Foliar Nematode Research Final Report: 2013-2015

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Overall Summary

We have successfully completed two years of research on foliar nematodes supported by the American Hosta Society. Thirteen different experiments were conducted. Here we report the methods and results in detail. Below is a list of our key findings:

1. Foliar nematodes do not overwinter as eggs in soil or in hosta plant tissues. They overwinter only as juveniles and adults in soil and in below ground hosta tissues (excluding roots) as we reported previously. They also overwinter in a desiccated form in the withered leaves.
2. Healthy hosta plants can get nematode infection from infested plants if the leaves of the two plants touch each other and there is sufficient moisture to allow nematodes to move from leaf to leaf.
3. Foliar nematodes move upward from overwintering sites onto hosta petioles in spring and early summer at or higher than 95% relative humidity (R.H.) but not at 65% R.H.
4. Two different experiments demonstrated that foliar nematodes do not actively migrate through the plant back into the soil with the onset of winter. However, frequent rains or sprinkler irrigation may dislodge the nematodes from infected leaves and move them to soil.
5. Twenty three different chemical products were evaluated in aqueous suspension for toxicity to foliar nematodes. The most effective products identified included: Grapefruit seed extract, Ammonia, Clorox, $KMnO_4$, NaDCC, Nemakill, Pylon and ZeroTol.
6. Growth chamber experiments were conducted on the effectiveness of soil drench treatments with selected chemicals and hot water to control foliar nematodes in soil in potted hosta plants. All treatments significantly reduced the number of foliar nematodes in soil, but Pylon, Nemakill, and hot water were the most effective treatments that virtually eliminated the nematodes from soil.
7. Leaf disc tests were conducted on the effectiveness of selected chemicals to kill foliar nematodes directly in hosta leaves. Spraying of nematode-infected leaf discs with Pylon or Nemakill solution resulted in 100% mortality of nematodes.
8. There was no evidence of phytotoxicity for Pylon or Nemakill treatments on healthy hosta plants.
9. Residual activity of Pylon in soil was up to 30 days compared to 20 days for Nemakill.
10. Treatment of nematode infested dormant hosta buds before they begin to emerge in the spring with Nemakill can significantly reduce potential for nematode infection of leaves in the summer season.

11. Overall, Nemakill, Pylon, ZeroTol, and hot water have potential for the management of foliar nematodes if used appropriately.

Experimental Procedures and Results

Note: the numbering of the following 13 experiments is arbitrary and not to suggest that they were performed sequentially. Some of the experiments were stand-alone experiments, while some were indeed sequential in nature. Where the results of one experiment were specifically used as a screening tool for another experiment, the relevant experiment number is noted.

Experiment 1. Determining whether foliar nematodes overwinter as eggs

One key bit of missing data in understanding the infection cycle of foliar nematodes is whether foliar nematodes overwinter in the form of eggs. In the early spring of 2013, soil, leaves, roots and overwintering hosta crowns were collected from Wooster, Ohio and examined to check for the overwintering foliar nematodes, especially for the overwintering nematode eggs. Nematodes were found in the soil, the leaves, and the crowns (buds) but not in the roots. Nematode eggs were not found in the soil and other samples, but we did not rule out the possibility that eggs may overwinter in the leaves and buds. In the early spring of 2014, hosta buds and soil samples were collected again from selected gardens in Tennessee and were examined for overwintering nematodes. Once again juvenile and adult nematodes were found in the soil and buds. Most nematodes in the buds were discovered when the outside layer was peeled off. From the 12 buds that we examined, some could hide 30-36 nematodes under the outside layer, but some buds only contained a few (4-6) under the outside layer. No eggs were found in the sampled soil or hosta buds. With a tissue staining technique (utilizing NaOCl as a pre-staining treatment for the plant tissue samples, followed by an acid-fuchsin staining and de-staining procedure), we confirmed that the nematodes do not overwinter as eggs in soil or plant tissues.

Experiment 2. Nematode transmission from infested to healthy hosta plants

We initially observed that if pots with healthy hosta plants were placed close to the pots with the nematode infested plants, the healthy hosta developed nematode infection symptoms. In a field experiment, we further determined that such transmission of infection occurred only when the leaves between the two plants overlapped or touched and plants were regularly watered by spraying from the top. In this experiment, we placed 3 healthy hosta plants in pots either adjacent to (touching each other) or 3-feet away from the nematode infected hosta plants growing the ground. Three such pairs of healthy-infected plants were set up and all plants were regularly watered from the top using a hose. We found that all healthy hosta plants that touched the infected hosta plants developed nematode infection symptoms and those 3-feet away did not. This suggests that healthy hosta plants can get nematode infection from infested plants if the leaves of the two plants touch each other and there is sufficient moisture to allow nematodes to move between leaves. As the same foliar nematode species can infect over 200 species of

ornamental plants, we predict that foliar nematodes from infested hosta plants may jump to other plant species such as ferns and *vice versa*.

Experiment 3. Determination of upward movement of foliar nematodes from soil to hosta leaves

This experiment was set up to determine the role of relative humidity (R.H.) in the potential upward migration of foliar nematodes from soil to cause infection of healthy leaves. In this experiment, we tested two relative humidity (R.H.) conditions: 65% and 95%. The 95% R.H. was achieved by bagging the hosta plants in a clear plastic bag. The nematode migration was confirmed by taking stem samples and by observing for the development of characteristic symptoms of nematode infection in the leaves once every two weeks.

Treatments:

1. 65% R.H.
2. 95% R.H.

