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Evaluation of botanical and chemical products for the control of foliar nematodes *Aphelenchoides fragariae*

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ABSTRACT

Foliar nematodes *Aphelenchoides* spp. Pose a serious threat to floriculture by causing significant economic losses in home gardens, production nurseries and arboretums, and threatening precious germplasm collections of many ornamental plants. Control of foliar nematodes has become difficult due to the loss of nematicides following the implementation of the U.S. Food Quality Protection Act. Herein, we used a three-stage approach to evaluate 24 products for their potential to control foliar nematodes *Aphelenchoides fragariae* in hosta. We first performed a direct contact assay in well-plates where *A. fragariae* nematodes were exposed to the aqueous suspension of high (2-fold dilution) or low (20-fold dilution) concentration of the test products. The products causing 100% nematode mortality at the low concentration were then tested through a drench application for their effectiveness against foliar nematodes in soil, followed by a leaf-disc test to determine their efficacy against nematodes in leaves as a spray application. Of the 24 products, Ammonia, Clorox, grapefruit seed extract, $KMnO_4$, NaDCC, Nemakill, Pylon and ZeroTol caused 100% nematode mortality in aqueous suspension at 20-fold dilution. Drench application of Ammonia, Clorox, $KMnO_4$, NaDCC and ZeroTol reduced over 85% and over 75% *A. fragariae* population in soil 7 and 42 days after treatment, respectively. Spray application of ZeroTol could reduce over 70% *A. fragariae* population in leaf discs, whereas Ammonia, Clorox, $KMnO_4$ and NaDCC caused about 50% reduction. Pylon (24% chlorfenapyr) and Nemakill (32% cinnamon oil, 8% clove oil, 15% thyme oil mixture) showed 100% mortality of *A. fragariae* in all three types of tests, and thus have great potential to serve as effective alternatives to manage foliar nematodes in floriculture.

1. Introduction

Foliar nematodes (*Aphelenchoides fragariae, A. ritzemabosi*, and *A. besseyi*) are unique plant-parasitic nematodes that cause serious damage to over 700 plant species including rice, strawberries, black currant, berries, alfalfa, and numerous ornamental plants such as hosta, chrysanthemums, ferns, lilies, begonia, etc (Kohl et al., 2010; Sanchez-Monge et al., 2015). Unlike most other plant-parasitic nematodes that infect root systems, foliar nematodes infect aerial plant parts. In the absence of host plant, foliar nematodes can also sustain in the soil by feeding on saprophytic fungi (Richardson and Grewal, 1993). Foliar nematodes overwinter in the soil or in plant parts including rhizomes, bulbs, and buds, but generally not in the roots (Jagdale and Grewal, 2006). In the spring, the overwintering nematodes move upward on the outer surface of leaves, stems, and petioles, eventually invading leaves (Grewal and Jagdale, 2001; Jagdale and Grewal, 2006; Wallace, 1959). The infection results in the formation of characteristic local lesions on leaves usually bounded by large veins that first turn yellow, then brown, and finally black (Jagdale and Grewal, 2006). Eventually the dead tissues dry and fall off, leaving large holes in the leaves, or entire leaflets may fall off in case of ferns leaving unsightly looking plants. While foliar nematodes account for economic losses in many plant species, they are particularly detrimental to the floriculture industry as the symptoms of nematode infection can directly decrease the market value of ornamental plants.

Hosta is one of the most popular ornamental plants throughout North America. 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 10 different hosta species and their hybrids (Grewal and Jagdale, 2001). As hosta foliage offers

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a variety of leaf shapes, textures, and colors, there is a growing concern among growers and nursery managers about the leaf damage caused by the foliar nematodes. In addition, nursery industry suffers losses due to quarantine restrictions on the trade of infected plants and bulbs from state to state and internationally. Control of foliar nematodes has been problematic as most of the standard chemical nematicides have been banned by the United States Environmental Protection Agency (US EPA) under the implementation of the Food Quality Protection Act (FQPA, 1996). Some effective nematicides and fumigants including methyl bromide have been phased out due to their broad spectrum toxicity and the threat to the degradation of the ozone layer.

