann funnel technique (Baermann, 1917). Nematodes were counted and expressed as numbers per 10 g of soil. Observations on plant-growth parameters (total plant biomass, number of leaves per plant, and leaf size) were recorded. In addition, the numbers of leaves per plant showing chlorotic lesions of nematode infection were recorded. Severity of nematode infection was assessed using a severity assessment scale of 0, 0.5, 1, 2, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100% on each leaf (Verreet et al., 1996). Severity of all leaves on a plant was assessed and expressed as mean severity per plant.

Effectiveness of boiling water drench: Two greenhouse trials—one on hosta (variety Patriot) and one on fern—were conducted in spring 2001 to evaluate the effectiveness of boiling water (100 °C) as a preventive treatment to control *A. fragariae* in plastic pots (346 cm^2 surface area). The trial on hosta was repeated in 2002. For the hot water treatment, 2 L water was heated until it reached 100 °C in an aluminum container and then 700 ml was drench-applied in the pots until the soil was saturated once every month for 3 consecutive months (April, May, June). Temperature in the center of the pot was recorded prior to and immediately after hot water drench. As a control treatment, 700 ml tap water (25 °C) was drench-applied per pot. All the pots were arranged in a randomized block design with eight replicates (pots) and two treatments (hot water and control).

Nematode populations in the infected leaves of hosta or fronds of fern were recorded 150 DAT as described above, except that the population in fronds was assessed from a 10-cm-long piece of a frond from each treatment. Total area of 10-cm-long frond piece was measured as described above. Nematodes from each frond were extracted as described above. In addition, nematode populations in the soil collected from both hosta and fern rhizospheres were assessed at 0, 30, 60, and 150 DAT as described above. Soil samples were collected 15 to 20 minutes in prior to each hot water drench (0, 30, 60 DAT). Observations on the plant-growth parameters including total plant biomass, number, and size of leaves or fronds were recorded. In addition, data on severity of nematode infection per plant and the numbers of leaves or fronds per plant showing chlorotic lesions were recorded.

Statistical analyses: Data on the soil and leaf nematode populations, plant-growth parameters, and arcsine-transformed values of percentage reduction in nematode population over control, number of infected leaves or fronds, and severity of nematode infection per plant were subjected to analysis of variance using the general linear models procedure (SAS Institute, Cary, NC). Significant differences between treatments were determined using the LSD test at $P < 0.05$. Changes in nematode populations in the soil overtime were compared using Tukey's mixed repeated measures analysis at $P < 0.05$.

Results

Effectiveness of hot water drench: Hosta plants treated with 90 °C water possessed lower ($P < 0.05$) numbers of nematode-infected leaves per plant than those treated with 25 °C and 70 °C water (Table 1). The population

Table 1. Effect of temperature of water as a soil drench on the growth characteristics of hosta and infection by *Aphelenchoide fragariae,* 300 days after treatment.[a]

Parameters	Water temperature (°C)		
	25	70	90
Biomass (g)	177.0 ± 17 A	168.2 ± 23 A	128.8 ± 30 A
Number of leaves	53.2 ± 10 A	41.5 ± 7 A	35.8 ± 3 A
Leaf size (cm^2)	75.6 ± 12 A	86.2 ± 11 A	15.9 ± 8 A
Number of leaves infected (%)	5.9 ± 0.7 A	6.5 ± 2.0 A	0.7 ± 0.7 B
Severity of nematode infection[b]	22.7 ± 5 A	22.4 ± 8 A	2.3 ± 2 A
Nematodes/cm^2 leaf	11.7 ± 0.5 A	13.5 ± 8.0 A	7.3 ± 7 A
Nematodes/10 g soil	217.5 ± 16 A	142.0 ± 29 B	127.5 ± 28 B

[a] Data are means of four replicates. Values in the same row followed by the same letters are not significantly different (LSD test, $P < 0.05$).

[b] Severity of nematode infection was calculated using a severity assessment scale of 0, 0.5, 1, 2, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100% on each leaf (Verreet et al., 1996).

