Exploring Novel Management Methods for Beech Leaf Disease, an Emerging Threat to Forests and Landscapes[1]

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Abstract

Beech leaf disease, caused by the foliar nematode *Litylenchus crenatae* ssp. *mccannii*, deforms leaves and causes defoliation in beech (*Fagus* spp.). We explored management of this nematode, which threatens the health of shade-tree, ornamental, and forest beech. Field and laboratory evaluations over three years demonstrated that properly timed foliar applications of fluopyram reduced counts of live nematodes by > 90%. *In vitro* bioassay of fluopyram yielded an EC_{50} of 1.2 ppm. Similarly, oxamyl was effective when applied via trunk injection or as a soil drench to trees with < 20 cm (8 in) trunk diameter early in the season, but due to a short residual, failed to protect buds from becoming colonized in the late season (i.e. fall). High mammalian and environmental toxicity of oxamyl may limit interest in its use to injection capsules. Root flare injection or soil application of abamectin, acephate, emamectin benzoate, or potassium phosphite were ineffective in suppressing nematode populations or protecting foliage. Effective treatments cannot improve the aesthetics of trees during the current season but may protect the health of the trees by limiting the numbers of nematodes that infect buds and cause damage to foliage the following season.

Species used in this study: American beech, *Fagus grandifolia* (Ehrh.); European beech, *Fagus sylvatica* (L.); North American beech leaf nematode, *Litylenchus crenatae* ssp. *mccannii* (Carta et al.).

Chemicals used in this study: abamectin (Aracinate and Lucid), acephate (Lepitect), emamectin benzoate (Mectinite); fluopyram (Broadform, Indemnify, and Luna Experience), horticultural oil (RES Hort Oil), oxamyl (Return), potassium phosphite (Polyphosphite 30), tebuconazole (Torque).

Index words: conservation, fluopyram, foliar nematode, invasive pest, IPM, nematode, oxamyl, SDHI.

Significance to the Horticulture Industry

Beech leaf disease (BLD), caused by an emerging and presumably introduced foliar nematode, threatens the health and future horticultural marketability of all beech species. Prior to this work, no effective treatments had been published. Damage to leaves occurs in developing buds before leaf expansion. Therefore, treatments that kill nematodes cannot improve aesthetic appearance in the year of treatment but can protect the next year's foliage if they suppress nematodes prior to them migrating and entering overwintering buds. Fluopyram, already registered for use on ornamental landscape, nursery, and beech nut (*Fagus grandifolia*) plantings as a fungicide, is an effective nematicide that kills BLD nematodes present in infected leaves. Based on this research, applying fluopyram as a foliar spray to infected trees just prior to the nematode dispersal period (late summer during high leaf-wetness events) when they migrate from leaves to developing buds can be an effective management tactic. It is also possible that other application timings could prove effective (e.g., shortly after full leaf expansion), but need validation. Measures to delay nematicide resistance to fluopyram may include application early in the season, when nematode populations are lowest, combining fluopyram with a conazole fungicide to inhibit metabolic detoxification, and rotation or combined treatments with other nematicidal chemistries representing alternative modes of action. The need for treatment can be assessed by monitoring foliage for characteristic symptoms (e.g., dark interveinal bands or distortion) and by extracting nematodes from dormant buds during the autumn, winter, or early spring.

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Introduction

Beech leaf disease (BLD) is a recently described foliar disease of American beech (*Fagus grandifolia*) in eastern North American forests that is caused by the phytophagous nematode *Litylenchus crenatae* ssp. *mccannii* (*Lcm*) (Burke et al. 2020, Carta et al. 2020, Ewing et al. 2019, Reed et al. 2020). This nematode is considered a subspecies of *Litylenchus crenatae* as described by Kanzaki et al. (2019) in association with gall-like leaf tissues of infected Japanese

beech (*F. crenata* Blume) in Japan. Although *L. crenata* was described as a new species in 2019, the symptoms of the gall-like tissues on leaves had been observed in Japan as early as 1988 on beech trees in natural stands of Japanese beech and cultivated stands of American and European beech (*F. sylvatica*) (Akimoto 2004, Kanzaki et al. 2019). Beech leaf disease was first detected in northeastern Ohio in 2012 and has since been detected in twelve additional states and one Canadian province (Volk and Martin 2023). The nematode has also been associated with disease in North America on planted European beech, Chinese beech (*F. engleriana* Seemen ex Diels), and oriental beech (*F. orientalis* Lipsky) (Burke et al. 2020).

Symptoms of BLD include interveinal banding, distortion, and thickening of leaves, as well as bud abortion (Carta et al. 2020, Ewing et al. 2019, Fearer et al. 2022). Inoculations of wounded leaves with *Lcm* failed to produce beech leaf disease symptoms, while inoculations of *Lcm* in wounded buds resulted in characteristic BLD symptoms (Carta et al. 2020). This suggests that infection and damage occur within the bud by *Lcm* prior to leaf emergence the following season. Visual foliar symptoms do not progress within a growing season once leaves have fully expanded; however, some buds fail to fully develop (Fearer et al. 2022). Over the course of a growing season, populations of *Lcm* in symptomatic leaf tissue are more prevalent in nematode extractions in late summer through fall (late July through October in Ontario and Ohio), and difficult to detect in symptomatic tissues in early spring (Reed et al. 2020). The timing of peak *Lcm* abundance in extractions coincides with bud maturation and development in beech. This window is putatively the dispersal period of *Lcm* moving from infected leaf tissue into the developing buds.

Foliar nematode diseases of woody plants are rare. Foliar nematodes can be inherently difficult to manage (LaMondia 1999, Oka 2020), and typically require an integrated approach involving plant host resistance, avoidance, sanitation, cultural practices that limit leaf wetness periods, and chemical applications (Fry 1982). Historically, nematicides and insecticides in the organophosphate and carbamate groups of chemistries have been used for plant parasitic nematode suppression, but many have been phased out due to their toxicity to humans and non-target organisms (Oka 2020). Newer chemistries with fewer non-target effects have been developed recently for agronomic crops and turfgrass, but few are labeled for use against nematodes in woody trees and shrubs (Jagdale and Grewal 2002, LaMondia 1999, Oka 2020).

American beech is an important late-successional hardwood tree species in eastern North America spanning as far north as Québec, Canada, south to the panhandle of Florida, and west to southeastern Texas (Tubbs and Houston 1990). American beech is an important tree species that provides overstory for regenerating shade-tolerant understory plants (Stephanson and Ribarik Coe 2017). Mature American beech produce large crops of nuts (mast) every two to three years, providing food for wildlife such as birds, rodents, deer and bears (McCullough et al. 2001, Tubbs and Houston 1990). Because of the wide range of growing habitats, production of shade through their canopy, ornamental value, and importance to wildlife, American beech trees are commonly planted in gardens and landscapes. BLD is likely to have lasting impacts on natural and urban forests, and management strategies are needed to slow the disease progress and severity to conserve and preserve beech species in North America.

Management of BLD with pesticides at the forest level is not economically feasible and could be ecologically damaging. On the other hand, selective management of individuals in forests, shade trees in residential landscapes, and specimen trees in arboreta could be responsible and achievable with an integrated pest management approach if effective tools were identified and registered. The aim of this study was to investigate chemistries that could be integrated into a BLD management strategy. Here, we report on efficacy trials of abamectin, acephate, emamectin benzoate, fluopyram, oxamyl, and polyphosphite in suppressing *Lcm* and reducing BLD symptoms of American and European beech in the northeastern United States.

Materials and Methods

Field trials 2021-2023. Several field trials were conducted to test the efficacy of various application methods, active ingredients, and timings in this manuscript. These trials are summarized with these methods in Table 1. They are expounded upon in the subsequent sections.

