A. Cover Page

OARDC Matching Grant Competition

New Submission

Infection behavior and management of foliar nematodes on hosta and other shade-tolerant ornamentals

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Beginning April 1, 2013 for 24 months
Amount Requested from OARDC: $50,000

Project Summary:

Foliar nematodes (*Aphelenchoides fragariae* and *A. ritzemabosi*) pose serious threats to the horticulture industry. These nematodes cause significant economic losses to hundreds of ornamental plant species, small fruits (e.g. strawberries), and some agricultural crops including faba beans. Control of foliar nematodes has proven difficult due the lack of understanding of their biology and loss of chemical nematicides due to the implementation of Food Quality Protection Act. Based on an invited request from the American Hosta Society, this project proposes to more thoroughly investigate the infection biology of *A. fragariae* on hosta and other shade-tolerant ornamental plants and develop a comprehensive strategy to manage the nematodes in home landscapes and nurseries. Specific objectives of the proposed project are to: (i) Elucidate the complete annual infection cycle of the foliar nematodes on hosta; (ii) Determine prevalence and mechanisms of spread of foliar nematodes from hosta to other shade tolerant ornamental plants and *vice versa* under landscape conditions; (iii) Develop a comprehensive integrated approach to manage foliar nematodes on hosta and other ornamentals for nurseries and home landscapes.

B. Resubmission Response

None

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D. Project Narrative

OBJECTIVES

Based on an invited request from the American Hosta Society, this project proposes to more thoroughly investigate the infection biology of the foliar nematode *Aphelenchoides fragariae* on hosta and other shade-tolerant ornamental plants and develop a comprehensive strategy to manage the nematodes in home landscapes and nurseries.

Specific objectives of the proposed project are to:

(i) Elucidate the complete annual infection cycle of the foliar nematodes on hosta

(ii) Determine the spread of foliar nematodes from hosta to other shade tolerant ornamental plants and *vice versa* under landscape conditions

(iii) Develop a comprehensive integrated approach to manage foliar nematodes on hosta and other ornamentals for nurseries and home landscapes

RATIONALE AND SIGNIFICANCE

Foliar nematodes (*Aphelenchoides fragariae* and *A. ritzemabosi*) cause significant economic losses to hundreds of ornamental plant species, horticultural crops such as strawberries, and agricultural crops such as alfalfa, faba beans, and others. These nematodes have unique biology as they infect aerial plant parts as opposed to most other plant-parasitic nematodes that infect roots. Foliar nematodes also differ from other plant-parasitic nematodes as they can also multiply on soil fungi in addition to their numerous plant hosts. The foliar nematodes overwinter in the soil or plant parts including rhizomes, bulbs, and buds, but generally not in the roots. In the spring, the overwintering nematodes move upward most-likely on the outer surface of leaves, stems, and petioles, eventually invading leaves (Grewal and Jagdale, 2001). The infection is believed to occur either through stomata or through direct penetration of epidermis in the upper or lower of the leaves. Once inside the leaf, the nematodes feed by inserting their stylets into the leaf cells. Nematode feeding results in the formation of local lesions that first turn yellow, then brown and finally black. The blackened areas sometimes fall off the leaves. The spread of infection is often limited by large veins, resulting in characteristic symptoms on different plant species depending upon the pattern of venation.

Control of foliar nematodes is difficult due to several reasons. First, the survival biology and infection cycle of the foliar nematodes are poorly understood and weakest links in the life cycle are unknown. Second, no sources of cultivar resistance have been reported. Third, almost all the chemical nematicides have been banned by the US EPA due to the implementation of the Food Quality Protection Act (FQPA). Fourth, the use of the most effective fumigant, methyl bromide, is phased out due to its broad spectrum toxicity and the threat to the degradation of the ozone layer. Fifth, the newer and more “softer” chemicals are only partially effective. As a result the foliar nematodes now pose a serious threat to both agriculture and horticultural industries and the nursery industry now looses thousands of dollars due to quarantine regulations and returned plant shipments by the customers. Therefore, greater understanding of the infection biology of foliar nematodes and more effective and sustainable methods of controlling the foliar nematodes are needed.

Based on a request from the American Hosta Society (see letter in Appendix), this project proposes to thoroughly investigate the infection biology of foliar nematodes (*Aphelenchoides fragariae*) on hosta and other shade-tolerant plants and develop a comprehensive and integrated strategy for the sustainable management of the nematodes in nurseries and home landscapes. Specific objectives of the proposed project are to: (i) Thoroughly elucidate the complete annual infection cycle of foliar nematodes on hosta; (ii) Determine prevalence and mechanisms of spread of foliar nematodes from hosta to other shade tolerant ornamental plants and vice versa under landscape conditions; (iii) Develop a comprehensive integrated approach to manage foliar nematodes on hosta and other ornamentals for nurseries and home landscapes.

Background and Preliminary Data

Foliar nematodes, *Aphelenchoides fragariae* and *A. ritzemabosi* cause serious damage to *Hosta* and other landscape, horticultural, and agricultural plants throughout United States. These nematodes have a wide host range with over 200 species serving as hosts including common landscape plants including *Achimenes, Anemone, Angigozanrthos, Begonia, Bergenia, Bouvardia, Chrysanthemum, Coleus, Columnea, Cyclamen, Dahlia,* ferns*, Ficus, Fuchsia, Gloxinia, Hibiscus, Irish, Phloxy, Lantana, Salvia, Saintpaulia, Sinningia, Streptocarpus, Viola,* and *Zinnia.* In the United States, the cultivation and production of hosta is a multimillion-dollar industry and nurseries grow and sell over a 1000 selections representing about 10 different hosta species and their hybrids. As hosta foliage offers a variety of leaf shapes, textures, and colors, there is a growing concern among the growers and nursery managers about the leaf damage caused by the foliar nematodes. In addition, the nursery industry suffers losses due to quarantine on the sales of infected plants and bulbs from state to state.

