



Thiabendazole as a Therapeutic Root Flare Injection for Beech Leaf Disease Management

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Abstract. Background: Thiabendazole (TBZ) has long been used in the arboricultural industry for tree injections as a key management tool of Dutch elm disease and sycamore anthracnose. This active ingredient is systemically distributed throughout the canopy and can offer multiple seasons of protection from these diseases. Well-studied in anthelmintic medicine, TBZ and other benzimidazole chemistries can be potent nematocides beyond their fungicidal use, disrupting microtubule assembly during mitosis with site-specific binding in some parasites. These nematocidal properties of TBZ allude to its potential in the management of beech leaf disease (BLD), caused by the foliar nematode *Litylenchus crenatae mccannii* (Lcm). Methods: To test TBZ for BLD management, symptomatic beeches were injected in Aurora and Chardon, OH, and Hillsborough, NJ. Treatments were evaluated using a combination of late-season dormant bud nematode counts and year-over-year change in canopy density and BLD symptom expression. Results: After 11- and 22-months post-treatment, trees significantly improved based on visual ratings, and Lcm was reduced in dormant buds of TBZ injected trees, while nontreated controls continued to have high disease severity and large numbers of Lcm in dormant buds. An injectable TBZ treatment for beech trees offers a new mode of action and application method against Lcm and a more appropriate tool where foliar applications are impractical or where environmental exposure is of concern. Conclusions: This study introduces a novel and effective tool that can be utilized in an integrated pest management program for BLD.

Keywords. *Fagus*; Integrated Pest Management; Invasive Species; Nematicide.

INTRODUCTION

Beech leaf disease (BLD) is an emerging threat to American beech (*Fagus grandifolia*) in Eastern forests, as well as for landscape European (*F. sylvatica*) and American beech in North America (Ewing et al. 2019). Since the initial detection of the disease in Lake County, OH, in 2012, the disease has spread to 14 additional states in the USA and to Ontario, Canada (Volk and Martin 2023). The disease is caused by the foliar feeding nematode *Litylenchus crenatae mccannii* (Lcm) (Carta et al. 2020). Symptoms of the disease include conspicuous, foliar interveinal dark green or chlorotic banding; crinkled, epinastic leaves; bud abortion; and eventual tree decline (Fearer et al. 2022). Foliar symptoms associated with the disease are the result of damage that occurs in buds prior to leaf emergence (Carta et al. 2020; Vieira et al. 2023; Fletcher et al. 2024). These symptoms can significantly reduce the photosynthetic potential of the canopy (McIntire

2023; Fletcher et al. 2024), resulting in a progressive depletion of carbohydrate reserves following successive years of infection (Skomarkova et al. 2006).

American beech is an important late-successional hardwood species in eastern North America ranging from as far north as Canada and south into Florida (Tubbs and Houston 1990). Beech are a mast seeding species that produce large quantities of nuts on a cyclical basis that provide a source of nutrition for wildlife such as birds, rodents, deer, and bear (Tubbs and Houston 1990; McCullough et al. 2005). In addition, the epithet of American beech '*grandifolia*' means large leaf and is in reference to the shade that beech provide as overstory. This shade can be critical for understory habitat for a variety of plant species and wildlife (Stephanson and Ribarik Coe 2017). In addition to the ecological importance of beech, American and European beech are important ornamental shade trees in North America due to their iconic smooth bark, fall

color characteristics, and historical use. Beech are also planted in landscapes, parks, and collections. Unabated, BLD is likely to have lasting impacts in natural and planted forest settings, as well as in ornamental and horticultural applications.

The development of management solutions for BLD is paramount, and utilizing pesticides in a management program is likely to be economically feasible for individual trees. Previously, fluopyram, a nematicidal and fungicidal active ingredient in the SDHI (succinate dehydrogenase inhibitor) family of chemistry in the ornamental labeled product Broadform (Envu, Cary, NC, USA) was found to be effective in suppressing numbers of Lcm and reducing symptoms of BLD (Lloyd et al. 2024). This product must be applied as a complete foliar cover spray to provide efficacy, which can be challenging for large shade trees. For large trees where sprays are not an option, a systemic treatment applied as a drench or root flare injection is needed. Emamectin benzoate and abamectin are 2 avermectin class pesticides with nematicidal properties that are active ingredients in products labeled for root flare injections of trees. However, neither are effective in reducing Lcm populations or reducing BLD symptoms (Lloyd et al. 2024).