Acephate field trials, 2021–2022. In 2021, 30 BLD-symptomatic *F. grandifolia* trees naturally occurring in a wooded lot of a municipal park in Guilford, CT and 29 BLD-symptomatic *F. sylvatica* and one *F. grandifolia* at Planting Fields Arboretum in Oyster Bay, NY, were selected for a soil-applied acephate efficacy trial to manage BLD. The trees were selected for the trial based on having minimal to no other biotic pests and no canopy dieback. In CT, the average DBH (diameter at breast height) of the American beech was 18.0 ± 10.7 cm (7.1 ± 4.2 in) (mean ± std. dev.), while the average DBH of the European beech in NY was 43.8 ± 14.3 cm (17.2 ± 5.6 in).

Trees were randomly assigned to three treatments including a non-treated control in a completely randomized design with 10 replications per treatment group including non-treated controls. The two acephate treatment timings were late summer vs. bud swell. Acephate applications were made with Lepitect [(97.4% soluble powder) Rainbow Ecosciences, Minnetonka, MN] solution consisting of 4.5 g product (4.4 g a.i.) mixed with 400 mL (0.16 oz mixed with 13.5 fL oz per inch of DBH) water per cm DBH, injected into the soil with high pressure (1034 kPa or 150 psi) using a soil injection probe. Prior to making treatment applications, trees were visually assessed by evaluating the percent of leaves in the canopy with BLD symptoms on 21 September 2021 at both trial locations and applications were made on the same day. On 7 March 2022 (Guilford, CT) and 15 March 2022 (Oyster Bay, NY), 10–12 twig samples, 15–20 cm (6-8 in) long with buds from the lower canopy from each tree were collected, bagged, and mailed by overnight delivery to a lab for dormant bud nematode extractions. From each sample, five

Table 1. A summary of the field trials to manage beach leaf disease (BLD) in various locations, with various active ingredients, and various application methods.

Study name	Year(s)	Tree species	Locations	N	Treatment	Application timing	Total applications	Application method	Trade name
Acephate field trials	2021–2022	F. grandifolia	Guilford, CT	10	Acephate	late winter	1	soil injection	Lepitect
			Guilford, CT	10		late summer			
			Guilford, CT	10	Non-treated	na		na	na
		F. sylvatica	Oyster Bay, NY	10	Acephate	late winter		soil injection	Lepitect
			Oyster Bay, NY	10		late summer			
			Oyster Bay, NY	10	Non-treated	na		na	na
Emamectin benzoate field trials	2021–2022	F. grandifolia	Broadview Heights, OH	10	Emamectin benzoate	late summer	1	root flare injection	Mectinite
				10					
				10	Non-treated	na		na	na
			Oyster Bay, NY	10	Emamectin benzoate	late summer		root flare injection	Mectinite
				10					
				10	Non-treated	na		na	na
			Mt. Kisco, NY	10	Emamectin benzoate	late summer		root flare injection	Mectinite
				10					
				10	Non-treated	na		na	na
Multi product field trial	2022–2023	F. grandifolia	Middlesex Co., CT	10	Oxamyl	late spring	1	soil drench	Return 2SL
				10				root flare injection	Return 2SL
				10	Fluopyram, paint			root flare paint	Indemnify
				10	Abamectin			root flare injection	Aracinate
				10	Polyphosphite			soil drench	Polyphosphite 30
				10	Non-treated	na		na	na
Fluopyram and abamectin field trial	2021–2022	F. sylvatica	Perry, OH	8	Fluopyram	late summer-early fall	4	foliar spray	Indemnify
				8	Abamectin + Hort Oil				Lucid + RES Hort Oil
				7	Non-treated	na		na	na

buds were removed from randomly chosen twigs, weighed, and then bud sheaths were opened with forceps. Opened buds were submerged in 10 mL of distilled water in 60 mm diameter petri dishes and held in the dark for 24 h. Nematodes were counted under a dissecting microscope using a mounted light below the petri dish. The extracted nematode counts were standardized by mass of buds used in the extraction. After one growing season post treatment, trees were visually rated on 13 July 2022 (Guilford, CT) and 23 August 2022 (Oyster Bay, NY) for percent canopy with BLD symptoms as done prior to treating in 2021.

Pre- and post-treatment disease severity measurements were analyzed using a restricted maximum likelihood (REML) mixed model across treatments independently with location as a random variable and treatment as a fixed effect in a general linear mixed model using JMP 16 (SAS, Cary, NC). Standardized dormant bud nematode counts were square-root transformed to normalize the data and analyzed using a REML mixed model across treatments as a fixed effect and location as a random effect. Means separations were calculated using Tukey's HSD for both analyses.

Emamectin benzoate field trials, 2021–2022. In 2021, 90 BLD-symptomatic *F. grandifolia* trees naturally occurring at three locations were selected to test the efficacy of emamectin benzoate in suppressing *Lcm* and reducing BLD severity. The locations of the three trials were Brecksville Reservation managed by Cleveland Metroparks in Broadview Heights, OH, a natural area of Planting Fields Arboretum in Oyster Bay, NY, and a natural area on a private residence in Mt. Kisco, NY. The trees were selected for the trial based on having minimal other biotic pests and no canopy dieback. The average DBH of the trees was 47.6 ± 13.0 cm (std. dev.) in Broadview Heights, OH, 34.5 ± 12.8 cm in Oyster Bay, NY, and 29.2 ± 7.6 cm in Mt. Kisco, NY. Within each of the three locations trees were randomly assigned to the following treatments with 10 replicates per treatment including non-treated controls at each site in a completely randomized design: 172 mg emamectin benzoate per cm of DBH (product rate: 10 mL per in DBH), 345 mg emamectin benzoate per cm of DBH (product rate: 20 mL per in DBH), and non-treated controls. The emamectin benzoate product used in these trials was Mectinite™ (soluble liquid concentrate), which is a 4% emamectin benzoate tree injection product (Rainbow Ecoscience, Minnetonka, MN). The emamectin benzoate injections were diluted in 118 mL distilled water per cm DBH to improve evenness of product distribution to the leaves and buds. The product was delivered into the root flare using the low volume manual macroinfusion kit from Rainbow Ecoscience following best management practices for root flare injections based on arboricultural industry standards with one injection site per 2.54 cm of DBH (Bernick and Smiley 2022). Treatments were deployed on 30 June – 1 July 2021 in Broadview Heights, OH, 16 August 2021 in Oyster Bay, NY, and 18 August 2021 in Mt. Kisco, NY.

At each site prior to treatment, the disease severity was visually evaluated for each tree by estimating the percentage of the canopy with BLD symptoms as observed from the ground. After full leaf expansion the following season in 2022, trees were rated again with the same methods on 15 September 2022 (Broadview Heights, OH), 19 July 2022 (Oyster Bay, NY), and 20 July 2022 (Mt. Kisco, NY).

On 14 March 2022 (Oyster Bay, NY) and 11 Apr 2022 (Broadview Heights, OH) arborists collected, bagged, and mailed overnight 10–12 twigs 15–20 cm (6-8 in) long with buds from the lower canopy from each tree at these two locations. Dormant bud samples were not collected from Mt. Kisco, NY. Nematode extractions from buds were performed as described in the previous section by opening buds and floating in water for 24 h, and counts were standardized by mass of buds. Pre- and post-treatment disease severity measurements and nematode counts from buds were statistically analyzed using the same methods as the acephate experiment.