Jagdale and Grewal (2006) studied the pathogenicity and overwintering survival of the foliar nematode, *A. fragariae*, infecting *Hosta* spp. Nematodes applied to either lower or upper sides of non-injured and injured hosta leaves were able to infect and produce typical symptoms on nine cultivars. Leaves of only four cultivars (Borschi, Fragrant Blue, Patomic Pride, and Olive Bailey Langdon) showed no symptoms of nematode infection. The nematodes overwintered as juveniles and adults in soil, dry leaves, and dormant buds, but not in roots. Nematode winter survival was higher in dormant buds and soil from the polyhouse than in an open home garden. Of the nematodes found in the dormant buds, 35% to 79% were located between the first two outside layers of the buds. The nematodes tolerated 8 hr exposure to 40°C and −80°C in leaf tissues. Relative humidity influenced nematode migration from soil to leaves. The presence of nematodes only on the outer surface of foliage (leaves and petioles) confirmed the migration of *A. fragariae* on the surface of the plants. Of the total number of nematodes found on the foliage, 25% to 46% and 66% to 77% were alive at 90% and 100% relative humidity, respectively, suggesting that high moisture is required for the survival and upward movement of nematodes. They concluded that adults and juveniles of *A. fragariae* overwinter in soil, infected dry leaves and dormant buds, and migrate in films of water on the outer surface of the plant during spring to leaves to initiate infection. **However, it is unknown whether the eggs of the foliar nematodes also overwinter. This is important because the eggs may withstand hot water and chemical treatments**. **Also unknown is the fact that how and when nematodes migrate back to the soil and crowns in the autumn for overwintering. This knowledge will enable the development of more effective strategies for managing the foliar nematodes.**

Kohl et al. (2010) studied population dynamics of *A. fragariae* over three growing seasons and during overwintering for naturally-infected, container-grown lantana (*Latana camara*) plants in a North Carolina nursery. During the growing season, the foliar nematode population in symptomatic leaves peaked in July each year then remained above 100 nematodes/g fresh weight into late summer. Foliar nematodes were also detected in asymptomatic and abscised leaves. Results suggest that leaves infected with foliar nematodes first develop symptoms at populations of about 10 nematodes/g. Foliar nematodes were detected in symptomatic and asymptomatic plant leaves and in abscised leaves during overwintering in a polyhouse, but the number of infected plants was low. A steep disease gradient was found for infection of lantana plants by *A. fragariae* on a nursery pad with sprinkler irrigation. When the canopies of initially healthy plants were touching the canopies of an infected plants, 100% of the plants became infected within 11 wk, but only 5 to 10% became infected at a canopy distance of 30 cm. Overwintering of *A. fragariae* in infected plants and a steep disease gradient during the growing season suggests strict sanitation and an increase in plant spacing are needed to mitigate losses from this nematode pest. **So what remains to be answered is whether nematodes infesting hosta plants can infect nearby non-hosta susceptible plants and *vice-versa*. Also it is unknown of there are any host-specific races in *A. fragariae*.**

Jagdale and Grewal (2002) studied 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 *A. fragariae* on the most popular ornamental, hosta (*Hosta* spp), for two consecutive years. They found ZeroTol (270 g liter-1 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-1 EC, trichlorfon 800 g kg-1 SP, ethoprophos 100 g kg-1 GR, oxamyl 100 g kg-1 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. **However, one problem with ZeroTol is its low persistence and incomplete control of the foliar nematodes. Therefore growers are looking for a more sustainable control alternative.**

Jagdale and Grewal (2004) evaluated the effectiveness of a hot water drench for the control of *A. fragariae* infesting hosta (Hosta sp.) and ferns (*Matteuccia pensylvanica*). Drenching with hot water at 70^{o}C and 90^{o}C in October reduced ($P < 0.05$) *A. fragariae* in the soil but not in the leaves relative to the control (25^{o}C) 300 days after treatment (DAT). Plants drenched with 90 degrees 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 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. They concluded 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. **However, field trials are still needed to demonstrate the safety and efficacy of the hot-water treatment on different species/varieties of hosta and other ornamental plants.**

Jagdale and Grewal (2006) studied the influence of entomopathogenic nematode *Steinernema carpocapsae* infected wax moth, *Galleria mellonella*, cadavers and their extracts on the foliar nematode *A. fragariae* on hosta in the greenhouse. Curative application of both cadavers and their extracts (prepared by crushing cadavers in water) in 1999 and only cadaver extract in 2000 significantly reduced *A. fragariae* numbers in leaves compared to the control 30 and 45 days after treatment (DAT). Both the cadavers and their extracts significantly reduced *A. fragariae* numbers in potting medium as compared to the control within 15, 30 and 45 DAT. Preventively applied cadavers also significantly reduced *A. fragariae* numbers in leaves compared to the control in 2001 but not in 2002. In both years, the numbers of *A. fragariae* infected leaves per plant decreased in the preventive cadaver treatment compared with the control. Preventively applied cadavers significantly reduced *A. fragariae* numbers in the potting medium compared to the control at 30 DAT in 2002 and 150 DAT in 2001. Dead (crushed or autoclaved) *S. carpocapsae* caused significant mortality at 4 and 8 DAT but live *S. carpocapsae* had no detrimental effect on *A. fragariae* in water suspension. Both *S. carpocapsae* infected cadavers and their extracts were toxic, causing over 75% *A. fragariae* mortality within 2 to 8 DAT. Toxicity of intact cadavers to *A. fragariae* increased with exposure time. They concluded that both curative and preventive applications of *S. carpocapsae* infected host cadavers can suppress *A. fragariae* populations on hosta. This suppressive effect can result from toxic metabolites produced by the nematodes or their symbiotic bacteria. **These results are summarized in Fig. 1 and Fig. 2.**