Experimental design: The pots with healthy hosta were examined first to ensure there were no foliar nematodes in the soil. The soil was then inoculated with the foliar nematodes at ~1500 nematodes in 30 ml water per pot. Three treated pots were separately bagged in clear plastic bags to maintain 95%-100% R.H. and the other three treated pots were kept in the growth chamber set at 65% R.H. The temperature in the growth chamber was set at 25^{o}C and it varied between 23 to 25^{o}C. The bags were opened for 5 minutes every day to allow air exchange and then closed back again to maintain the desired R.H.

Number of replications: There were three hosta plants per R.H. treatment.

Observation: Every two weeks after the foliar nematode inoculation in the soil, the leaves were visually checked for symptoms of nematode infection in the leaves, and a small piece of stem was taken to soak in water in a petri-dish to check for the presence of the foliar nematodes.

Results:

1. At four weeks post-foliar nematode application, foliar nematodes were detected on the petiole of the plants in the 95% R.H. but not in the 65% RH treatment.
2. At ten weeks post-treatment, leaf infection was observed on the plants in the 95% R.H. treatment but not in the 65% R.H. treatment, and the infection was confirmed by soaking the infected leaves in water in a petri-dish for 24 hours, by observing the presence of nematodes under the microscope.

Experiment 4: To determine whether foliar nematodes migrate into the soil with the onset of winter

A fully-replicated pot experiment was set up to determine whether foliar nematodes actively migrate into the soil when the temperatures begin to fall with the onset of winter. The detailed

descriptions of the treatments, experimental set up, and data recording procedures are given below.

Procedure:
1. Three artificially-infected and three healthy hosta plants grown in the pots in the growth chamber were selected for the experiment. The plants used for this test were checked for the presence of nematodes in the leaves before setting up this test and only nematode-free plants were used.
2. All pots were drench treated with 0.5% (v/v) Nemakill solution to kill the foliar nematodes in the soil, carefully avoiding contact of the chemical solution with the leaves. The soil surface in the pots was covered with plastic wraps to prevent the nematode-infected leaves from falling into the pot. This was done in the middle of September, 2014.
3. The pots were then transferred to the open garden area in October, 2014.
4. The soil was carefully sampled every two weeks to check the population of foliar nematodes until the snow fall in January.

Results and interpretation:
No nematodes were detected in the soil during the four-month observation period. This suggests that foliar nematodes do not actively migrate through the plant into the soil with the onset of winter. The nematodes may actually undergo desiccation (anhydrobiosis) in the drying leaves and then passively reach soil through the direct contact of leaves with the soil. Alternatively, frequent overhead watering/sprinkler irrigation or rain may prevent nematodes to undergo desiccation and may even enable them to fall to the ground in water/rain droplets. However, no studies on nematode desiccation or rehydration were conducted.

Experiment 5: To determine whether foliar nematodes move downwards actively or only passively through overhead sprinkler irrigation

A fully-replicated field experiment was set up in early November to determine whether foliar nematodes move downwards to the leaf bottom actively or only passively through overhead sprinkler irrigation. The detailed descriptions of the treatments, experimental set up, and data recording procedures are given below.

Procedure:
1. Two infected and two healthy hosta plants grown in the field were selected for the experiment.
2. Four leaves from each of the selected hosta plants were chosen for the treatment.
3. For the treatment, two of the selected leaves from each plant were sprayed with water every morning for a week to mimic overhead sprinkler irrigation or frequent rains and the other two leaves received no water treatment.
4. The four leaves from each plant were removed from the plant and were checked for the presence and the number of foliar nematodes. The leaves were cut and grouped into three parts: the leaf palm, the middle part of the leaf (5-cm long), and the bottom part of the leaf (5-cm long). The leaf sections were further thinned into strips, placed into 10-cm Petri-dishes and soaked in the water for 48 h. The presence of foliar nematodes in each section was then checked under microscope.

Results and Interpretation:
We did not detect nematodes from the healthy plants, but detected foliar nematodes in all the leaf sections from the infected plants. Notably, for the bottom section, significantly more number of foliar nematodes were detected when the leaves were treated with water every morning. Such results suggest that the foliar nematodes do not move actively downwards with the onset of winter. The detection of more nematodes in the bottom section when the leaves were sprayed with water indicates that the foliar nematodes may be washed down the leaves with sprinkler irrigation or rain.

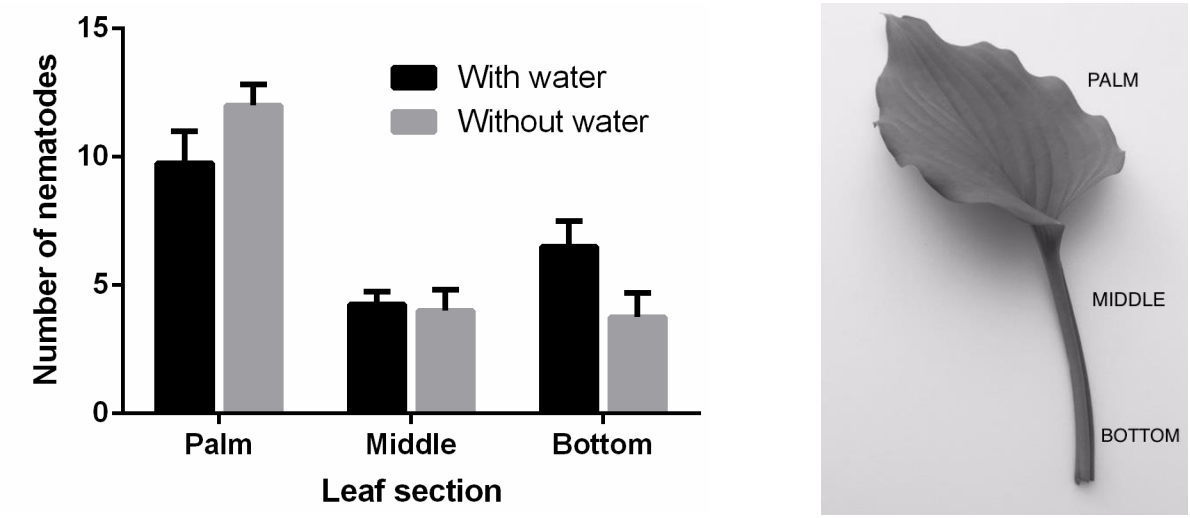

Fig. 1. Number of nematodes on various parts of the leaf when infected leaves were either sprayed with water daily or not.