Multi-product efficacy trial CT, 2022. A trial was conducted on private land in Middlesex County, Connecticut, at a site with mixed hardwood forest containing abundant American beech. Candidate beech trees were selected based upon presence of foliage that could be accessed from the ground and trunk diameter ranging from 12–40 cm (5-16 in) DBH. Tree diameter was measured with a DBH tape to the nearest cm, and geographical coordinates (latitude and longitude) recorded, as measured by a smart phone application (Coordinates GPS Formatter, Mapnitude, Bangkok, Thailand). These coordinates were found to be of high precision (nominally ∼10 cm) but with significant drift (∼5 m accuracy) varying by day. Because the coordinates were recorded on one day, they were useful for assessing the distance between trees (via a customized spreadsheet program) to assure that no two treated trees were closer than 10 m to each other. Adequately spaced trees then constituted a subset of eligible trees for the study. Trees were then ordered from smallest to largest DBH, outliers for size were discarded, and groups of six trees each for blocks averaging 13, 16, 18, 19, 20, 21, 24, 26, 29, and 37 cm DBH were chosen for a randomized complete block design with six treatments and ten replicates.

Treatments consisted of (1) a non-treated control, (2) a soil drench with potassium polyphosphite (solution) (Polyphosphite 30, 0-0-27 fertilizer, Plant Food Company, Cranbury, NJ) applied diluted 60 mL (51 g a.i.) into a total of 470 mL per 2.5 cm DBH (2 fl. oz. into one pint per inch DBH), drenched from the root flare to 50 cm outwards, (3) oxamyl (soluble liquid) (Return 2 SL, Rotam North America, Greensboro, NC), applied as a trunk injection of 2 g active ingredient (a.i., 8.3 mL product) diluted into a total volume of 50 mL injected per 2.5 cm DBH, (4) oxamyl applied as a root crown drench, diluting 2 g a.i. into 500 mL and with drench placement the same as the potassium polyphosphite treatment, (5) fluopyram (suspension concentrate) (Indemnify 3.34 SC, Bayer Environmental Science, Cary, NC) diluting 0.79 mL product (316 mg a.i.) into 20 mL of 5% Silwet L-77 Surfactant (Helena Agri-Enterprises, Collierville, TN) in water per 2.5 cm DBH, painted onto the root flare, and (6) abamectin (Aracinate 2%, Rainbow Ecoscience water soluble) diluted 2.5 mL (50 µL of a.i.) into 25 mL total per 2.5 cm DBH applied through root flare injection. Root flare injections (oxamyl and abamectin) were made to the ridges of root flares at

the base of the trunk, using one injection site per 5 cm DBH and a Q-Connect system (Rainbow Ecoscience). All treatments were applied on 25 April 2022, prior to bud break, with temperatures of approximately 18 C (64 F) and fair weather. The soil drench with potassium polyphosphite was reapplied on 7 July 2022, using the same dosage and methods as the first application.

Trees were evaluated for their condition on 8 July 2022 by three observers. Two ratings were made. The first used a rating system developed by Dr. D. Herms of Davey Tree Expert Company (pers. comm., D. Herms, 18 April 2022): 1, asymptomatic; 2, leaf banding is present, green transitioning to yellow within leaves, no canopy thinning evident; 3, leaf distortion and some twig death was present, 5–35% canopy thinning; 4, severe leaf distortion, 35–75% canopy thinning; 5, 75–95% canopy transparency; 6, tree death. The second rating system counted the number out of ten clusters of leaves that exhibited BLD symptoms. Each individual conducting assessments inspected clusters of leaves from the lower to the upper canopy. Entire clusters of leaves originating from an overwintering bud were evaluated as a unit because damage is aggregated based upon whether the bud from which leaves emerged had been infected: leaves originating from an overwintering bud either have no damage or are nearly all damaged. For each rating system, each of three individuals provided ratings for a tree; these were averaged before being subjected to statistical analyses.

Nematode population measurements were made from leaves sampled on 19 (odd-numbered replicates) and 22 (even numbered replicates) of August 2022. Ten symptomatic leaves were collected from each tree, enclosed in a plastic bag, and brought to the laboratory. Because our first sampling date was a Friday and nematode condition can deteriorate following emergence in water, sampled leaves (from both dates, to be consistent) were held for two days at ∼20 C before extraction. The leaves were first weighed, cut with scissors into ∼5 mm wide strips, mixed thoroughly, then a 2.0 g (fresh weight) composite sample placed into a 25 cm diameter aluminum pie tin and covered overnight with ∼300 mL water containing 20 µL Tween 20 (Fisher Scientific, Pittsburgh, PA) surfactant. Leaf fragments and nematode suspension were then washed and sieved using 21 cm diameter #80 and #400 U.S.A. Standard Test Sieves (180 and 37 µm openings, respectively; Fisher Scientific). The nematodes were gently washed with a minimal volume of water from the sieves into gridded plastic dishes for counting under a dissecting microscope at 40–80× magnification. Samples with excessive nematodes for counting were diluted to a volume of 15 mL, stirred, and a 1 mL subsample counted with a compound microscope at 40× magnification on a nematode counting slide (Chalex Corp., Centreville, MD). Nematode counts were $\log(x + 1)$-transformed to establish homogeneity of variance prior to conducting analysis of variance using Statistix 9 software (Tallahassee, FL).

Nematode counts from buds were assessed by collecting 10-cm long shoots from the ground and with a pole pruner, up to a height of 7 m. Six shoots were sampled from around each tree on 27 February 2023 and transported in a cooler to the lab for temporary storage in a refrigerator. On three successive days (27 February, 1 March and 2 March 2023), sets of 20 samples were processed to count nematodes from buds. Six buds from each tree (one from each shoot) were weighed, then teased open with a probe and submerged in 20 mL of water in a 30-mL disposable plastic cup. Nematodes were allowed to emerge overnight and were counted the next day without sieving after removing the buds while rinsing with a fine stream of water. Nematodes were counted as with the foliar samples had been previously, using gridded dishes or a counting slide. The nematodes per gram of buds did not require transformation prior to conducting analysis of variance.

Fluopyram and abamectin foliar application efficacy field trial, 2021–2022. On 2 Jul 21, 24 European beech trees representing seven cultivars (‘Copper’, ‘Dawyk Gold’, ‘Purple Fountain’, ‘Pendula’, ‘Red Obelisk’, and two unknown selections) with BLD were inventoried, tagged, and evaluated based on percent canopy with BLD symptoms as described in the acephate and emamectin benzoate field trial sections. Trees were 6–8 cm DBH, planted in rows at a private field-grown nursery in Perry, Ohio. To reduce pesticide drift effects, experimental units were spaced to maximize available distance (closest space ∼ 3 m) from neighboring trees. Three treatments including a non-treated control were assigned to trees according to a completely randomized design. Fluopyram was applied as Indemnify (suspension concentrate) (Bayer Environmental Science, Cary, NC) at 0.7 mL (316 mg a.i.) per L (8.5 fl oz per 100 gal), abamectin was applied as Lucid (emulsifiable concentrate) (Rotam North American Inc., Miami, FL) at 0.6 mL (11 mg a.i.)/L (8 fl oz per 100 gal) mixed with 0.5% v/v of RTSA Horticultural Oil (Rainbow Ecoscience, Minnetonka, MN), and non-treated controls were left unsprayed. Foliar applications were made on 30 July 21, 19 August 21, 10 September 21, and 30 September 21 with a 15 L (4 gal) battery-powered backpack sprayer (Husqvarna, Charlotte, NC) when windspeeds were less than 16 km per hr.

On 13 April 22, 6–8 twigs 15–20 cm long that were flagged as having leaves with symptoms in 2021 were harvested from each plant, bagged, and shipped overnight to the lab. Nematode extractions from buds were performed as described previously in the acephate and emamectin benzoate field trial sections. Nematode counts were standardized by mass (g) of five buds. On 13 June 22 after leaves had fully expanded on the trees, disease severity was evaluated as percent canopy with BLD symptoms as done initially.

Disease severity for 2021 and 2022 was subjected to analysis of variance in SAS JMP 16 using percent canopy with BLD symptoms as the response variable and treatment as the fixed effect for each year independently; data did not require transformation. Mean separations were calculated with Tukey’s HSD in JMP 16. Total *Lcm per* g of bud tissue was log(x+1)-transformed to normalize variance and subjected to analysis of variance in JMP 16. Mean separations of nematode counts were calculated using Dunnett’s test to compare both foliar treatment groups to the non-treated control.