Jagdale et al. (2009) further investigated the effects of *S. carpocapsae* and its symbiotic bacterium, *Xenorhabdus nematophila* applied to the potting medium on pyrogallol peroxidase (P-peroxidase), guaiacol peroxidase (G-peroxidase) and catalase activities in *Hosta* sp and *Arabidopsis thaliana* leaves as components of induced systemic resistance. They found that P-peroxidase activity was significantly higher in the leaves from hosta plants treated with *S. carpocapsae* infective juveniles (IJs) and *S. carpocapsae* infected insect cadavers than in the leaves from the control plants 2 weeks after treatment. The G-peroxidase activity was significantly higher in *S. carpocapsae* infected cadaver and *X. nematophila* treatments 10 and 15 DAT and in *S. carpocapsae* IJs treatment 5 and 15 DAT. The catalase activity in hosta leaves was significantly higher in *S. carpocapsae* infected cadaver and *X. nematophilus* treatments compared with the control 5 and 15 DAT and in *S. carpocapsae* IJs treatment 5 and 10 DAT. Further, the catalase activity in *A. thaliana* leaves was significantly higher in *S. carpocapsae* IJs treatment than in the control 7 DAT. They also determined the effects of *S. carpocapsae* infected cadavers and *S. carpocapsae* IJs on *PR*1-gene expression in transgenic *A. thaliana* leaves through GUS (β-glucuronidase) activity assay and found that the *PR1*-gene was expressed in leaves from all treatments except the control. They concluded that the EPNs and their symbiotic bacteria induce systemic resistance in hosta, reducing foliar nematode infection and reproduction in hosta leaves. **These results are summarized in Fig. 3. We now need to evaluate the effectiveness of the EPN-induced systemic resistance approach in the field.**

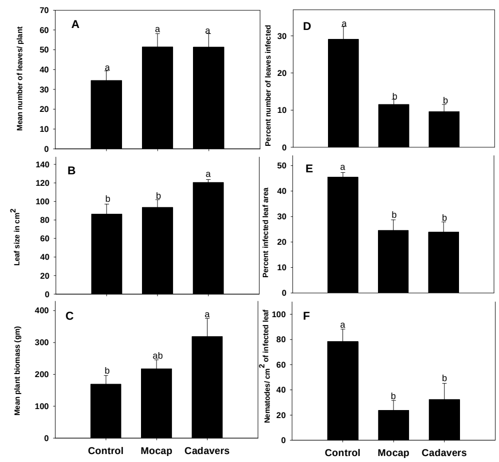

Fig. 1. Effect of *S. carpocapsae* infected wax moth cadavers, tap water (control), ethoprophos on total number of leaves per plant, leaf size, plant biomass, the numbers of nematode-infested leaves and percent chlorotic area caused by nematode infection and the number of *A. fragariae* in hosta leaves 150 days after treatment. Each bar represents a mean (± SE) of eight replicates. Bars with the same lower case letter are not significantly different by LSD test, *P*< 0.05. Preliminary data and evidence for induced resistance

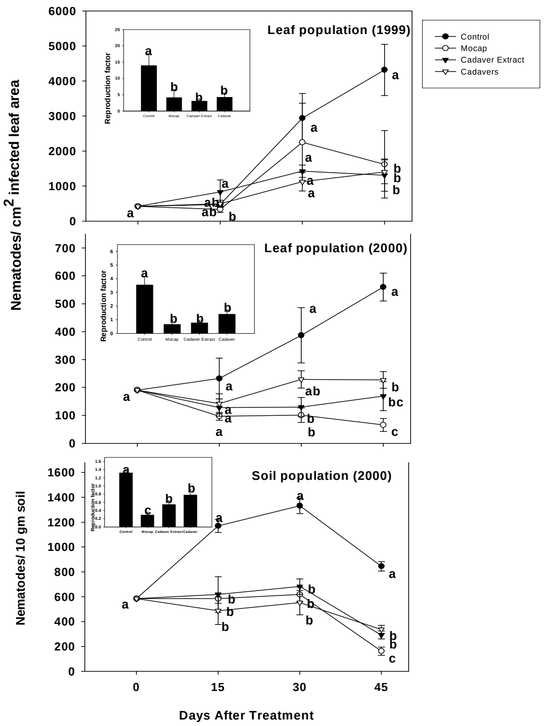

Fig. 2. Effect of *S. carpocapsae* infected wax moth cadavers, their extract, tap water (control), and ethoprophos on the population of *A. fragariae* in hosta leaves (1999 and 2000) and in the soil (in 2000) 0, 15, 30 and 45 days after treatment. Data point in each time interval in the line graph and also each bar represents a mean (± SE) of four replicates. The data points in each time interval in the line graph and bars with the same lower case letter are not significantly different by LSD test, *P*< 0.05. Reproduction factor = p_f (Final population) ÷ (Av. 449.2 and 199.3 initial population for 1999 and 2000 respectively.

Fig. 2. Jagdale et al.

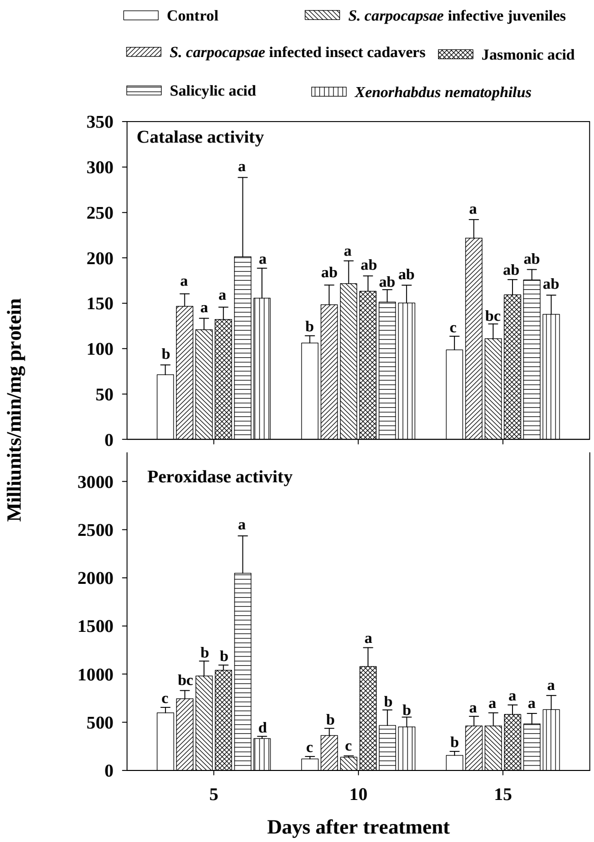

Fig. 3. Effect of *Steinernema carpocapsae* infective juveniles, *Steinernema carpocapsae* infected wax moth (*Galleria mellonella*) cadavers, jasmonic acid, salicylic acid and symbiotic bacteria, *Xenorhabdus nematophilus* on the activities of both catalase and G-peroxidase enzymes in the intact hosta leaves. Bars (mean ± SE) in the same and between the days with same letter(s) are not significantly different according to Tukey's mixed repeated measure procedure ($P < 0.05$).