Experiment 6. Evaluation of 23 chemical products against foliar nematodes in aqueous suspension laboratory bioassays

Laboratory bioassays were conducted in 24-well plates to test the effects of the 23 candidate products on the foliar nematode *Aphelenchoides fragariae* in aqueous water suspension. A high and a low concentration of each compound were tested along with a no-chemical (water) control. Four wells from a 24-well plate were assigned to each treatment at each time of sampling. Each of the four wells was considered as a separate replicate and was composed of an aliquot (500 µl) of nematode suspension containing about 500 juvenile and adult nematodes (70:30). For the high concentration treatment, 500 µl of each product were added to the 500 µl nematode suspension. For the low concentration, a solution of 50 µl of the product was diluted with 450 µl of water and then added to the nematode suspension. The nematode mortality was recorded 24, 48 and 72 h after exposure to the compounds. At each observation, a thoroughly mixed 200 µl sub-sample from each well of 24-well plate was transferred into a 5-cm Petri dish containing 5 ml water and held at room temperature for the recovery of nematodes for 72 h. Numbers of live and dead

nematodes were counted after concentrating the suspension to 1 mL. Non-motile nematodes were still considered alive if they responded to prodding by a fine probe.

Results:

The most effective products identified from the laboratory bioassay included: Grapefruit seed extract, Ammonia, Clorox, $KMnO_4$, NaDCC, Nemakill, Pylon and ZeroTol (**Tables 1** and **2**).

Table 1: Mortality of *Aphelenchoides fragariae* after exposure to the tested products in water at high concentration

Product	Dilution	Mean mortality (Mean ± SEM, n=4) (%)		
		24 h	48 h	72 h
Bayer Veg-Garden Insect Spray	50% (v/v)	5±2	8±1	8±1
Sevin	50% (v/v)	12±6	44±2	48±2
Bayer Multi-Insect Control	50% (v/v)	15±1	27±6	28±4
Permethrin	50% (v/v)	16±4	41±3	40±2
Acetamiprid	50% (v/v)	24±2	29±5	29±4
KONTOS	50% (v/v)	33±1	40±0	42±0
Merit	50% (v/v)	36±1	51±1	70±1
Cygon	10^{-3}% (w/v)	57±1	63±1	68±1
AzaMax	50% (v/v)	65±4	75±4	77±5
Abamectin	2% (w/v)	72±2	77±2	77±1
Clothianidin	10^{-3}% (w/v)	73±1	78±0	80±0
Oregano Oil	50% (v/v)	79±1	85±1	96±0
Ortho Bug B Gon	50% (v/v)	87±4	86±1	87±2
Spectracide Insect Killer	50% (v/v)	90±3	92±3	92±2
Neem Oil	50% (v/v)	91±3	99±1	100±0
Ammonia	20% (v/v)	100±0	100±0	100±0
Clorox	20% (v/v)	100±0	100±0	100±0
GFS Extract	50% (v/v)	100±0	100±0	100±0
$KMnO_4$	2% (w/v)	100±0	100±0	100±0
NADCC	2% (w/v)	100±0	100±0	100±0
Nemakill	50% (v/v)	100±0	100±0	100±0
Pylon	50% (v/v)	100±0	100±0	100±0
ZeroTol	50% (v/v)	100±0	100±0	100±0

Table 2: Mortality of *Aphelenchoides fragariae* after exposure to the tested products in water at low concentration

Product	Dilution	Mean mortality (Mean ± SEM, n=4) (%)		
		24 h	48 h	72 h
Bayer Veg-Garden Insect Spray	5% (v/v)	5±1	8±2	8±1
Sevin	5% (v/v)	6±2	7±2	7±2
Bayer Multi-Insect Control	5% (v/v)	7±2	13±4	16±3
Oregano Oil	5% (v/v)	7±1	11±2	12±2
Acetamiprid	5% (v/v)	8±2	9±2	7±1
Permethrin	5% (v/v)	8±3	22±5	23±4
Merit	5% (v/v)	9±0	9±0	9±0
KONTOS	5% (v/v)	23±0	26±0	27±0
Ortho Bug B Gon	5% (v/v)	27±3	50±3	49±2
AzaMax	5% (v/v)	33±4	51±12	52±11
Neem Oil	5% (v/v)	36±8	71±12	72±12
Cygon	10^{-4}% (w/v)	53±2	55±3	62±4
Abamectin	0.2% (w/v)	59±1	60±0	63±1
Clothianidin	10^{-4}% (w/v)	62±1	61±1	58±1
Spectracide Insect Killer	5% (v/v)	62±3	68±4	66±4
GFS Extract	5% (v/v)	94±2	100±0	100±0
GFS Extract	0.5% (v/v)	86±1	92±1	100±0
Ammonia	5% (v/v)	100±0	100±0	100±0
Clorox	5% (v/v)	100±0	100±0	100±0
$KMnO_4$	0.5% (w/v)	100±0	100±0	100±0
NADCC	0.5% (w/v)	100±0	100±0	100±0
Nemakill	5% (v/v)	100±0	100±0	100±0
Nemakill	0.5% (v/v)	100±0	100±0	100±0
Pylon	5% (v/v)	100±0	100±0	100±0
Pylon	0.5% (v/v)	100±0	100±0	100±0
ZeroTol	5% (v/v)	100±0	100±0	100±0
ZeroTol	0.5% (v/v)	96±2	100±0	100±0

Experiment 7. Evaluation of chemical and hot-water soil drench treatments for the control of foliar nematodes infesting potted hosta plants

A fully-replicated pot experiment was set up to determine the effectiveness of selected chemical treatments and a hot-water soil drench to control foliar nematodes infesting potted hosta plants. The chemical treatments were selected based on our laboratory bioassay tests (reported above in Experiment 6). In addition a behavior modifying compound Clothianidin was also included based on the Hosta Societyôs request. The detailed descriptions of the treatments, experimental set up, and data recording procedures are given below.