Late season fluopyram spray to protect dormant buds. This experiment's goal was to determine whether late-season application of fluopyram could affect survival of *Lcm* that had already entered dormant buds. The experiment was conducted using infected American beech in Hartford Co., CT. Trees exhibited an early stage BLD condition showing considerable leaf banding and yellowing sections within leaves, but the trees hadn't experienced significant defoliation, bud mortality, or branch dieback. Individual shoots of 20–30 cm were flagged in an alternating pattern around four trees, with a total of eight pairs of branches to match treated and non-treated control shoots in a completely randomized design. Fluopyram in a formulation registered for use on ornamental trees (Broadform (suspension concentrate), containing 252 $g \cdot L^{-1}$ each of fluopyram and trifloxystrobin) was applied 11 Oct 22 at the highest labeled fluopyram concentration of 157 ppm (8 fl oz per 100 gal) in a mixture containing 0.063% v/v (8 fl oz per 100 gal) of a spreader sticker (Tactic, Loveland Products Inc., Greeley, CO) to the shoot of each pair designated for treatment. The spray was applied with the fingertip fine mist sprayer (Model S703BK, Container and Packaging, Eagle, ID) to wet the upper and lower surfaces of leaves and the bark.

Shoots were collected on 31 October (20 d after treatment). Individual buds were longitudinally bisected from the apical tip to their base, then teased open with fine forceps to expose the leaf primordia before cutting off the bud and submerging the tissues overnight in 15 mL water containing 0.01% Tween 20, held in 30 mL disposable plastic containers. The resulting extracted nematodes were counted without sieving, using the same methods as for the Connecticut multi-product efficacy trial. Data were analyzed in Statistix 9 as a paired t-test following $\log(x + 1)$ transformation to normalize variance.

Fluopyram bioassay with Luna Experience, 2022. This experiment determined whether there might be synergism between fluopyram and a conazole fungicide when these products are applied as a foliar spray to *Litylenchus*-infected beech leaves. Shoots (25 cm long) from infected American beech were obtained on 30 September 22 from the same forest site described earlier in Hartford Co., CT. Thirty-two branches were numbered at the time of collection, and each was randomly assigned to one of eight treatment combinations in a 4 (dosage) × 2 (presence/absence of tebuconazole) level factorial arrangement with four replicates in a completely randomized design. Fluopyram was either from the Indemnify formulation (one active ingredient), or from Luna Experience (suspension concentrate) (two active ingredients, fluopyram and tebuconazole, each present at 200 $g \cdot L^{-1}$). Fluopyram concentrations were chosen to reflect the amount of active ingredient expected to be applied with one, two, or four applications at the labeled rate. Fluopyram levels were (1) non-treated check, (2) low concentration (152 ppm), (3) medium concentration (304 ppm), and (4) high concentration (608 ppm). The fluopyram + tebuconazole treatment combinations presented both products at the same concentration. The tebuconazole-alone treatment used Torque (suspension) (432 $g \cdot L^{-1}$, Cleary Chemical, Alsip, IL) at a concentration of 608 ppm. All treatment combinations also included the organosilicone surfactant Silwet L-77 at 300 $\mu L \cdot L^{-1}$. Products were sprayed onto upper and lower surfaces of foliage to the point of runoff with a fingertip fine mist sprayer (Berlin Packaging Model X351, Chicago, IL). The surfaces of leaves were allowed to dry, then the treated shoots for each treatment combination were grouped together in a plastic container with cut ends in water. Treated branches were held for 4 d under low natural light conditions at ~18 C while enclosed in a large plastic bag to prevent desiccation. Sprayed leaves were removed on 4 October 22, weighed, and then processed for nematode extraction and counting using the same methods as for the efficacy trial in the multi-product Connecticut trial. Mobile and immobile nematodes were counted. Due to high counts necessitating sample dilution and use of the nematode counting slide, nematodes were classified as mobile or immobile, and responsiveness to probing was not evaluated for immobile nematodes. Counts were adjusted to represent the number expected from 2 g of leaf tissue and log-transformed to normalize variance before conducting analysis of variance using Statistix 9.

Fluopyram bioassay with Broadform, 2022. On 10 October 22, 60 twigs, 20–30 cm long with leaves from a BLD-infected American beech tree in Plymouth, MA were collected from, cut ends wrapped in a moistened paper towel and then aluminum foil, bagged and shipped overnight to the lab in a cooler with an ice pack. A fresh cut was made on the proximal end of the twig and provided 20 mL of distilled water in a 50 mL conical tube. Pre-treatment nematode populations were quantified from ten random hole punches from symptomatic leaf bands for each twig taken with a 6-mm diam hole punch, then placed in 10 mL of distilled water for 24 hr to extract nematodes. Nematodes were counted as previously discussed and standardized based on surface area of the leaf punches to calculate *Lcm per* cm^2 of symptomatic leaf tissue. In addition to live nematodes, the number of immobile or dead nematodes were also counted to calculate a ratio of live:dead.

Each twig was randomly assigned to four treatments and 10 replicates in a completely randomized design. Foliar spray treatments to cuttings were (1) fluopyram + trifloxystrobin applied as Broadform at a rate of 0.6 mL/L (8 fl oz per 100 gal, 150 ppm fluopyram), (2) fluopyram applied as Indemnify at a rate of 0.7 mL per L (8.5 fl oz per 100 gal, 280 ppm fluopyram), (3) fluopyram + trifloxystrobin as Broadform applied at 0.6 mL per L tank-mixed with tebuconazole applied as Torque at a rate of 0.5 mL (216 mg a.i.) per L (6 fl oz per 100 gal), and (4) a distilled water control. All treatments, including the water control, were applied with the addition of Lesco Spreader/Sticker (Lesco, Cleveland, OH) at a rate of 0.5 mL per L (6 fl oz per 100 gal), and each were individually mixed in 500 mL solutions in a 2 L hand-pressurized sprayer (Itisll, ZheJiang, China) where solutions were applied to foliage of each treatment group until run-off. Shoots were held in tubs with lids and maintained indoors in low light.

Treatment effects were evaluated with nematode extractions 2 and 5 d post treatment (dpt) using leaf punches taken adjacent to the pretreatment samples and using the same extraction methods. Nematodes were counted as alive if they were moving after watching under the microscope

Table 2. **Summaries of acephate and emamectin benzoate efficacy trials, 2021 and 2022, on beach leaf disease (BLD) caused by *Litylenchus crenatae* ssp. *mccannii* (*Lcm*).**

Experiment	Treatment Rate (per cm dbh)	Treatment Season	N[z]	Disease Severity (% canopy with BLD + se)[y] 2021	2022	Δ	*Lcm per* g bud[x]
Acephate soil injection[w]	4.5 g	Late summer[v]	20	32 (6)	75 (4)	43	3476 (1195)[u]
	4.5 g	Late winter (bud swell)[t]	20	32 (8)	69 (5)	37	1501 (427)
	Non-treated	Non-treated	20	35 (6)	79 (4)	44	1064 (253)
Emamectin benzoate root flare injection[s]	172 mg	Late summer	30	29 (5)	59 (5)	30	1631 (516)
	345 mg	Late summer	30	27 (4)	55 (4)	28	404 (151)
	Non-treated	Non-treated	31	31 (5)	60 (5)	29	589 (232)

[z]Combined replicates from each trial location, and analyses considered location as a random variable.

[y]Disease severity estimated visually as percent canopy with BLD symptoms and presented as averages across treatments and experiments with the standard error of the mean in parentheses. 2021 measurements were made on 9/21/21 (Acephate trials both locations) and 6/30/21 and 7/1/21 (Broadview Heights, OH), 8/16/21 (Oyster Bay, NY) and 8/18/21 (Mt. Kisco, NY) for the emamectin benzoate trials. 2022 measurements were made on 7/13/2022 (Guilford, CT) and 8/23/2022 (Oyster Bay, NY) for the acephate trials and 9/15/22 (Broadview Heights, OH), 7/19/22 (Oyster Bay, NY) and 7/20/22 (Mt. Kisco, NY) for the emamectin benzoate trials.