APPROACH

Objective 1: Elucidate missing links in the infection cycle of the foliar nematodes on hosta

Sub-objective (A) Determine if foliar nematodes overwinter as eggs: In our previous research, we observed that the adults and juveniles of foliar nematodes can overwinter in the soil and hosta crowns. However, it is unknown whether nematode eggs also overwinter. This information is critical as eggs may withstand the hot water and chemical treatments that are effective against the nematode juveniles and adults. Therefore, soil and hosta crown samples will be collected from foliar nematode infested nurseries at monthly intervals from December to April. At each sampling time, ten separate soil and ten crown samples will be collected from three different nurseries and nematode eggs will be extracted using a centrifugation floatation technique and counted. Viability of the extracted eggs will also be determined by checking the hatching of the eggs in water at 25°C over a two-week period. To capture the influence of any differences in the severity of winter conditions, the experiment will be conducted twice (once each in year 1 and year 2) and the daily winter temperatures will be obtained from the OARDC weather station.

Sub-objective (B) Determine factors affecting the upward movement of overwintering nematodes to leaves in the spring and summer and downward movement back to the crowns and soil in the autumn: We have already demonstrated that overwintering adult and juvenile nematodes move upward in the spring on the outside of growing plant and infect only the fully expanded leaves late in the summer, but factors affecting this upward movement are unknown. Also unknown is how and when nematodes migrate back to the soil and crowns in the autumn for overwintering and what factors affect this downward movement. Therefore, we plan to evaluate the influence of two major environmental factors, temperature and humidity, on upward and downward migration of the nematodes during differences phases of plant growth in growth chamber experiments. Nematode-infested hosta crowns will be planted in pots and held at three different Relative humidity conditions: 85%, 90%, 95%, and 100%, in different growth chambers. Samples of growing buds, petioles and leaves will be collected at 15 day intervals until appearance of symptoms of nematode-infection on the leaves. Plant samples will be rinsed in water to examine external migration of foliar nematodes on surfaces. The rinsed plant samples will be then stained with acid-fuchsin to examine internal migration of nematodes towards leaves through plant tissues. Ten plants will be examined at each relative humidity treatment and the experiment will be repeated in year 1 and 2.

Objective 2: Determine prevalence and mechanisms of spread of foliar nematodes from hosta to other shade tolerant ornamental plants and vice versa under landscape conditions

A plant-to-plant dispersal study will be conducted to determine if the nematodes can move from one con-specific host plant to another and between hetero-specific host plants. We will use hosta and fern plants planted either singly or together at three different spacings: 0-cm (touching), 30-cm, and 100 cm apart from each other in a nursery setting. At each spacing one infected and one healthy plant will be planted in one-gallon pots. Spacing (three) and plant species (infected hosta with healthy hosta; infected hosta will healthy fern; infected fern with healthy hosta) be replicated five times, resulting in a total of 45 plants. This experiment will be repeated at a different location in the same year. Plants will be water daily with a hose except when there is sufficient rain. Leaves will be observed for nematode symptoms at weekly intervals starting late in the summer until the autumn. If no symptomatic leaves were observed, the non-symptomatic leaves will be randomly collected and examined under the microscope for nematode infection.

Objective 3: Develop a comprehensive approach to manage foliar nematodes on hosta and other ornamentals for nurseries and home landscapes

Sub-objective (A) Evaluate the efficacy of different chemical products on foliar nematodes in laboratory bioassays: Laboratory bioassays will be conducted in 24-well plates to test the effects of imidacloprid, DiSyston, Cygon, Pylon, AzaMax on *A. fragariae* at 25 ± 2^{o}C as described by Jagdale and Grewal (2002). A high and a low concentration of each compound will be tested along with a no-chemical (water) control. ZeroTol (hydrogen dioxide) will be included as chemical standard. Four wells from a 24-well plate will be assigned for each treatment, and each well will be considered as a separate replicate. A 500 µl solution of each concentration will be transferred into each well and a 500 µl suspension containing about 1500 nematodes will be added to each well to achieve a desired % active ingredient (a.i.) for each product. Observations on percent nematode mortality will be recorded 24, 48 and 72 h after exposure. At each observation, a thoroughly mixed 200 µl sub-sample from each well of 24-well plate will be transferred into a 5 cm dia. dish containing 5 ml water and held at room temperature for the recovery of nematodes for 72 h. Numbers of live and dead nematodes will be counted after concentrating the suspension to 1 mL. Non-motile nematodes will be considered alive if they respond to prodding with a fine probe.

Sub-objective (B) Evaluate the effectiveness of cultural, biological, and chemical treatments to manage foliar nematodes on hosta and ferns in greenhouse experiments: The effectiveness of cultural (hot water treatment), biological (entomopathogenic nematodes) and chemical (pesticides) treatments targeted at soil and crown application to prevent foliar nematodes from migrating upwards and producing leaf symptoms will be evaluated in pots containing naturally infested hosta and fern plants in a green house. Treatments will include: (i) live infective juveniles of the entomopathogenic nematode, *S. carpocapsae*; (ii) *S. carpocapsae* infected waxworm cadavers, (iii) hot water drench (cultural practice); (iv) the most effective pesticide identified in sub-objective 3(A) above; (v) ZeroTol (a Standard); and (vi) an untreated control. All treatments will be applied immediately prior to sprouting of the plants to avoid plant injury and the pots will be arranged in a randomized complete block design. The rates of application and water temperature will be based on the outcome of the sub-objective 3(A) above and on our previous studies reported in the Background and Preliminary Data section. Initial nematode population in soil will be recorded just before the application of the treatments and thereafter at every 30 day intervals. Visual inspection of leaves for symptoms of nematode infection will be carried out at weekly internals until the end of the season. Nematode population in symptomatic leaves will also be quantified at the end of the season to compare the treatments.