Treatments:

1. Ammonia (2% v/v)
2. Nemakill (0.5% v/v)
3. ZeroTol (1% v/v)
4. Sodium dichloroisocyanurate (NaDCC) (0.5% w/v)
5. Pylon (Chlorfenapyr) (1% v/v)
6. Potassium permanganate (0.5% w/v)
7. Clothianidin (10 ppm)
8. Hot water drench (boiling water ~100C)
9. Untreated control

Experimental design: Randomized Block Design

Number of replications: 6 pots per treatment (two sets of three pots (blocks) per treatment)

Foliar nematode culture and inoculation: Foliar nematodes *Aphelenchoides fragariae* were cultured on agar plates with the soil-inhabiting saprophytic fungus *Rhizoctonia solani*. The nematodes were extracted from the agar plates using the Baermann funnel technique and ~800 juvenile and adult nematodes (2:1 ratio) were inoculated per pot in all the pots.

Environmental conditions: Pots were kept in a controlled environment growth chamber set at 25C and 85% Relative Humidity and pots were watered daily. The test was conducted in June and July, 2014.

Treatment application method and timing: All treatments were applied as soil drench one-week after foliar nematode inoculation.

Sampling for nematode population assessment: soil samples (10 g) were collected from each pot prior to foliar nematode inoculation and then every 15 days after the application of the treatments to assess the effect on nematode survival and multiplication in soil to determine the rapid kill and residual effects of the treatments. The nematodes were extracted from the soil samples using the Baermann funnel technique over a 72-h period, heat-killed, and counted under the microscope. Only living nematodes are extracted using the Baermann funnel technique but they have to be killed to be properly identified and counted. Sampling over times allowed us to determine if the nematode population was increasing/multiplying over time.

Results: All treatments significantly reduced the number of foliar nematodes in soil, but Pylon, Nemakill, and hot water were the most effective and virtually eliminated nematodes from soil (Tables 3, 4 and 5; Figures 2, 3, and 4).

Table 3: Number of foliar nematodes in soil (per 10 g) 7 days after the drench treatments

Treatment	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6
Control	76	42	65	36	65	82
Ammonia	7	7	8	9	6	8
Nemakill	0	0	0	3	0	0
ZeroTol	1	14	3	3	2	2
NaDCC	4	15	4	9	4	8
Pylon	0	0	1	1	2	0
$KMnO_4$	5	7	6	12	9	8
Boiling water	1	0	0	0	0	0
Clothianidin	20	22	15	16	21	34

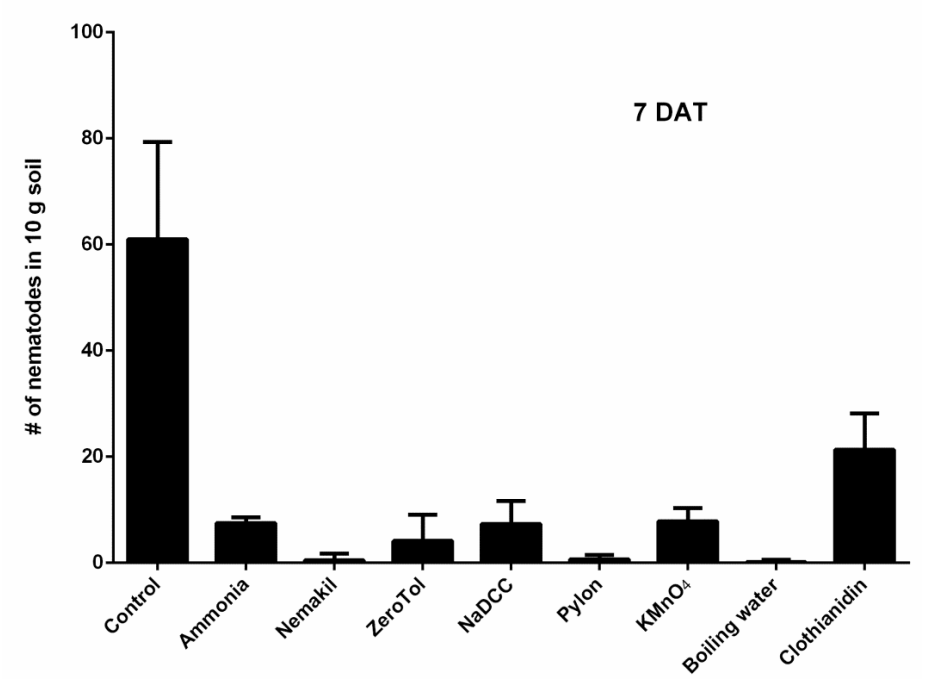

Fig. 2: Number of foliar nematodes in soil (per 10 g) 7 days after the drench treatments

Table 4: Number of foliar nematodes in soil (per 10 g) 14 days after the drench treatments