[x]Average *Litylenchus crenatae mccannii* nematodes per g of dormant bud tissue calculated from extractions of six buds from each individual tree followed by standard error of the mean in parentheses.

[w]Acephate injections were applied with the product Lepitect with a high-pressure soil injection probe evenly around the base of the trunk in a solution diluted with water at 400 ml/cm DBH.

[v]Applications made on 9/21/21 in Guilford, CT and Oyster Bay, NY.

[u]Dormant bud extractions from trees in the late summer treatment timing of the acephate experiment had statistically higher (P=0.02) nematodes compared to the other treatments, but disease severity one growing season post treatment was no different (P=0.22).

[t]Applications made on 3/7/22 (Guilford, CT) and 3/15/22 (Oyster Bay, NY).

[s]Emamectin benzoate root flare injections were applied with the product Mectinite with a small volume macroinjection with low pressure diluted in 118 ml of distilled water per cm DBH.

for 2–3 sec. Dead nematodes were identified and counted if they were immobile and having a rigid appearance. Nematode counts were standardized by total number of *Lcm* per cm^2 of tissue. Data required log transformation to normalize variance. The log(total *Lcm*/cm^2 + 1) and log[(live *Lcm*/cm^2 + 1)/(dead *Lcm*/cm^2 + 1)] data were subjected to ANOVA in JMP 16; transformed *Lcm* counts were analyzed separately for each evaluation date.

In-vitro fluopyram dose-response, 2022. The sensitivity of *Lcm* to direct exposure to fluopyram or a fluopyram + tebuconazole mixture was conducted to estimate their EC_{50} and EC_{90} responses. Nematodes were extracted in bulk from heavily infected foliage obtained 17 October 22 from the same site in Hartford Co., CT as used in the fluopyram bioassay with Luna Experience. Leaves were removed from branches in the field and brought back to the laboratory in a plastic bag. To create a *Lcm* nematode suspension, shredded leaves (150 g, torn by hand) were immersed in 8 L of tap water with two aquarium air stones positioned at the bottom of the container delivering a total of 2 $L \cdot min^{-1}$ of air. After 4 h, 5 mL aliquots of the *Lcm* suspension were added with vigorous mixing to an additional 5 mL solution containing either fluopyram or fluopyram + tebuconazole (Indemnify and Luna Experience, respectively) held in a 30-mL disposable plastic serving container with lids (Solo Soufflés, Dart Container, Mason, MI), where the bioassay also took place. The initial concentrations of fluopyram were twice the targeted concentration, so the addition of 5 mL of *Lcm* suspension brought the test concentrations to 1.00, 1.26, 1.59, 2.00, 2.52, 3.17, and 4 ppm.

Starting at 20 h after initial exposure and continuing for the next 4 h, samples of nematodes (18–45 individuals) from each concentration and replicate were evaluated by counting mobile and immobile individuals, using a compound microscope and a nematode counting slide. One sample of immobile nematodes from each concentration × tebuconazole treatment combination were further evaluated by rolling them with an eyelash probe and observing under a dissecting microscope whether they were still capable of moving. Data were analyzed graphically with log concentration vs. probability scale, using SigmaPlot (for averages), and also analyzed via nonlinear regression and analysis of variance, using the solver function from Excel (Microsoft, Redmond, WA) to calculate expected values, deviations, and sums of squared deviations, using all data. A two-parameter logistic model with the slope and EC_{50} was used, where predicted mortality = $100/(1 + \exp(-1 * ((\log(conc) - \log(EC_{50})/k)))$, where k is the reciprocal of the slope.

Results and Discussion

Acephate efficacy field trials, 2021–2022. Soil injected acephate applications, whether applied in late summer or at bud swell in both trial locations had no effect on the disease severity of the treated trees or on the overwintering nematode population within buds ($P>0.05$, Table 2). The tree condition worsened from the 2021 pretreatment to the 2022 post-treatment evaluations for all treatment groups including the non-treated control. There were slightly more nematodes found in overwintering buds from trees that were treated with acephate in the late summer ($F_{(2,56)} = 4.0$, $P=0.024$)

compared to other treatment groups, but this did not appear to have any implications on BLD severity as no differences were found across treatments in the post treatment evaluation ($F_{(2,56)} = 1.6$, P=0.22).

Nematicides in the organophosphate and carbamate group chemistries have historically been used for managing nematodes by inhibiting acetylcholinesterase activity in the central nervous system (Oka 2020). Unlike many of the other organophosphate chemistries such as chlorpyrifos or diazinon that are now restricted or banned from use in landscape settings, acephate is an active ingredient that is still registered for use in landscapes. Acephate had efficacy in previous trials investigating nematode suppression (Baheti et al. 2015, Jagdale and Grewal 2002, Meena et al. 2016). Acephate resulted in 73.6 and 54.7% reduction in *Aphelenchoides fragariae* populations in hostas (*Hosta* spp. Tratt) relative to non-treated controls in Ohio in 1999 and 2000, respectively (Jagdale and Grewal 2002). Similarly, seed treatments with acephate in maize (*Zea mays* L.) combined with foliar applications of neem resulted in 53–76% reduction relative to non-treated control plants infested with maize cyst nematode (*Heterodera zeae*) in India (Meena et al. 2016). Although there was a reduction in phytophagous nematodes found in these other pathosystems when acephate was applied, soil applications of acephate applied to American and European beech during the dispersion period of *Lcm* (late summer) or at bud swell (late winter), prior to leaf emergence, had no effect on BLD severity or *Lcm* populations relative to the non-treated controls (Table 2).

Emamectin benzoate efficacy field trials, 2021–2022. Disease severity as measured by the percent of canopy showing symptoms increased among all treatment groups irrespective of emamectin benzoate macroinjection treatments or treatment rates ($F_{(2,56)} = 1.0$, P=0.37 for post-treatment evaluation), and the number of nematodes extracted from overwintering buds did not differ among treatments ($F_{(2,56)} = 2.8$, P=0.07); Table 2). We conclude that emamectin benzoate macroinjections in late summer are not effective in suppressing *Lcm* or for protecting beech trees from BLD.

Emamectin benzoate is an insecticide in the avermectin class of chemistry, which is a group of chemistries that includes nematicides, miticides and insecticides discovered as products of a soilborne actinomycete bacterium *Streptomyces avermitilis* (Burg et al. 1979). Emamectin benzoate has been shown to be effective at preventing infestation of *Bursaphelenchus xylophilus,* the causal agent of pine wilt disease, and its vectors (*Monochamus* spp.) (Sousa et al. 2013, Takai et al. 2003). When injected at a dose of 10 g ai per cubic meter of wood tissue, emamectin benzoate protected *Pinus densiflora* Siebold & Zucc. or *P. thunbergii* Parl. (91–100% protection) for three years from pine wilt disease introduced via inoculation of *B. xylophilus,* whereas the majority (70–100%) of non-treated controls died in the same period (Takai et al. 2003). Similarly, Sousa et al. (2013) demonstrated that *Pinus pinaster* Aiton trees injected with emamectin benzoate at 32 or 64 mg $\cdot$ cm^{-3} of wood resulted in no mortality from pine wilt disease, while 33% of the non-treated controls died from pine wilt. In laboratory tests (*data not shown*) emamectin benzoate treated *Lcm* were killed from exposure to 43, 430, 4300, and 43,000 ppm emamectin benzoate after 1 hour of exposure. It is possible that too low of a concentration of emamectin benzoate reached the leaves from our macroinjections due to emamectin benzoate having low water solubility (Takai et al. 2003). Ash injected with emamectin benzoate for emerald ash borer prevention had foliar residues averaging 6 ppm (McCullough et al. 2011), and it is possible that emamectin benzoate could be less mobile in beech than in ash (*Fraxinus* spp. Tourn. ex L.). More water-soluble formulations of this class of chemistry applied as a root flare injection could be successful in targeting *Lcm* and should be pursued further.