Data Analysis and Interpretation: All data will be subjected to appropriate statistical analyses. Specifically multivariate procedures of the SAS (SAS Institute, 2008) will be used for data analysis and interpretation. Since the experiment will be conducted on different sites or years, data will be analyzed for spatial/temporal variability effects. These procedures will provide the most up-to-date capabilities for repeated measures of analysis of variance and analysis of covariance to separate simple and interactive effects of predictor variables on dependent variables using the F-protected Least Significant Difference (LSD) test.

Pitfalls and limitations

No major pitfalls are expected, except for the availability of greenhouse/growth chamber space. Requisition for space will be made as soon as the proposal is approved for funding.

Means of applying results

The results will be shared with the members of the American Hosta Society through annual reports and presentations at their annual national and regional meetings.

Schedule of Activities

Due to the annual life cycle of the foliar nematodes most experiments will be conducted simultaneously taking advantage of the natural infestation/migration/dispersal of the nematodes

Schedule of Major Tasks:

Task	Year 1	Year 2
1. Objective 1	|--	---------------♦
2. Objective 2	I----------♦	I-----------♦

Role of Investigators

Dr. Grewal will serve as a lead coordinator of this project and will be responsible for all aspects this project and reports. He will also take a lead on soil food web, weed, pest, and biocontrol studies proposed. A post-doc will be hired to carry out all the sampling and analysis and to write reports, popular and per reviewed articles.

E. References

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SAS Institute (2008) SAS User's guide: Statistics. Version 9.13. SAS Institute, Cary, NC.

F. Signature Areas

The proposed project directly addresses the Food Security, Production, and Human Health signature area of CFAES.

G. Collaborative Arrangement

None

H. Strategy for Acquiring Future Funding

We plan to use the preliminary data obtained in this project for seeking funding from several federal sources. Specifically, we will submit a proposal to the National Institute of Agriculture's Specialty Crops Research Program and another one to the Organic Transitions Programs focused of strawberries.

I. Previous SEEDS Funding: Grewal

Category: Matching
Period of Support: April 2009- September 2011
Title of the project: Effect of organic and inorganic fertilizers on turfgrass ecosystem health
Summary of objectives: The overall objectives of this field plot study was to assess the effects of organic and inorganic fertilizers on multiple biological and ecological aspects of turfgrass ecosystem, including overall turfgrass aesthetic quality (greenness), plant health (shoot nutrient, alkaloid, and free amino acid contents), turfgrass resistance to weed, insect, and disease infestation, soil health (relative concentrations of soil nutrient contents including NH_4-N and NO_3-N [two major forms of N utilized by the plants], dissolved organic nitrogen [important N reservoir], soil organic matter [a key indicator of overall soil quality], soil microbial biomass [a key indicator of the microbial activity and nutrient cycling efficiency], and soil nematode community structure [a bio-indicator of the overall condition and functioning of the soil food web]).
Results: The study was conducted on tall fescue plots established in 2006 on either subsoil or topsoil. The recommended annual rate of each fertilizer was applied in two separate applications, one in June/July and the other in September/October. Turfgrass visual quality was recorded before the start of the trial in July 2008 and then twice or three times each year immediately before and then one week after each fertilizer application. Soil samples were collected once in the spring and once in the fall annually to determine soil health via a comprehensive nematode community analysis. All soil nematodes were extracted using the Baerman funnel method and identified to genus level. The results indicate that both turfgrass quality and soil health remained higher in the plots established on topsoil compared to those established on subsoil. Overall, both fertilizers had small but positive effect on turfgrass quality and the two fertilizers generally did not differ from each other. The effect of the two fertilizers on the soil health was a bit more variable. Overall, the Nature's Touch fertilizer caused greater reductions in populations of plant-parasitic nematodes and had more positive effect on the non-plant-parasitic nematodes compared to the Scotts fertilizer.
Publication: Cheng, Z., Salminen, S. & Grewal, P. S. 2010. Effect of organic fertilizers on the greening quality, shoot and root growth, and shoot nutrient and alkaloid contents of turf-type endophytic tall fescue, *Festuca arundinacea*. *Ann. Appl. Biol.* 156, 25-37.

Additional funding received: Full matching and additional funding in the amount of $150,000 was received from MTD Corporation.

J. Matching Funding

This proposal has been developed based an invitation from the American Hosta Society who has committed to providing the matching funds from the society (see Attached letter from Vice President, Rob Mortko) and assisting with the acquisition of funds from other relevant plant societies including the American Fern Society, the American, the American Isis Society, the National Chrysanthemum Society, and the North American Strawberry Growers Association.

K. Curriculum Vitae

PARWINDER S. GREWAL

Education:
Ph.D. (Zoology), 1990, Imperial College, University of London, UK
MS (Nematology), 1983, Punjab Agricultural University, India
BS Agriculture (Honors in Plant Protection), 1981, Punjab Agricultural University, India

Employment and Professional History:

2011-	University Distinguished Scholar, The Ohio State University (OSU)
2008-	Director, OARDC Research Internships Program, OSU
2006-	Professor, Department of Entomology, OSU
2004-	Director, Center for Urban Environment & Economic Development, OARDC
2003-	Director, Urban Landscape Ecology Program, OARDC
2002- 2005	Associate Professor, Department of Entomology, OSU
1997-2001	Assistant Professor, Department of Entomology, OSU
1995 - 1997	Research Group Leader, Biosys, Inc., Columbia, MD
1993 - 1995	Senior Scientist and Research Manager, Biosys, Inc., Palo Alto, CA
1991 - 1993	Post-doctoral Research Associate, Entomology, Rutgers University, NJ
1987 - 1991	Higher Scientific Officer, Entomology, HRI, Littlehampton, England
1984 - 1987	Scientist, Entomology and Nematology, NCMRT, ARS, Solan, India

Recent Honors and Awards:

2011	University Distinguished Scholar Award, The Ohio State University
2011	OARDC Innovator of the Year Award, The Ohio State University
2009	Fellow, Society of Nematologists, USA
2009	OARDC Distinguished Senior Faculty Award, Ohio State University
2008	Recognition Award in Urban Entomology, Entomological Society of America (ESA)
2003	Award for Excellence in Integrated Pest Management, North Central Branch, ESA.
2002	Syngenta Crop Protection Award, Society of Nematologists, USA.
2002	OARDC Distinguished Junior Faculty Award, The Ohio State University