Thiabendazole is in the benzimidazole class, FRAC group 1, and is the active ingredient in Arbotect 20-S (Syngenta, Basel, Switzerland). It is a broad-spectrum fungicide containing 26.6% active ingredient thiabendazole hypophosphite (TBZ). This product is routinely used to treat and prevent Dutch elm disease and anthracnose of sycamores when applied as a root flare injection in trees (Stipes 1973; Stennes and French 1987; Himelick and Neely 1988). Although routinely used in the arboriculture industry for these fungal diseases, thiabendazole was originally discovered and classified as a potent anthelmintic for use in treatments against livestock parasites (Brown et al. 1961) and only later found to have antifungal properties (Staron and Allard 1964). In fact, a single dose of 50-mg TBZ/kg body weight eliminated more than 95% of gastrointestinal parasitic nematodes representing 10 different genera in sheep (Brown et al. 1961). Since its discovery, TBZ has been used in veterinary medicine (Kelly et al. 1981; Njanja et al. 1987; Williams and Broussard 1995), agriculture (Vitti et al. 2014), and commercial mushroom production (McLeod 1973) in suppressing parasitic nematodes. It was approved by the United States Federal Drug Administration in

1967 as a human medication (Mintezol; MERCK & CO., Inc., Rahway, NJ, USA) for treatment of strongyloidiasis (threadworm) and several other parasitic infections (NCATS 2024) until eventual replacement with newer medications. The objective of this study was to determine if thiabendazole applied as a root flare injection to beech trees could suppress the population of Lcm and provide therapy to trees suffering from BLD.

MATERIALS AND METHODS

Study Sites and Experimental Design

Three natural areas on private properties with mixed hardwood forests including American beech as a dominant species in Aurora, OH; Chardon, OH; and Hillsborough Township, NJ, were selected for trialing Arbotect 20-S for the management of BLD. At each site, trees were inventoried on 2022 August 7 (Aurora, OH); 2023 July 11 (Chardon, OH); and 2023 August 30 (Hillsborough, NJ). Inventories included visually rating BLD symptoms as percent of the canopy with foliar symptoms, visual ratings of a percentage of fine twig dieback throughout the canopy, and diameter at breast height (DBH). Visual ratings were based on a consensus of at least 3 ISA Certified Arborists[®]. Between 20 to 22 beech trees with BLD symptoms were inventoried at each site. Half of the population was treated with Arbotect 20-S and the remaining trees were nontreated controls. All experiments were set up as a completely randomized design. On average, the DBH of trees were 36.1 (SE = 2.3) cm (Aurora, OH); 28.4 (SE = 2.3) cm (Chardon, OH); and 44.3 (SE = 3.2) cm (Hillsborough, NJ).

TBZ Treatments

Thiabendazole treatments were administered to trees as root flare injections using macroinjection equipment from Rainbow Ecoscience (Minnetonka, MN, USA). Treatments were made on the same day as the initial inventory for all locations. Best management practices for root flare injections were followed to reduce phytotoxicity and increase uptake efficiency by using high helix 6.0-mm (15/64-inch) diameter drill bits and a tank pressure of 103 to 138 kPa (15 to 20 psi) (Bernick and Smiley 2022). One injection site per 2.54-cm (1-inch) DBH was drilled perpendicularly into the root flares of each tree to a depth of approximately 3 cm (1.2 inches). At the Aurora, OH, and Hillsborough, NJ, sites, trees were treated according

to the Arbotect 20-S label under the “1 year growing season rate” for elms (*Ulmus* spp.) at a rate of 4.7-mL Arbotect 20-S/cm DBH diluted in 118 mL of distilled or deionized water/cm DBH (“low rate”). At the Chardon, OH, site, trees were treated according to the Arbotect 20-S label under the “3 year growing season rate” for sycamores (*Platanus* spp.) at a rate of 18.6 mL of Arbotect 20-S/cm DBH diluted in 590 mL of deionized water/cm DBH (“high rate”). “Low” and “high” rates, described in the previous sentences, are arbitrary categories in this manuscript that differentiate the 2 Arbotect 20-S rates used in these trials. Non-treated controls were left undisturbed at all locations.

Efficacy and Injection Site Damage Assessments

The season following injections at all sites, disease severity was assessed similarly to our initial ratings, where the percent of canopy with BLD symptoms and fine twig dieback was visually estimated by 3 ISA Certified Arborists for each tree. In addition, assessments were conducted 2 growing seasons after injections at the Aurora, OH, site. Assessments were performed on 2023 June 13 and 2024 June 13 (Aurora, OH); 2024 June 12 (Chardon, OH); and 2024 June 10 (Hillsborough, NJ). In addition, injection sites were assessed for exterior damage by recording the number of sites that showed any level of cracking of bark or fluxing or discoloration from previous fluxing at the injection sites. In 2024, at all sites a conditional consensus wound closure rating was also recorded, where 1 = injection sites were not closing; 2 = injection sites were actively closing; and 3 = injection sites were closed.