Multi-product efficacy trial CT, 2022. The visual ratings of each tree's condition on 8 July 22 disclosed no significant differences among treatments ($F_{(5,\ 44)} < 1.0$, $P>0.5$) for both assessment methods. Averages of tree ratings using the 1–6 condition scale (1 = no damage and 6 = mortality) for treatments ranged from 2.9 to 3.4 (SE $\pm$ 0.17–0.27); the non-treated control was rated 2.9. The count of symptomatic per ten leaf samples ranged from 7.1 to 8.8 (SE $\pm$ 0.31–0.94); the non-treated control was rated 7.4. The change in symptom expression within the same growing season of treatment is not likely because damage would have occurred prior to leaf emergence and symptom expression does not change in individual leaves after leaf out (Carta et al. 2020, Fearer et al. 2022).

In contrast to the damage ratings, there were highly statistically significant differences in the numbers of nematodes extracted from late summer foliage within the season of treatment among treatments ($F_{(5,44)} = 8.45$, $P<0.0001$). The only two treatments significantly different from the non-treated control were the oxamyl root flare injection treatment, with population reductions (relative to the non-treated control) of about 98.8%, and the oxamyl soil drench treatment, with population reductions of about 88% (Table 3). Reductions in nematode populations (log-transformed counts) resulting from the oxamyl soil drench were inversely correlated with the diameter of the tree (r = 0.80, P=0.01). The average of a 48% reduction in the populations of nematodes from use of the phosphite drench was not significantly different from either the non-treated control or the oxamyl soil drench treatment due to high variability in amount of nematodes extracted from each sample. Since beech leaf disease symptoms do not change in one growing season (Fearer et al. 2022), it is not surprising that the symptoms did not differ across treatments since ratings were done in the same season of applications. However, when buds were collected from these trials in the late winter of 2023 there were no differences in nematode populations. This is not surprising since the trees were in a forest dominated by beech and oxamyl has very low residual activity. While it suppressed nematodes in spring and late summer, the residual activity was not long lived to protect against the inundation of inoculum in the late summer through the fall.

Although abamectin has been shown to be effective on managing many types of nematodes (Cayrol et al. 1993, Crow et al. 2017, Stretton et al. 1987), the root flare injections with Aracinate (a.i.: abamectin) were not effective

Table 3. **Summary of multi-product efficacy trial, Middlesex Co., CT, 2022. Treatments were applied 25 April 2022. *Litylenchus crenatae* ssp. *mccannii* (*Lcm*) populations in leaves were assessed in late August; buds were collected 27 February 2023.**

Product, application method	Application rate ercm dbh	N	*Lcm per* 2 g leaves[z] mean (± se)	*Lcm per* g bud mean ± se
Non-treated	n.a.	10	631 (388, 1030) a	11,000 ± 3,250
Oxamyl, drench	0.8 g a.i.	9	77.2 (29.9, 197) b	11,800 ± 3,270
Oxamyl, injection	0.8 g a.i.	10	6.3 (3.10, 12.2) c	9,600 ± 2,860
Fluopyram, paint	0.13 g a.i.	10	553 (297, 1030) a	8,700 ± 3,540
Abamectin, injection	0.02 g a.i.	10	518 (356, 753) a	7,600 ± 2,600
Polyphosphite, drench[y]	24 mL product	10	330 (172, 632) ab	5,400 ± 1,630

[z]Means back-transformed from log-transformed data are given; mean minus and plus standard error is given in parentheses. Means followed by the same letter are not significantly different, Tukey's HSD, $P < 0.05$.

[y]A second application of polyphosphite was applied at the same rate on 7 Jul 2022.

for reducing *Lcm* populations. This could be similar in nature to the emamectin benzoate injections, which were also not effective, in that these products have very low water solubility, resulting in poor movement either to foliage or to the buds. Similarly, the potassium polyphosphite treatments had little effect in reducing the *Lcm* populations, but this product may not have direct effects against the nematode. Oka et al. (2007) found that phosphite treatments did not prevent infection or initiation of giant cell formation in root tissue caused by *Meloidogyne marylandi,* but it did inhibit the development of the giant cells. Given the limited understanding of the biology of *Lcm* and the factors involved in causing beech leaf disease, it is unclear if this mechanism would be effective. Research currently is underway investigating this active ingredient to combat BLD (D. Herms, C. Hausman, and D. Volk, personal communication 2022).

Oxamyl is a carbamate nematicide and insecticide with high mammalian toxicity (LD_{50} 5.4 $mg{\cdot}kg^{-1}$) (Oka 2020) and has been phased out for use in many sectors of agriculture due to non-target effects (LaMondia 1999). In addition, oxamyl is within a group of chemistries that has high leaching potential in soil, implying a higher likelihood of ground water contamination (Oka 2020). Root flare injections could mitigate the risk of soil and water contamination because the product is directly injected into the tree. If injection products were to be labeled for managing BLD, it would be advisable to formulate them into enclosed capsules to minimize risk of exposure to the applicator. Some of the trees receiving root flare injections exhibited slime flux exuding from the injection sites. Special attention would have to be given to surface disinfect injection sites and injection equipment to reduce the likelihood of an introduction of bacteria or fungi into the interior of the trees. Given the efficacy of oxamyl against *Lcm* early in the life cycle, a later timing of application should be investigated for preventing successful colonization of buds. An early treatment timing could be effective for treating relatively isolated trees with limited risk of recolonization. Due to the systemic activity of oxamyl, this treatment would create an opportunity for treating large trees that are in areas where foliar applications are difficult due to drift management concerns. All of this is contingent upon new manufacturing and EPA approval.

Fluopyram and abamectin foliar application efficacy field trial, 2021–2022. Pretreatment assessment of disease severity measured as percent canopy with BLD symptoms in late July 2021 did not statistically differ among treatment groups (Table 4; $F_{(2,19)} = 0.6$; P=0.56). Disease severity significantly declined in the trees treated with fluopyram in the 2022 flush of leaves but increased in non-treated controls and abamectin tank-mixed with horticultural oil. The nearly 40% reduction in symptomatic canopy compared to the previous year was statistically significant ($F_{(2,19)} = 8.2$, P=0.002). Extractions of nematodes in dormant buds paralleled the foliar symptoms with respect to treatment efficacy: average *Lcm*/g of dormant bud tissue in fluopyram-treated trees was statistically different

Table 4. **Summary of efficacy trial for beach leaf disease (BLD) in Perry, OH evaluating fluopyram and abamectin tank mixed with horticultural oil foliar applications during the dispersal period of *Litylenchus crenatae* ssp. *mccannii* (*Lcm*).**

Product[z]	Rate (mL per L)	N	Disease severity[y] 2021	2022	Δ	*Lcm per* g bud[x]
Fluopyram	0.7	8	48 (10)	10 (5)[w]	−38	25 (6)[v]
Abamectin + hort oil	0.6 + 5	8	31 (10)	53 (10)	22	679 (450)
Non-treated	0	7	35 (11)	51 (11)	16	493 (242)

[z]Foliar applications were made on 7/30/21, 8/19/21, 9/10/21 and 9/30/21 with a backpack sprayer with full coverage and until leaf runoff.

[y]Average percent canopy with BLD symptoms followed by standard error of the mean in parentheses.

[x]Average *Litylenchus crenatae mccannii* nematodes per g of dormant bud tissue calculated from extractions of six buds from each individual tree followed by standard error of the mean in parentheses.