TABLE 2. Effect of boiling water on the growth characteristics of hosta and population of *Aphelenchoide fragariae* in hosta leaves at 150 days after treatment.[a]

Parameters	2001		2002	
	Boiling water	Control	Boiling water	Control
Biomass (g)	99.2 ± 26 A	169.4 ± 27 A	110.9 ± 28 A	167.5 ± 20 A
Number of leaves	30.5 ± 5 A	34.5 ± 6 A	30.9 ± 5 B	55.8 ± 8 A
Leaf size (cm^2)	75.6 ± 12 A	86.2 ± 11 A	22.3 ± 8 B	58.5 ± 5 A
Number of leaves infected (%)	9.0 ± 1 B	29.0 ± 4 A	3.9 ± 2 B	11.7 ± 1 A
Severity of nematode infection[b]	14.0 ± 3 B	45.5 ± 2 A	7.7 ± 4 B	29.3 ± 2 A
Nematodes/cm^2 leaf	23.1 ± 5 B	78.5 ± 9 A	10.8 ± 6 A	6.1 ± 2 A

[a] Data are means of eight replicates ± SE. Values in the same row for same year followed by the same letters are not significantly different (LSD test, $P < 0.05$).
[b] Severity of nematode infection was calculated using a severity assessment scale of 0, 0.5, 1, 2, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100% on each leaf (Verreet et al., 1996).

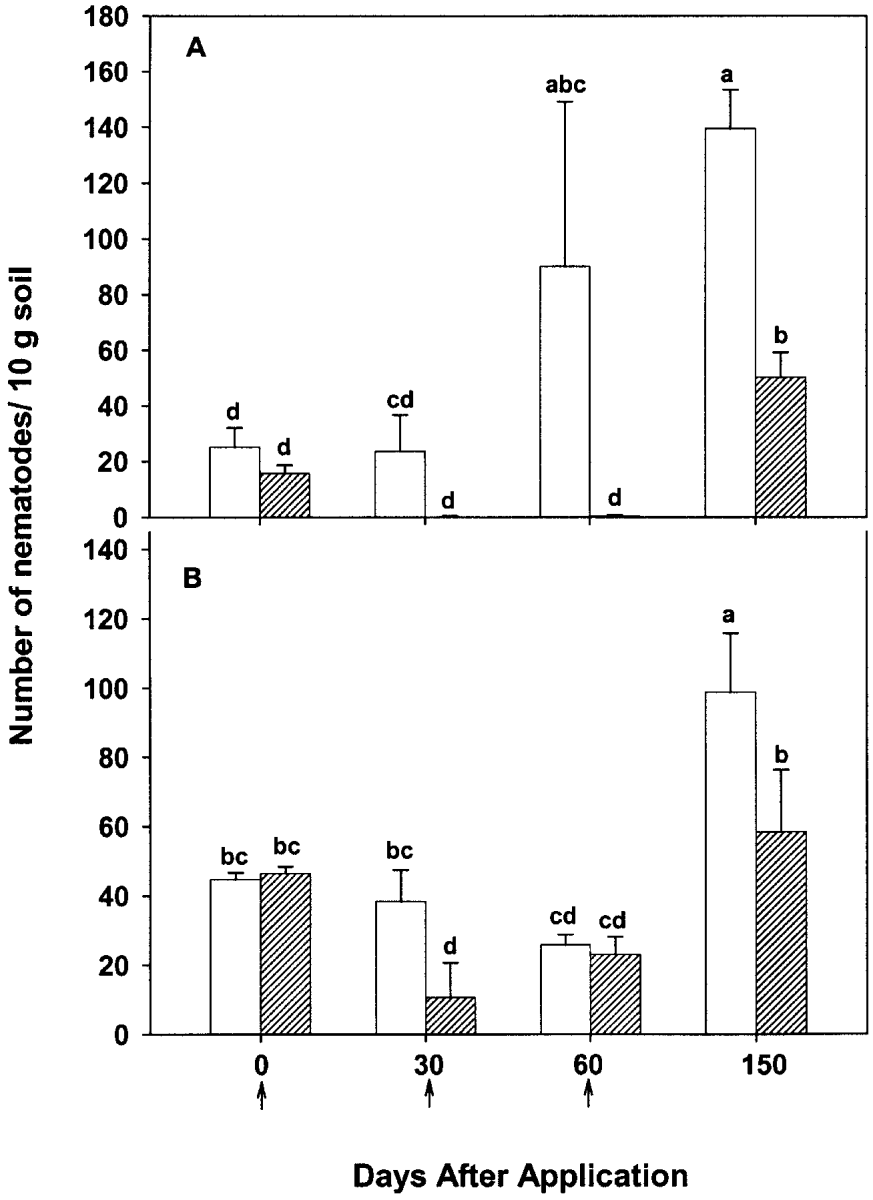

FIG. 1. Effect of boiling water on the population of *Aphelenchoide fragariae* in the soil in 2001 (A) and 2002 (B) 0, 30, 60, and 150 days after treatment with tap water (□) and boiling water (▨). Data are means of eight replicates. Bars (mean ± SE) in the same and between the time intervals with same letter(s) are not significantly different, according to Tukey's mixed repeated measure procedure ($P < 0.05$). Arrows indicate times of boiling water drench.