Treatment	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6
Control	36	72	34	66	82	31
Ammonia	17	9	9	9	6	8
Nemakill	0	0	0	1	0	0
ZeroTol	9	14	7	11	9	11
NaDCC	6	15	12	9	11	9
Pylon	0	0	1	0	0	0
$KMnO_4$	11	8	6	12	9	8
Boiling water	0	0	0	0	0	0
Clothianidin	18	25	9	19	25	18

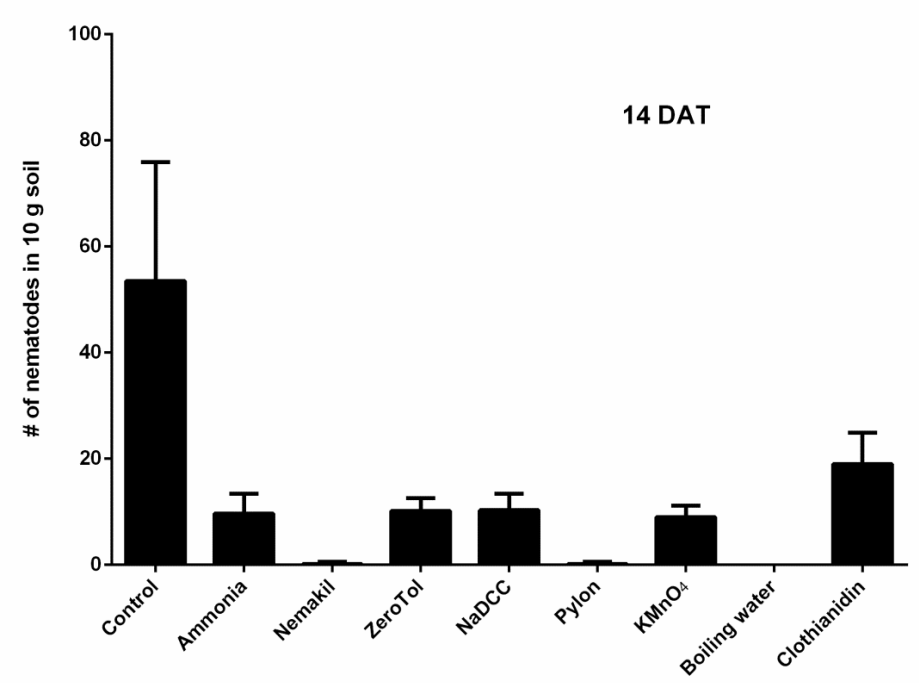

Fig. 3: Number of foliar nematodes in soil (per 10 g) 14 days after the drench treatments

Table 5: Number of foliar nematodes in soil (per 10 g) 42 days after the drench treatments

Treatment	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6
Control	54.	38.	72.	46.	59.	38.
Ammonia	12.	9.	18.	7.	12.	15.
Nemakill	0.	0.	0.	0.	0.	0.
ZeroTol	9.	8.	8.	9.	12.	3.
NaDCC	14.	15.	9.	12.	15.	3.
Pylon	0.	0.	0.	0.	0.	0.
$KMnO_4$	8.	14.	9.	11.	9.	8.
Boiling water	0.	0.	0.	0.	0.	0.
Clothianidin	18.	32.	11.	9.	18.	12.

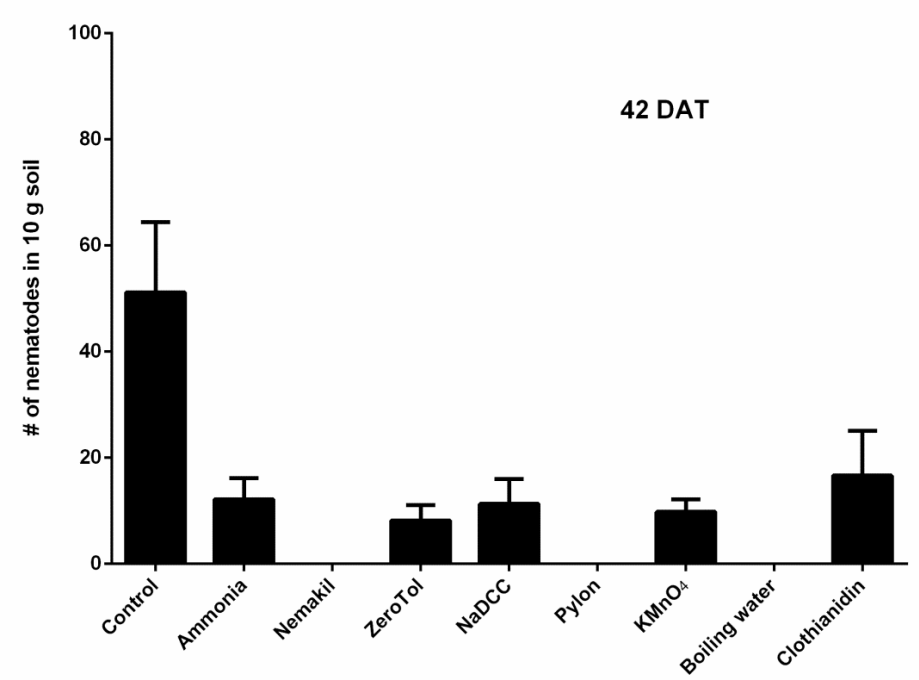

Fig. 4: Number of foliar nematodes in soil (per 10 g) 42 days after the drench treatments

Experiment 8. Evaluation of selected chemical products for the control of foliar nematodes in hosta leaves: a leaf disc test

A fully-replicated Petri-dish experiment was set up to determine the effectiveness of selected chemical treatments to control foliar nematodes in hosta leaves. The chemical treatments were selected based on our laboratory bioassay tests (reported above in Experiment 6). We used a new leaf disc approach in which we ensured that the chemicals were in contact with the nematode infested leaf discs. We determined nematode mortality 24 h after treatment of leaf discs by soaking the treated and untreated leaf discs in water to release the nematodes.