[w]Disease severity in 2022 was statistically different in trees treated with fluopyram (P=0.002) with Tukey's HSD mean separations.

[v]Average *Lcm* counts were statistically different in dormant buds of trees treated with fluopyram (P=0.04) with Dunnett's mean separation test compared to the non-treated controls.

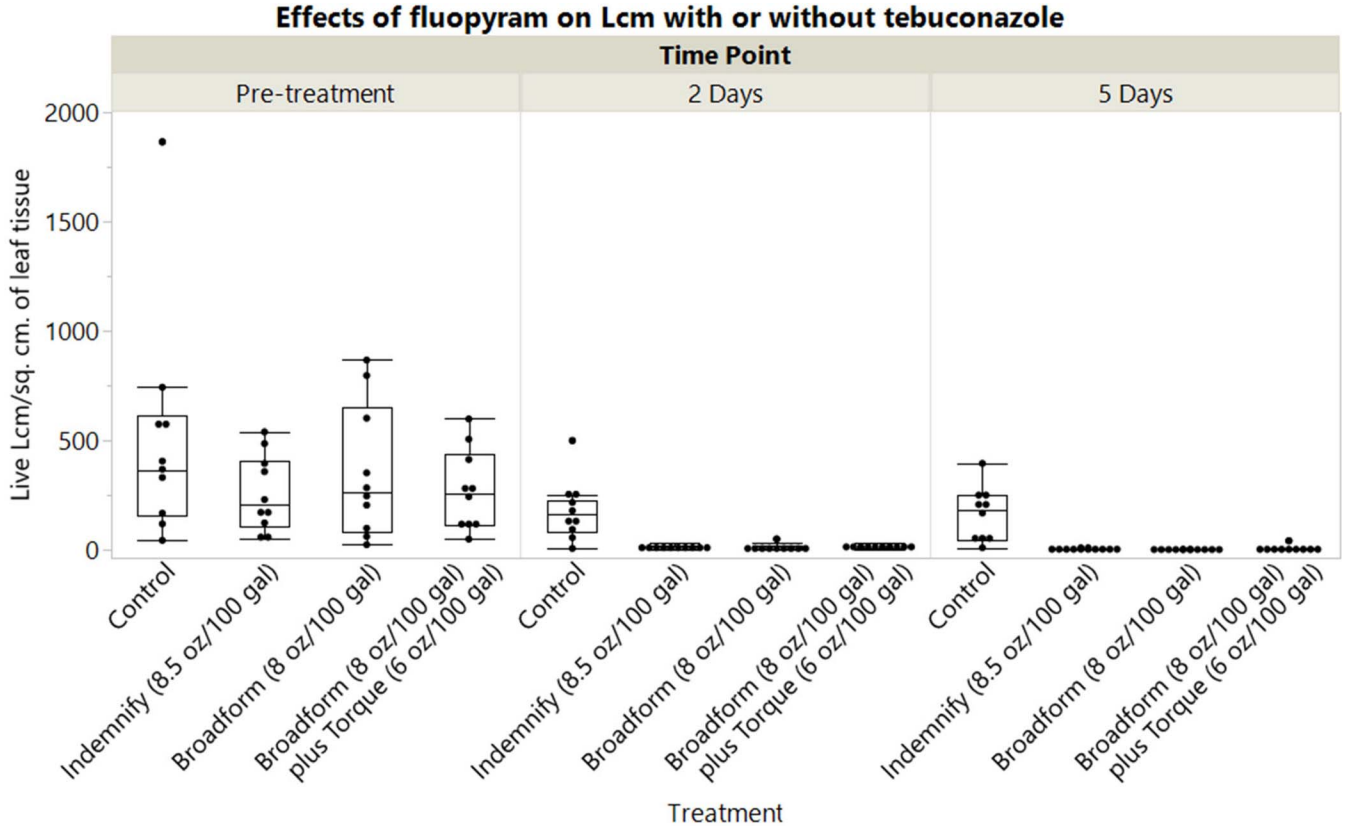

Fig. 1. Efficacy of the fluopyram products including Indemnify and Broadform applied in a laboratory bioassay with or without the tank mix of tebuconazole applied as Torque. Live *Litylenchus crenatae* ssp. *mccannii* (*Lcm*) expressed as *Lcm* · cm^{-2} of leaf tissue across all respective treatments over a time series of pretreatment, two, and five d post treatment. Treatment rate equivalents were as follows: Indemnify at 251 ml per 379 L (8.5 fl oz per 100 gal), Broadform at 237 ml per 379 L (8 fl oz per 100 gal), and Broadform plus Torque at 237 ml per 379 L (8 fl oz per 100 gal) and 177 ml per 379 L (6 fl oz per 100 gal), respectively.

than the non-treated control trees (P=0.04) but did not differ among the non-treated controls and abamectin tank-mixed with horticultural oil treatments. Compared to other trials where we extracted *Lcm* from American beech, the European beech trees in this trial had fewer *Lcm* extracted, which could be due to this site being recently infested (< 2 years prior to starting experiment, personal communication with nurseryman), or perhaps reduced suitability of European beech for *Lcm* population development.

Late season fluopyram spray to protect dormant buds. The number of nematodes extracted from individual buds ranged from 2 to 4,650 (eggs were present in some samples but were not counted). Spraying leaves and branches late in the season with fluopyram had no observable effect on the number of nematodes extracted from the dormant buds ($t_{(7)}$ = 0.82, P=0.44). These applications likely were after the peak dispersal period of the *Lcm* moving from symptomatic leaves to overwintering buds. Further, we suspect that this chemistry does not readily move into buds.

Fluopyram foliar bioassay including Luna Experience, 2022. Populations of mobile nematodes were dramatically reduced 4 d after fluopyram had been sprayed on leaves. There was a large proportion of immobile nematodes present, even in the non-treated control (77%), and so there were no significant differences found among treatments with respect to the numbers of dead nematodes observed. The counts of mobile nematodes, however, were dose-dependent with back-transformed means of 1,580, 14.4, 9.7, and 4.4 nematodes respectively for the 0, 152, 304, and 608 ppm fluopyram treatment combinations (combined across the tebuconazole factor). This resulted in an overall reduction of mobile nematodes of approximately 99% for all fluopyram treatments. The addition of tebuconazole had no significant effect or interaction with fluopyram with respect to the numbers of live nematodes extracted ($F_{(1,27)}$ = 0.24; P=0.63).

Fluopyram foliar bioassay including Broadform, 2022. The total live *Lcm per* cm^2 did not statistically differ in the pre-treatment counts across treatment groups, including the water and spreader sticker controls ($F_{(3,36)}$ = 0.59, P=0.63), suggesting that there were similar initial nematode counts. The total number of dead *Lcm per* cm^2 slightly differed among treatments at 5 dpt ($F_{(3,36)}$ = 3.6, P=0.02), but there were no differences in this variable across treatments at other time points (pre-treatment and 2 dpt). On the other hand, the mean live *Lcm per* cm^2 were greatly reduced in all treatments relative to the control at two and five dpt ($F_{(3,36)}$ = 13.6 and 46.6, respectively, P<0.0001) (Fig. 1) with no differences between the various fluopyram treatments. The live:dead transformed *Lcm* counts were statistically significant for two