Selected Professional Service:

2008-2011	President, President Elect, and Vice President, Society of Nematologists, USA
2006-2008	Chair of the Regional Project S-1024 on Microbial Control
2011-2012	Panel Member, NIFA-Pre-doctoral and Post-doctoral Fellowships Program
2006	Panel Manager, USDA-NRI Integrative Biology of Arthropods and Nematodes
2005	Panel Member, USDA-NRI Integrative Biology of Arthropods and Nematodes
2001	Panel Member, USDA-NRI Entomology/Nematology Competitive Grants
2005-2008	Editorial Board, *Annals of Applied Biology (UK)*
2001-2006	Editorial Board, *Biocontrol Science and Technology (UK)*
2001-2004	Editorial Board, *Biological Control (USA)*
1999-2001	Editor, *Journal of Nematology (USA)*
1991-	Editorial Board, *International Journal of Nematology (UK)*

Recent Funding History:

Grewal, P.S., Islam, K.R., McCoy, E.L., Kumarappan, S., and Sundermeier, A. Assessing, modeling, and maximizing ecosystem services in long-term and transitioning organic farming systems. NIFA Organic Transitions Program, 2012-2015, $750,000

Grewal, P.S., Gao, G., Vickner, S. & Zhu, H. Sustainable Management of grape root borer in vineyards, USDA-PMAP, 2012-2014, $200,000

Tiniano, J., Jones, K.B., Grewal, P.S., Rabe, M., Wagenbrenner, M. An agrarian urbanist overlay for central city development, US Department of Housing & Urban Development, 2011-2013, $870,000

Walton, B.M., Mach, J.J., Schwarz, T., Grewal, P.S., and Beach, D. Urban parks and vacant lands as mechanisms of ecological and social stability in the Cleveland Urban Ecosystem, NSF-ULTRA-EX, 2009-2012, $279,500

Recent Publications:

Chapters in edited books [Invited, peer reviewed: Total 21]

Grewal, P.S. 2012. Entomopathogenic nematodes as tools in integrated pest management. In: *Integrated Pest Management: Principles and Practice* (D. P. Abrol, Ed.), CABI Publishing, Wallingford, UK, 162-236.

Grewal, P.S. 2012. From IPM to ecosystem management: the case of urban lawn. In: *Integrated Pest Management: Principles and Practice* (D. P. Abrol, Ed.), CABI Publishing, Wallingford, UK, 450-488.

Grewal, P.S., Bai, X., Jagdale, G.B. 2011. Longevity and stress tolerance of entomopathogenic nematodes. In: *Molecular and Physiological Basis of Nematodes Survival* (R. N. Perry and D. Wharton, Eds.), CABI Publishing, Wallingford, UK, pp. 157-181.

Recent Refereed Publications (Career Total 175)

Grewal, P.S. & Grewal, P.S. 2012. Can cities become self-reliant in energy? *Cities*, in press

Knight, A., Cheng, Z., Grewal, S.S., Islam, K.R., Kleinhenz, M.D., and Grewal, P.S. 2012. Soil health as a predictor of lettuce productivity and quality: A case study of urban vacant lots. *Urban Ecosystems* (in press)

Park, S., Taylor, R.A.J. & Grewal, P.S. 2012. Taylor's power law describes life history characteristics: An empirical analysis of spatial distribution of soil nematode community in urban landscapes. *Applied Soil Ecology* (in press)

An R. & Grewal P.S. 2012. Differential gene expression in closely-related bacterial species by comparative hybridization. Methods in Molecular Biology, 815, 103-119.

An, R. & Grewal, P.S. 2012. Selective capture of transcribed sequences: a promising approach for investigating bacterium-insect interactions. *Insects* 3, 295-306.

An, R., Voss, M. Jagdale, G.B. & Grewal, P.S. 2012. Differences of immune defense in selected inbred lines of *Heterorhabditis bacteriophora* in two white grub species. *Insects* 3, 378-389.

Blaine, T.W., Clayton, S., Robbins, P. & Grewal, P.S. 2012. Homeowner attitudes and practices towards residential landscape management. *Environ. Manag*. 50, 257-271.

Grewal, S.S. and Grewal, P.S. 2012. Can cities become self-reliant in food? *Cities* 29, 1-11.

Yadav, P., Duckworth, K. & Grewal, P.S. 2012. Habitat structure influences below ground biocontrol services: A comparison between urban gardens and vacant lots. *Landscape and Urban Planning* 104, 238-244.

Yadav, P., Mitch, B., Foster, W. & Grewal, P.S. 2012. Factors affecting mosquito populations in the constructed and created wetlands. *Urban Ecosystems* 15, 499-511.

An, R. & Grewal, P.S. 2011. PurL gene expression affects biofilm formation and symbiotic persistence of *Photorhabdus temperata* in the nematode *Heterorhabditis bacteriophora*. *Microbiology* 157, 2595-2603.

Briar, S.S., Miller, S.A., Stinner, D., Kleinhenz, M.D., & Grewal, P.S. 2011. Effect of different organic transition strategies for peri-urban vegetable production on soil properties, nematode community, and tomato yield. *Appl. Soil Ecol*. 47, 84-91.

Deol, Y.S., Jagdale, G.B., Canas, L. & Grewal, P.S. 2011. Delivery of entomopathogenic nematodes directly through commercial growing medium via the inclusion of infected host cadavers: A novel approach. *Biol. Contr.* 58, 60-67.

Grewal, S.S., Cheng, Z., Wolboldt, M., Masih, S., Knight, A., Huda, M. & Grewal, P.S. 2011. An assessment of soil nematode food webs and nutrient pools in community gardens and vacant lots in two post-industrial American cities. *Urban Ecosystems* 14, 181-190.

Maneesakorn, P., An, R., Daneshvar, H., Taylor, K., Bai, X., Adams, B.J., Grewal, P.S. & Chandrapatya, A. 2011. Phylogenies and co-phylogentic relationships between the entomopathogenic nematodes (*Heterorhabditis*: Rhabditida) and their symbiotic bacteria (*Photorhabdus*: Enterobacteriacae). *Mol. Phylog. Evol.* 59, 271-280.