Treatments:

1. Ammonia (2% v/v, commercial rate)
2. Nemakill (0.5% v/v)
3. ZeroTol (1% v/v)
4. Sodium dichloroisocyanurate (NaDCC) (0.5% w/v)
5. Pylon (Chlorfenapyr) (1% v/v)
6. Potassium permanganate ($KMnO_4$) (0.5% w/v)
7. Untreated control

Experimental design: Leaves were collected from the nematode-infested hosta plants, and leaf discs (2.5-cm radius) were prepared from the nematode infested areas of the leaves.

Number of replications: Six leaf discs per treatment (two sets [blocks] of three discs per treatment)

Treatment application method: The leaf disc was held with forceps and treatment was applied with a sprayer by spraying two times on each side of the disc in a fume hood. The discs were held in Petri discs for 24 h at 25C. This test was conducted in October.

Nematode population assessment: At 24 h post-treatment, each leaf disc was soaked in water in a petri-dish for an additional 24 h, and the numbers of living nematodes emerging from the discs were then counted under a microscope.

Results: Nematode-infected leaf discs soaked in Pylon and Nemakill showed 100% mortality of nematodes (Table 6; Figure 5).

Table 6: Number of living foliar nematodes per leaf disc 24 h after treatment

Treatment	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6
Control	36	34	45	36	40	48
Ammonia	18	26	16	20	18	24
Nemakill	0	0	0	0	0	0
ZeroTol	14	12	10	13	10	15
NaDCC	10	8	12	9	14	13
Pylon	1	0	0	0	0	0

$KMnO_4$	12	12	9	14	11	15

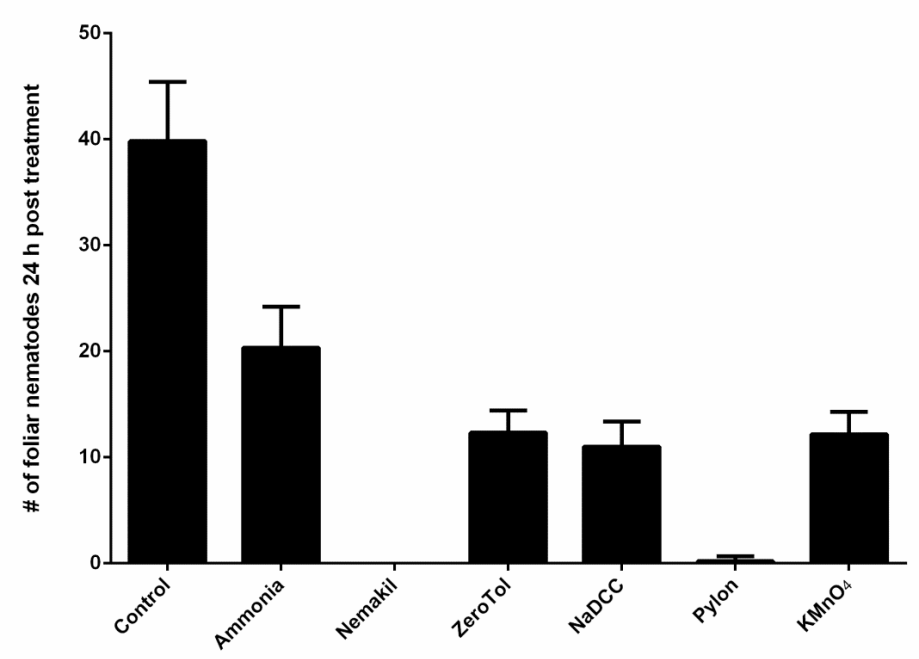

Fig. 5: Number of living foliar nematodes per leaf disc 24 h after treatment

Experiment 9. Determination of phytotoxicity of Pylon and Nemakill to healthy hosta plants

In the soil drench experiments described above (Experiment 7), we did not see any phytotoxicity by Pylon or Nemakill to the hosta plants. We conducted an additional greenhouse experiment to examine the phytotoxicity of these two products to healthy hosta plants when applied as a foliar spray. The plants were arranged in a randomized block design with two sets of three replications for each treatment. Pylon and Nemakill were prepared in water at the low concentration as used in the leaf-disc assay and sprayed onto each plant for three consecutive days. Phytotoxicity symptoms, if any, were recorded every 7 days after the application of the products. Within 8 weeks, we did not find any negative impacts of Pylon and Nemakill on the hosta leaves compared to the control plants.

Experiment 10. Determination of residual activity of Pylon and Nemakill in soil

This experiment was set up to determine the persistence of residual activity of Pylon and Nemakill in soil when used as a soil drench. As these two chemicals were very effective in killing foliar nematodes in soil, it is necessary to know how long the residual effect would last in the soil and how frequently the nematicide needs be reapplied for continued nematode control.

Treatments:

1. Soil drenched with Nemakill (0.5% v/v)
2. Soil drenched with Pylon (1% v/v)
3. Untreated soil

Experimental design: Soil in a 1-oz cup was treated with Nemakill, Pylon, and water separately. At 0, 10, 20, 30, and 40 days post the treatment, about 100 foliar nematodes in 0.2 ml water were added to each cup. The viability of foliar nematodes in soil was then assessed 72 hours later.

Number of replications: Three cups at each time point for each treatment.

Nematode population assessment: At 72 h post nematode inoculation, the soil from the cups was placed into Baermann funnels to extract the surviving nematodes, and the numbers of extracted living foliar nematodes were then counted under a microscope.