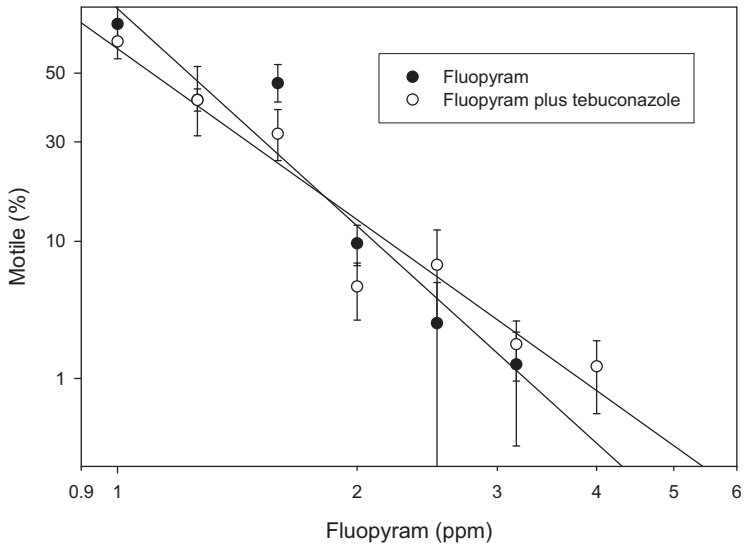

Fig. 2. In vitro response of *Litylenchus crenatae* ssp. *mccannii* (*Lcm*) nematodes extracted from beech leaves to 20 – 24 h exposure of fluopyram. Nematode mobility (mean ± se) was assessed visually for 2 – 3 sec on 20 – 40 nematodes (n = 5). Subsamples of nematodes determined that 75% of nematodes classified as immobile could move when probed, irrespective of fluopyram concentration.

and five dpt ($F_{(3,36)} = 15.6$ and 21.7, respectively, $P<0.0001$), but not pre-treatment level counts ($F_{(3,36)} = 0.8$, $P=0.5$), further supporting the efficacy of fluopyram against *Lcm*.

In-vitro fluopyram dose-response. There was a strong linear dose-response [using log(dose) vs. probability(mortality) scaling] for immobilization of extracted nematodes with increasing concentrations of fluopyram ($R^2 = 0.93$, 0.94, and 0.96 for regressions on fluopyram, fluopyram + tebuconazole, and combined data, respectively, Fig. 2). All nematodes exposed to fluopyram became sluggish or were immobilized, whereas the nematodes present in water were vigorously moving. Among nematodes exposed to fluopyram, the proportion rated as immobile that were still capable of moving when probed was 0.75, which did not vary with concentration. For fluopyram and fluopyram + tebuconazole, respectively, the EC_{50} (1.25 and 1.17) and slopes (8.0 and 7.7) were nearly identical. There was no significant difference between responses with the addition of tebuconazole ($F_{(1,66)} = 1.57$, $P=0.21$).

Fluopyram was originally registered and used as a fungicide with a succinate dehydrogenase inhibition mode of action, but later was found to have nematicidal properties. Several commercially available products target nematodes, including Indemnify, but none are explicitly labeled for nematodes with a residential or forestry use site (Oka 2020). This chemistry inhibits respiration in nematodes as it does in fungi (Heiken 2017, Oka 2020). Fluopyram has been effective in turfgrass for select phytophagous nematodes and for targeting agricultural crop root-infecting nematodes (Crow et al. 2017, Heiken 2017, Oka 2020, Petelewicz et al. 2020). Total galls per 100 shoots caused by the Pacific shoot-gall nematode on annual bluegrass (*Poa annua* L.) putting greens were reduced and the turf quality was improved when treated with fluopyram (Petelewicz et al. 2020). Crow (2017) found fluopyram effective for reducing damage caused by sting, root-knot, and ring nematodes, but was ineffective against lance nematodes on bermudagrass (*Cynodon dactylon* L.) putting greens.

While three formulations of fluopyram (Broadform, Indemnify, and Luna Experience) were effective in our laboratory bioassays, only two are currently labeled for use on trees (Broadform and Luna Experience). Broadform (fluopyram plus trifloxystrobin) is labeled for ornamental and shade trees and Luna Experience (fluopyram plus tebuconazole) has an agricultural use label for beech nuts. The foliar applications of fluopyram in the bioassays resulted in significant mortality of *Lcm* relative to the spreader-sticker controls. Similarly, in the nursery trial, fluopyram applied as Indemnify reduced the *Lcm* population in overwintering buds when applied prior to and throughout the *Lcm* dispersal period. Importantly, these applications also resulted in a significant reduction in disease severity the following season. Although late season (mid-October) applications of fluopyram did not reduce nematode survival in buds, it is possible that the fluopyram cannot penetrate buds and these nematodes were already present in the buds prior to making these applications. The bark applications of fluopyram proved ineffective, but fluopyram may be like many fungicides in having difficulty penetrating the bark and translocating to the foliage. Fluopyram represents a ready-to-use tool for integrated pest management of beech leaf disease in the landscape.

Because fluopyram has a site-specific mode of action, we should be concerned about the likelihood that *Lcm* could evolve resistance to this active ingredient. Two mechanisms dominate evolution of pesticide resistance: target site insensitivity and metabolic detoxification. Relative to the *Lcm*/fluopyram interaction, target site insensitivity would occur if a mutation in the succinate dehydrogenase enzyme leads to a poor fit of fluopyram to this enzyme. Two options for preventing resistance due to target site insensitivity are (1) use of the product when the populations are at their lowest to limit the probability that a chance mutation for resistance will be present in the treated

population, and (2) use of mixtures or rotation of products with different modes of action. Currently, we do not have rotation partners defined for use in resistance management of BLD or data to support early season application efficacy, but these will be foci for future research.

Metabolic detoxification could cause fluopyram to degrade rapidly enough in the nematode to prevent intoxication. The enzyme system most commonly implicated in metabolic detoxification is the cytochrome P450 or mixed function oxidase family. Curiously, demethylation inhibitor fungicides such as conazoles block this class of enzymes (Wilkinson et al. 1972), blocking the synthesis of ergosterol, which is lethal to many fungi. It is fortuitous that the Luna Experience product is a premix of fluopyram and tebuconazole, which may block the detoxification route of nematicide resistance development of *Lcm* to fluopyram. This mixture was undoubtedly designed to combine two modes of action for fungicide resistance management purposes. When targeting *Lcm*, the role of tebuconazole would not be that of an additional toxicant, but as a synergist to reduce the likelihood of metabolic detoxification. At this moment, there is no evidence that mixed function oxidases play any role in metabolizing fluopyram in *Lcm;* the dose response lines for fluopyram and fluopyram plus tebuconazole do not differ. These dose-response data will provide a useful baseline for comparison of *Lcm* populations in the future, to monitor whether resistance is occurring.

In summary, although acephate, emamectin benzoate, abamectin, and potassium polyphosphite did not prove effective in field trials, fluopyram and oxamyl were both effective. We demonstrated their efficacy when applied as foliar sprays (fluopyram) or root flare injections and drenches (oxamyl). Oxamyl is currently not labeled for use in forest or landscape trees and given the potential non-target and applicator effects of this carbamate nematicide it would be advisable, if labeled, to be manufactured as closed system injection capsules to limit the exposure to the applicator and environment. In addition, future studies need to investigate timing of application of oxamyl for best efficacy.

In our foliar field trial using fluopyram as Indemnify timed to provide overlapping applications during the *Lcm* dispersal period, treated trees had reduced numbers of overwintering nematodes in buds and a significant reduction in BLD severity compared to non-treated controls. This active ingredient is an effective tool for managing *Lcm* and suppressing BLD symptoms in plants but is only a component in a comprehensive disease management program. It is not economically feasible or possible to treat entire forests of beech with pesticides, and other research will be needed to find additional management tactics. On the other hand, beech trees in nurseries, landscapes, municipal plantings, gardens, and arboreta can be managed effectively with this chemistry to prolong the health of trees that become infected with *Lcm*. The rates used in these studies were from the higher end of the range of the labels (tank rate of 153 ppm), which can be cost prohibitive. Based on our dose response assay *Lcm* is quite sensitive to fluopyram at concentrations as low as 1.2 ppm, which is two orders of magnitude lower than the concentration mixed in the tank. Future studies need to focus on optimal timings and concentrations of the fluopyram products for management of *Lcm*. This is the first peer-reviewed publication to document successful management of beech leaf disease.

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