Morris, E.E & Grewal, P.S. 2011. Susceptibility of the adult Japanese beetle, *Popillia japonica* to entomopathogenic nematodes. *J. Nematol.*43, 190-194.

Zhu, H., Grewal, P.S. & Reding, M. 2011. Development of a desiccated cadaver delivery system to apply entomopathogenic nematodes for control of soil pests. *Appl. Eng. Agric*. 27, 317-24.

An, R. Bai, X. & Grewal, P.S. 2010. Reliable fusion PCR using Taq polymerases and pGEM-T easy vectors. *World J. Microbiol. Biotechnol*. doi: 10.1007/s11274-010-0498-0.

An, R. & Grewal, P.S. 2010. Molecular mechanisms of persistence of mutualistic bacteria *Photorhabdus* in the entomopathogenic nematode host. *PLoS ONE* 5: e13154.

An, R. & Grewal, P.S. 2010. *Photorhabdus flourescens kleinii* subsp. nov. (Enterobacteriales: Enterobacteriaceae). *Cur. Microbiol.* 62, 539-545.

An, R. & Grewal, P.S. 2010. *Photorhabdus temperata stackebrandtii* subsp. nov. (Enterobacteriales: Enterobacteriaceae). *Cur. Microbiol.* 61, 291-297.

Maneesakorn, P., Grewal, P.S. & Chandrapatya, A. 2010. *Steinernema minutum* sp. nov. (Rhabditida: Steinernamatidae): A new entomopathogenic nematode from Thailand. *Int. J. Nematol.* 20, 27-42.

Park, S., Cheng, Z., McSpadden Gardener, B.B. & Grewal, P.S. 2010. Are nematodes effective bioindicators of soil conditions and processes along distance from roads and age of development in urban areas? *Journal of Environmental Indicators* 5, 28-47.

Park, S., Cheng, Z., Yang, H., Morris, E. E., Sutherland, M., McSpadden Gardener, B.B. & Grewal, P.S. 2010. Soil chemical properties vary with distance to roads and age of urban development. *Urban Ecosystems* 13(4), 483-497, DOI: 10.1007/s11252-010-0130-y

Cheng, Z., Salminen, S. & Grewal, P.S. 2010. Effect of organic fertilizers on the greening quality, shoot and root growth, and shoot nutrient and alkaloid contents of turf-type endophytic tall fescue, *Festuca arundinacea*. *Ann. Appl. Biol.* 156, 25-37.

Williams, R.N., Fickle, D.S., Grewal, P.S. & Dutcher, J. 2010. Field efficacy against the grape root borer, *Vitacea polistiformis* and persistence of *Heterorhabditis zealandica* and *H. bacteriophora* in vineyards. *Biol. Contr.* 53, 86-91.

Yang, H., McCoy, E.L., Grewal, P.S. & Dick, W.A. 2010. Dissolved nutrient and atrazine removal by column-scale monophasic and biphasic rain garden model systems. *Chemosphere* 80, 929-934.

L. Current and Pending Support

CURRENT & PENDING SUPPORT

Name: Parwinder S. Grewal

NAME (List/PD #1 first)	SUPPORTI NG AGENCY	TOTAL $ AMOUN T	EFFECTIVE DATES	% OF TIME COMMIT TED	TITLE OF PROJECT
	Current:				
Walton B.M., Mach J.J., Schwarz T., Grewal P.S. & Beach D.	NSF-ULTRA	$279,500	2009-2012 Ends December 2012	5%	Urban parks and vacant lands as mechanisms of ecological and socia stability in the Cleveland urban ecosystem
Hoy C.W. & Grewal P.S.	USDA-NRI	$327,450	2009-2013 Ends June 2013	5%	Increasing invertebrate services in agroecosystems
Grewal P. & L. Phelan	OARDC Small Industry	$6,000	2012-2012	2%	Integrated control of pests and diseases in tomatoes
Grewal P.S. & van der Knaap	OARDC Seeds	$50,000	2012-2013	5%	A novel approach to plant protection through durable systemic resistance
Parwinder Grewal, Khandakar R. Islam, Edward L. McCoy, Alan Sundermeier, Subbu Kumarappan	USDA	$750,000	September 2012-August 2015	3%	Assessing, Modeling, and Maximizing Ecosystem Services in Long-term Organic and Transitioning Farming Systems
	Pending:				
Grewal P.S.	OARDC SEEDS	$50,000	2013-2015	5%	Infection behavior and management

	Matching Grant (This proposal)				of foliar nematodes on hosta and other shade-tolerant ornamentals

M. Budget

SEEDS BUDGET FORM

Investigator's Name: Grewal				
Department: Entomology				
	Year 1 SEEDS	**Year 2 SEEDS**	**Year 1 Match (if applicable)**	**Year 2 Match (if applicable)**
A. Salaries and Wages				
1. Research Associates / Post Doctorates	2750	2750	17,500	17,500
2. Graduate Students **Number of Semesters 4**	16,200 2semesters	16,200 2 semesters		
3. Undergraduate Students				
4. Other				
Total Salaries and Wages	18,950	18,950	17,500	17,500
B. Fringe Benefits (student 10%; Post-doc 36.1%)	2673	2673	6318	6318
C. Total Salaries, Wages and Fringe Benefits (A plus B)	21623	21623	23,818	23,818
D. Nonexpendable Equipment				
E. Materials and Supplies	2,277	2,277	1000	1000
F. Travel	500	500	112	112
G. Publication Costs				
H. Other (Describe in Budget Justification - Consultant)				
Total (C through H)	25,000	25,000	25,000	25,000

CFAES Graduate student, # of semesters requested: Year one__2__ Year two__2__

N. Budget Narrative
The funds requested will be used for the salary and benefits of a part-time post-doctoral researcher for two years and a graduate student for 5 semesters. All the required equipment for this project is available in Dr. Grewal lab and greenhouse space is available in Entomology Greenhouses. Growth chamber space is also available but will be reserved. and with the collaborating farmer. Budget requested for the materials and supplies will be used for purchasing laboratory chemicals and other necessary supplies for analyzing soil and plane samples for foliar nematodes, purchase of pots and plant material. Travel budget is requested to visit nurseries for the collection of soil and plant samples and to set up field experiments.