Results: Both products show high residual activity during the first 10 days after application to the soil, but nematode mortality rate then falls off quickly. Residual activity of Pylon in soil persisted up to 30 days compared to 20 days for Nemakill (Fig. 6).

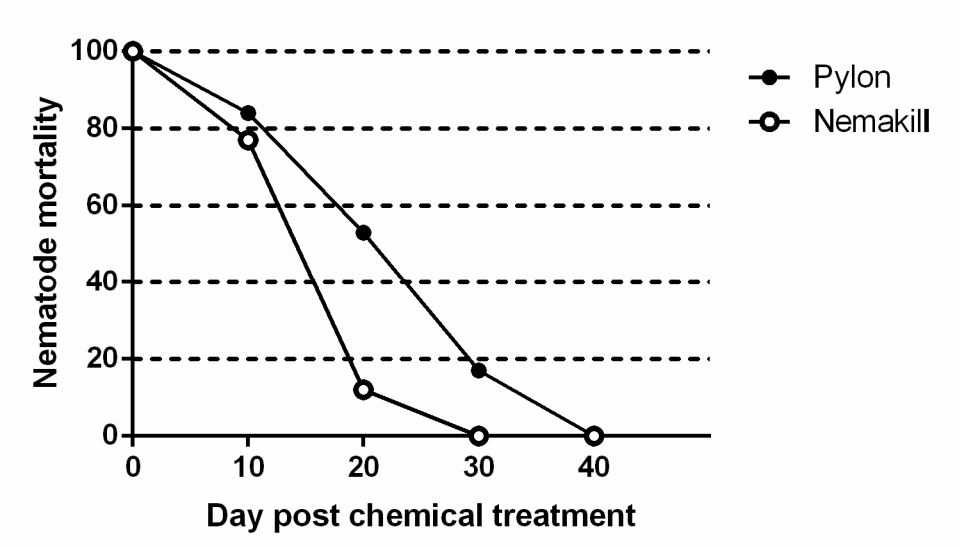

Fig. 6: Mortality (%) of foliar nematodes in the soil at 0, 10, 20, 30 and 40 days post the chemical treatment

Experiment 11. Determine effectiveness of treatment of nematode-infected hosta buds with 0.5% (v/v) Nemakill in preventing nematode infection (symptom development) in leaves later in the summer/fall season.

Procedure:
The Nemakill treatment was given to the buds of 12 dormant nematode-infected plants grown in the ground at a garden at the end of February 2015 in Knoxville, TN. There were 12 control plants that were not treated with Nemakill. All plants were spaced at least 50 cm far apart from each other. Each plant was treated with 1 liter of 0.5% (v/v) Nemakill solution prepared in tap water which was slowly drench applied to the center of the crown. The control plants received 1 liter of tap water. The plants were examined for possible nematode infection symptoms visually every month until the end of the growing season.

Results and Interpretation:
By the end of July, all untreated control plants showed nematode infection symptoms on at least 3-4 leaves. None of the treated plants had any nematode infection symptoms except one that showed restricted foliar nematode infection symptoms on two leaves. These two leaves were removed, cut into small pieces and soaked in water for 24 h in a petri dish. The leaf pieces were then removed and the water was examined under a microscope for the presence of nematodes. The total number of nematodes from these two infected leaves was found to be 12. No evidence of phytotoxicity was observed on the plants treated with Nemakill.

These results indicate that a single application of Nemakill treatment of buds of the hosta plants before they emerge from dormancy in the spring can significantly reduce the potential for nematode infection in the summer/fall season.

Experiment 12: To determine whether entomopathogenic nematode (EPN) treatment affects the population of foliar nematodes in soil and in hosta leaves

A fully-replicated field experiment was set up in October to determine whether EPN (*Steinernema carpocapsae*) treatment affects the population of foliar nematodes in soil and in hosta leaves. The detailed descriptions of the treatments, experimental set up, and data recording procedures are given below.

Procedure:
1. Foliar nematodes *Aphelenchoides fragariae* were cultured on agar plates with the soil-inhabiting saprophytic fungus *Rhizoctonia solani*. The nematodes were extracted from the agar plates using the Baermann funnel technique and diluted to ~500 nematodes per 10 ml solution.
2. Eight hosta plants grown in the garden were selected for this experiment, with four plants for the treatment and the other four plants for the control.
3. Soil around the roots of each selected plant was drenched with 20 ml of the prepared nematode solution to establish a uniform nematode population.
4. Rhizosphere soil samples (5 g) and leaf samples (10 leaf discs of 1-cm radius cut from two randomly selected leaves) were collected from each plant to examine the initial nematode

population one-week after inoculation of foliar nematodes in soil but one day before treatments. The nematode population in soil was examined using the Baermann funnel technique over a 72-h period. The leaf samples were checked after soaking the leaf discs in water for 48 h in a Petri-dish.
5. Four plants were treated with EPN-infected waxworm (*Galleria mellonella*) cadavers placed subsurface around the roots, and the other four plants were treated with freeze-killed waxworm cadavers as the control. Each plant was provided with three waxworm cadavers.
6. At 7 and 14 days after the cadaver placement, rhizosphere soil samples (5 g) and leaf samples were collected again from each plant to examine the nematode population.

Results & Interpretation:
EPN-applied in soil did not significantly affect foliar nematode numbers in leaves either 7 or 14 days after treatment. The number of foliar nematodes in soil was significantly lower in the EPN treatment 14 days after treatment, suggesting that over time EPN treatment had a suppressive effect on the population of foliar nematodes in the soil.