TABLE 3. Effect of boiling water on the growth characteristics of fern and population of *Aphelenchoide fragariae* in fern fronds at 150 days after treatment.[a]

Parameters	2001	
	Boiling water	Control
Biomass (g)	29.8 ± 9 A	36.0 ± 4 A
Number of leaves	12.0 ± 2 A	12.9 ± 2 A
Leaf size (cm^2)	15.9 ± 8 A	34.6 ± 8 A
Number of leaves infected (%)	22.6 ± 1 A	45.4 ± 1 A
Severity of nematode infection[b]	30.8 ± 2 A	63.1 ± 2 A
Nematodes/cm^2 leaf	87.1 ± 5 A	153.0 ± 6 A

[a] Data are means of eight replicates ± SE. Values in the same row followed by the same letter are not significantly different (LSD test, $P < 0.05$).

[b] Severity of nematode infection was calculated using a severity assessment scale of 0, 0.5, 1, 2, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100% on each leaf (Verreet et al., 1996).

of *A. fragariae* in the leaves and severity of nematode infection at 300 DAT did not differ among treatments (Table 1). Application of 70 °C and 90 °C water reduced ($P < 0.05$) the population of *A. fragariae* in the soil at 300 DAT relative to the control (Table 1). When compared with the control, plants treated with 70 °C and 90 °C water showed no adverse effects on the total biomass, number of leaves, and their size at 300 DAT (Table 1).

Effectiveness of boiling water drench:

Hosta. Boiling water reduced ($P < 0.05$) the numbers of nematode-infected leaves per plant and severity of the nematode infection at 150 DAT relative to the control in both years (Table 2). Overall, the population of *A. fragariae* in the leaves of hosta was higher in 2001 than in 2002. Boiling water reduced ($P < 0.05$) *A. fragariae* populations in the leaves at 150 DAT by 67% relative to the control in 2001 but not in 2002. Although boiling water drench had no adverse effect on the total plant biomass relative to the control, it reduced ($P < 0.05$) the total numbers of leaves and their size in 2002 (Table 2). Initial population of *A. fragariae* in the soil was higher in 2002 than in 2001 (Fig. 1A,B). In the soil, application of boiling water reduced ($P < 0.05$) the population of *A. fragariae* relative to the control, causing an overall 61% to 98% reduction throughout the experiment in 2001 and 75% in 2002 within the first 30 DAT (Fig. 1A,B).

Fern. Total number of nematode-infected fronds per plant, severity of nematode infection, and number of nematodes in frond leaflets were unaffected by the application of boiling water (Table 3). Boiling water treatment had no adverse effect on either total plant biomass, numbers of fronds per plant, or the leaflet size at 150 DAT (Table 3).

DISCUSSION

Use of hot water as a preventive treatment to manage foliar nematodes may provide an environmentally safe alternative to nematicides. We found that boiling water drench consistently reduced the numbers of nematode-infected leaves and the size of chlorotic lesions on hosta in both years. The heat generated from the hot water dissipated through the potting medium and to the layers of dormant crowns, thus killing nematodes and reducing initial inoculum level. The boiling water had a temperature of 100 °C. Our data suggest that the actual temperature range that caused 67% reduction in nematode population in the hosta leaves or 98% in the soil was between 44 °C and 58 °C. Previous studies have demonstrated that the hot water dips at temperatures between 44.4 °C and 47.7 °C were effective in controlling *A. fragariae* in strawberry runners (Qiu et al., 1993) and above 48.88 °C in Rieger begonia (Powell and Riedel, 1973). MacLachlan and Duggan (1979) reported that the strawberry plants were unable to tolerate exposure to water at 50 °C.

We found that both hot (70 °C or 90 °C) and boiling (100 °C) water treatments had no adverse effect on the survival and sprouting of dormant crowns of hosta. Also, these treatments did not affect total biomass of hosta. However, as opposed to 90 °C water, 100 °C water reduced the numbers of hosta leaves per plant and their size relative to the control in 2002. We believe that the third boiling water drench in June 2002 that increased the soil temperature to 58 °C may have reduced the number and size of hosta leaves. Qiu et al. (1993) reported that hot water treatment of strawberry crowns at 49.4 °C for 5 minutes reduced plant growth and number of flowers. We also found that the application of both 90 °C and 100 °C water treatments was equally effective in reducing *A. fragariae* population in the leaves. Thus, these results suggest that the application of a 90 °C water drench, as a preventive treatment in autumn or spring could prove effective in reducing foliar nematode infection of hosta without affecting plant vigor. In the case of fern, boiling water treatment neither reduced the nematode infection/population nor showed damaging effects on the growth parameters, suggesting that different hot water treatment protocols may be needed for each ornamental plant species.

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