O. Service as an Investigator
PI is eligible to serve as an investigator on federal grants

P. Conflict of Interest

CONFLICT OF INTEREST LIST

Name: Parwinder S. Grewal

Name	Co-Author	Collaborator	Advisees/ Advisors	Other – Specify Nature
Adams, Byron	X			
Adhikari, B. N.	X			
Alumai, Alfred	X			
An, Ruisheng			X	
Bai, Xiaodong	X			
Briar, Shabeg			X	
Bussaman, P.	X		X	
Byrne, Loren	X		X	
Cardina, John	X			
Chandrapatya, A.	X			
Chaston, J. M.	X			
Cheng, Zhiqiang	X		X	
Ciche, Todd	X			
Daneshvar, H.	X		X	
Denardo, Elizabeth A.	X		X	
Dick, W. A.	X	X		
Dillman, A.	X			
Dutcher, J.	X			
Fickle, D. S.	X			
Fife, Jane	X		X	
Gaugler, Randy	X	X	X	
Georgis, Ramon	X			
Goodrich-Blair, Heidi	X			
Grunkemeyer, Mark	X			
Hogenhout, Saskia	X	X		
Hoy, Casey W.	X	X		
Jagdale, Ganpati B.	X		X	
Kovach, Joseph	X			
Kunkel, Brian A.	X		X	
Lewis, Edwin E.	X			
Li, Tan	X		X	
Lin, C.Y.	X			

Maneesakorn, P.	X		X	
McCartney, D.	X			
McCoy, E. L.	X	X		
Miller, Sally A.	X			
Nahar, M.S.	X	X		
Power, Kevin T.	X		X	
Richmond, Douglas S.	X		X	
Rimelspach, Joseph	X			
Saeb, Amr	X		X	
Salminen, Seppo	X		X	
Sandhu, Sukhinder	X		X	
Shapiro-Ilan, David	X			
Shetlar, D. J.	X	X		
Sreevatsan, S.	X			
Sternberg, P. W.	X			
Stock, Patricia	X			
Taylor, K.	X		X	
Williams, R. N.	X			
Yang, H.	X		X	

Q. List of Potential Reviewers

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Department of Plant Pathology

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Phone: (865) 974-7947
Fax: (865) 974-4744
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Dr. Larry Duncan
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Citrus Research and Education Center
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E-mail: lwduncan@ufl.edu

R. Discovery Themes at The Ohio State University

This application directly addresses the Energy and Environment discovery theme of the Ohio State University.

Grewal, Parwinder

From: Made in the Shade Gardens [Rob@HostaGuy.com]
Sent: Wednesday, August 29, 2012 2:31 PM
To: Grewal, Parwinder
Subject: Proposed Foliar Nematode Research Project for the American Hosta Society
Attachments: Alternate Nematode Control.pdf

Dear Dr. Parwinder Grewal,

I am the current Vice President - Genus Hosta for the AHS, responsible for scientific research. While we are currently midway in sponsoring and executing a hosta virus research project at the University of Minnesota, my AHS executive board has encouraged me to accelerate the pursuit of a concurrent foliar nematode research program. As a result I would like to request a formal proposal from you and your colleagues at the Ohio State University to lead this research effort. Our goal would be to kick off the research effort as soon as possible – even as soon as spring 2013 assuming adequate funding has been raised. Our fall AHS executive board meeting is scheduled in late October which would be a very convenient time to present your proposal.

While I don't want to reinvent the wheel here, I would like us to validate certain key findings of prior research. In my mind there also still remain critical gaps in our understanding of the entire life cycle and physical movement of foliar nematodes. These gaps would seem to be best addressed before we begin testing and defining an effective treatment protocol.

Your requested scope of research would include:

- Document other shade perennials that are affected by foliar nematodes (i.e. shade tolerant plants that would normally be grown in close proximity of hostas)
- Confirm key aspects of prior research including: the upward migration of nematodes in spring and summer and under what specific circumstances this occurs (temperature, humidity, plant growth phase, etc); do they migrate solely on the exterior surface of the plant; how do they enter the leaf – stoma only or other pathway as well; do some nematodes remain behind in the soil/crown; in what state (worms, eggs) do nematodes overwinter
- Document downward migration of nematodes in late summer/fall (note that many of us have tested for the presence of nematodes on an affected leaf late in the year and could not find any)
- Document when/where eggs are laid and if they can exist in a "dormant" state
- Document movement of nematodes from one plant family/genus to another when planted in close proximity
- Efficacy testing of treatments and existing available pesticides (also examine effect of application timing based on life cycle understanding from above) including (but not necessarily limited to): Thermal treatment (both hot air and hot water), Imidacloprid, Di-Syston, Cygon, Pylon, Zerotol, AzaMax, chitinous material (see page 5 of the attached publication on Alternate Nematode Control), and other chemicals that you might recommend for testing
- A final written report along with semi-annual progress reports

As part of your proposal I would request that you also take the lead in the writing and submission of grant applications that would help underwrite the cost of this research. Since a number of other plants are also affected by foliar nematodes, this research program would be expected to receive broad support. Specifically the Perennial Plant Association would be a likely target for a grant application. Other relevant plant societies and organizations might include: the American Fern Society, the American Iris Society, the National Chrysanthemum Society and the North American Strawberry Growers Association. You may have other ideas and contacts as well. Depending on the support received from these various groups, our testing of specific plants may need to be expanded beyond just hostas. As a very general target the American Hosta Society might fund 50% of your research with grant money from these other societies and organizations funding the other 50%. Assuming grant monies are received as described, it is conceivable that total research funding around $50,000 would be achievable. Similar research efforts undertaken by the American Hosta Society have involved a nominal 3 year duration.

Please let me know if there are any questions or comments that we might need to address. I can be reached by e-mail, at home (913-829-0760) or by cell (913-206-6939).

I hope this proposed research project will be of interest to you and your colleagues. On behalf of the American Hosta Society I look forward to receiving your proposal. Please let me know when we can expect your proposal.

Thanks,
Rob Mortko
American Hosta Society
Vice President, Genus Hosta

Spam
Not spam
Forget previous vote