Since the last decade, efforts have been made to search for alternative products for management of foliar nematodes affecting ornamental and horticultural crops. Jagdale and Grewal (2002) tested a biological agent (*Pseudomonas cepacia*), two plant products (clove extract and Nimbecidine) and twelve chemical pesticides for the control of *A. fragariae*. They found that only diazinon EC, trichlorfon SP, oxamyl GR and ZeroTol were effective in reducing nematode population in soil and leaves. However, diazinon EC, trichlorfon SP and oxamyl GR were banned by the US Environmental Protection Agency. ZeroTol was therefore suggested as a useful product for managing foliar nematodes in soil, however, it did not provide acceptable levels of control in the leaves because of limited contact with nematodes and its short persistence (Jagdale and Grewal, 2002). Jagdale and Grewal (2004) also tested hot water drench for the control of *A. fragariae*. They noted that drenching of hosta crowns with hot water (70–100 °C) could significantly knock down the population of *A. fragariae* in the soil, but it caused reduction in the number and size of hosta leaves developing from the treated crowns. As yet, there are only a few products recommended for use on foliar nematodes. Besides ZeroTol, Pylon has been recommended for foliar nematode control (Crow and Dunn, 2005; Gill et al., 2004; Lamondia, 2012), although efficacy of Pylon has not been investigated in detail. In addition to chemical products, essential plant oils have been observed also carrying inhibitory activity against foliar nematodes. Previous studies in other nematodes have suggested that essential oils and their components are able to inhibit the root-knot nematode *Meloidogyne incognita* (Oka et al., 2000) and the pinewood nematode *Bursaphelenchus xylophilus* (Kang et al., 2013). It was recently reported that treatment with essential oils in the grass *Brachiaria brizantha* could reduce foliar nematode *A. besseyi* inoculums in seeds (Monteiro et al., 2014). While essential plant oils have shown their nematicidal potentials, more studies are needed to investigate the potential of these botanicals to control foliar nematodes.

In this work, 24 organic and chemical products were tested, including two registered chemical pesticides ZeroTol and Pylon via a three-stage evaluation process to identify the most promising products for the control of foliar nematodes.

2. Materials and methods

2.1. Foliar nematodes

Nematode-infected hosta plants were obtained from a commercial nursery and from several home gardens. Foliar nematodes *A. fragariae* were extracted from infected hosta leaf tissues by soaking in water for 48 h and distinguished at high power (400 X) using an inverted microscope. The identified nematodes were washed in 0.85% saline solution and propagated on a soil-inhabiting fungus *Rhizoctonia solani* cultured on potato dextrose agar medium in 10-cm Petri dishes at 25 °C. For assays described below, the nematodes were extracted from the plates using Baermann funnel technique 2 days before each experiment.

2.2. Botanical and chemical products

A total of 24 candidate products were tested for their efficacy against *A. fragariae* as suggested by the American Hosta Society. Trade names, active ingredients and sources of the products tested in are provided in Table 1. The powdered product formulations (Cygon, NaDCC, $KMnO_4$, Abamectin, and Clothianidin) were first mixed in double-distilled water to prepare a solution based on the concentrations of active ingredient indicated in Table 1.

2.3. Aqueous suspension bioassays

Aqueous suspension bioassays were conducted in 24-well plates to determine the effects of the candidate products (Table 1) on mortality of *A. fragariae* with direct contact at 25 °C. Each product was tested at 2- and 20-fold dilutions along with a non-chemical control (water) following the procedure described by (Jagdale and Grewal, 2002). Four wells from a 24-well plate were randomly assigned for each treatment and for each sampling time point. Each well was considered a separate replicate and was composed of an aliquot (500 μL) of nematode suspension containing a mixture of about 500 juvenile and adult nematodes (~70:30). For the 2-fold dilution treatment, 500 μL of each product was added to the nematode suspension. For the 20-fold dilution treatment, a solution of 50 μL of the product plus 450 μL of water was added to the nematode suspension in each well. When necessary, 5 μL of a solution composed of DMSO and Tween 20 (0.5%) was added (with 5 μL of water reduced correspondingly) to dissolve the organic compounds present in some products. Nematode mortality was recorded 24, 48 and 72 h after exposure to the products. At each time point, a thoroughly mixed 200 μL sub-sample from each of the four wells for each treatment 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. Live and dead nematodes were counted using a 1-mL Peter's nematode counting slide after concentrating the nematodes into 1 mL suspension. All inactive nematodes were prodded to determine whether they were alive or dead, and nematodes were considered alive if they responded to a fine probe. Both the low and high concentration experiments were repeated once using a different set of nematode inoculum.

2.4. Soil drench assays

The products causing 100% mortality at 20-fold dilution in the well-plate contact bioassays were selected for a pot experiment for drench application to determine their effectiveness for the control of foliar nematodes in the soil. Briefly, hosta plants newly divided in June were maintained in 1.72-L pots (15.24 cm-diameter X 14.5 cm-height) with soil potting mix (Moisture Control Potting Mix Miracle-Gro, Scotts Company, Maryville, Ohio) in a walk-in growth chamber at 25 °C with 85% R.H. and were watered daily. The pots were inoculated with freshly extracted *A. fragariae* at 800 nematodes per pot at 90 days after division. The pots were drenched with the selected products one-week after the nematode inoculation, and the control pots were drenched with tap water. The products selected for the soil drench assay were: Ammonia (2% v/v), Nemakill (0.5% v/v), ZeroTol (1% v/v), NaDCC (0.5% w/v), Pylon (1% v/v), and $KMnO_4$ (0.5% w/v). In addition, Clothianidin (0.2% w/v) that caused over 50% mortality of *A. fragariae* in aqueous suspension was included besides the boiling water (~100 °C) treatment (Jagdale and Grewal, 2004) and a control tap water treatment. The soil drench treatments were applied until all the soil in the pot was fully saturated. The treated pots (each pot considered as a replication) were arranged in a randomized design with three pots per treatment. Nematode survival and multiplication in soil were assessed