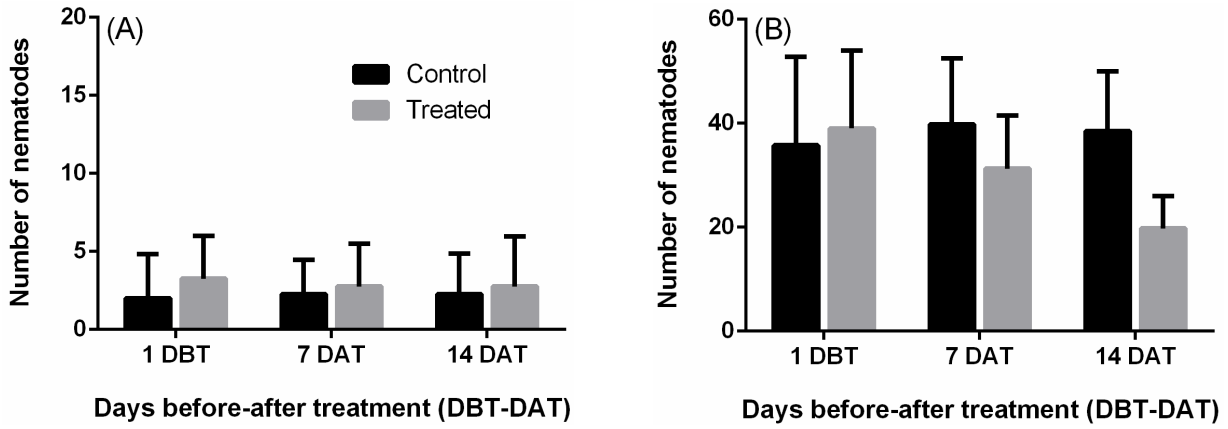

Fig. 7: Number of foliar nematodes per leaf disc (A) and in the soil (B) at 1 day before treatment and 7 and 14 days post treatment with entomopathogenic nematodes

Experiment 13: To determine whether chitinous material treatment affects the population of foliar nematodes in soil and in hosta leaves

A fully-replicated field experiment was set up in October to determine whether chitinous material treatment affects the population of foliar nematodes in soil and in hosta leaves. The detailed descriptions of the treatments, experimental set up, and data recording procedures are given below.

Procedure:

1. Foliar nematodes *Aphelenchoides fragariae* were cultured on agar plates with the soil-inhabiting saprophytic fungus *Rhizoctonia solani*. The nematodes were extracted from the agar plates using the Baermann funnel technique and diluted to ~500 nematodes per 10 ml solution.
2. A 1% (wt/wt) solution of chitin from shrimp shells (Sigma-Aldrich) was prepared in 1% acetic acid.
3. Eight hosta plants grown in the garden were selected for this experiment, with four hosta plants for the chitin treatment and the other four plants for the control.
4. Soil around the roots of each selected plant was drenched with 20 ml of the prepared nematode solution to establish a uniform nematode population in the soil.
5. Rhizosphere soil samples (5 g) and leaf samples (10 leaf discs of 1-cm radius cut from two randomly selected leaves) were collected from each plant to examine the initial nematode population one-week after inoculation of foliar nematodes in soil, but one day before the chitin treatment. The nematode population in soil was examined using the Baermann funnel technique over a 72-h period. The leaf samples were checked after soaking the leaf discs in water for 48 h in a Petri-dish.
6. Four plants were drench treated with chitin solution around the roots, and another four plants were treated with 1% acetic acid solution as a control. Each plant was provided with 500 ml of the prepared chitin solution.
7. At 7 and 14 days after the treatments, rhizosphere soil samples (5 g) and leaf samples were collected again from each plant to examine the nematode population.

Results & Interpretation:
We did not detect any significant influence of chitin on the population of foliar nematodes either in leaves or in soil 14 days after treatment. These results suggest that there is no direct toxic effect of chitinous material on nematodes. However, longer observations may reveal nematode suppressive effects due to other mechanisms.

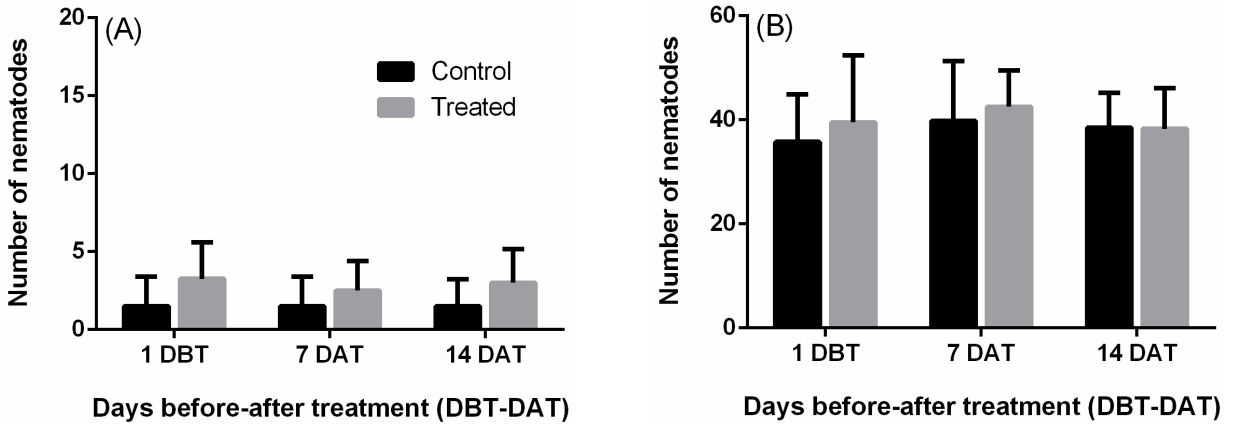

Fig. 8: Number of foliar nematodes per leaf disc (A) and in the soil (B) at 1 day before treatment and 7 and 14 days post treatment with chitin.