Lcm Quantification from Dormant Buds Post-Treatment

On 2023 February 27 and 2024 March 30 (Aurora, OH); 2024 March 28 (Chardon, OH); and 2024 March 14 (Hillsborough, NJ), arborists climbed each tree and collected 10 to 12 live, 15- to 20-cm long twigs evenly distributed from the entire canopy in each tree, which were bagged and mailed on ice overnight to the Bartlett Tree Research Laboratories (Charlotte, NC). Prior to processing, twig samples were stored in a 4 °C refrigerator for less than one week. From each sample, 6 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 hours. 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. These procedures were based on a previously published method (Lloyd et al. 2024).

Statistical Analyses

To examine treatment effects, a repeated measure (within subject) analysis of variance (ANOVA) (Aurora, OH) or paired *T*-test (Chardon, OH, and Hillsborough, NJ) was conducted to determine if there was a treatment effect in change in percent canopy with BLD foliar symptoms and percent canopy with fine twig dieback over the years that trees were evaluated. Each location and treatment group were analyzed independently comparing the first-year pre-treatment ratings with post deployment ratings. Locations were not combined due to a location effect in an exploratory analysis, and at each location initial disease severity ratings across the 2 treatment groups (TBZ and nontreated) were statistically similar, allowing us to confidently use the aforementioned statistical approach. In all analyses, if there was a significant treatment effect, means were separated using Tukey’s HSD or Student’s *T*-test for when there was more than 2 years or less than 2 years, respectively, in the analyses. Dormant bud nematode counts per gram of bud tissue were $\log(x + 1)$ transformed to normalize variance prior to conducting an ANOVA where treatment was a fixed effect and means were separated with Student’s *T*-test. Frequency of injection damage for all sites is presented for approximately 11 months post-treatment (mpt) for all sites and for 22 mpt for the Aurora, OH, site only. All analyses were conducted in JMP 17.2 (Cary, NC, USA).

RESULTS

Initially, all trees at each location had on average 64 ± 7 (Aurora, OH); 65 ± 4 (Chardon, OH); and 42 ± 4 (Hillsborough, NJ) percent canopy with BLD symptoms and 10 ± 3 (Aurora, OH); 20 ± 2 (Chardon, OH); and 25 ± 2 (Hillsborough, NJ) percent canopy with fine twig dieback. The following growing season post-treatment, there was a significant ($P < 0.05$) reduction in the percent canopy with BLD symptoms in trees treated with TBZ at the Aurora and Chardon, OH, sites where disease (percent canopy with BLD) was on average reduced by 70% (Aurora, OH) and

85% (Chardon, OH). After 2 growing seasons post-treatment, TBZ-treated trees at the Aurora, OH, site again had significantly lower percent canopy with BLD symptoms than pretreatment ratings, with no increase in disease severity compared to one year post-treatment. At both the Aurora and Chardon, OH, sites, the percent of the canopy with fine twig dieback did not significantly ($P > 0.05$) change over time post-treatment for nontreated control or TBZ-treated trees.

At the Hillsborough, NJ, site, percent canopy with BLD symptoms significantly increased ($P < 0.05$) in trees treated with TBZ, where treated trees had on average a 66% increase of the canopy with BLD symptoms. However, the percent canopy with fine twig dieback significantly improved ($P < 0.05$) in the TBZ-treated trees by 71%, while the percent canopy with fine twig dieback significantly increased by 95% in the nontreated control trees.

At all sites and for both pre- and post-treatment years, nontreated control trees had either similar percent canopy BLD symptoms ($P > 0.05$)(Aurora and Chardon, OH) or significantly increased in percent

canopy BLD symptoms ($P < 0.05$)(Hillsborough, NJ) (Table 1).

On average, there were significantly ($P < 0.05$) lower numbers of Lcm in dormant bud tissues in TBZ-treated trees compared to the nontreated control trees at the Chardon, OH, and Hillsborough, NJ, sites, and although not significant, a trend of lower quantities of Lcm in dormant buds from the Aurora, OH, site (Table 2). There was significant variation in the Lcm/g of bud tissue. Overall, there was an 86% (Aurora, OH); 99% (Chardon, OH); and 70% (Hillsborough, NJ) reduction in Lcm across TBZ-injected trees compared to nontreated controls.

While there was some damage associated with the injection sites, the frequency of cracked and fluxing sites was generally low across all study locations (Table 3). The average percentage of cracked injection sites per tree across all locations 11 mpt was 19%. The average percentage of injection sites per tree showing evidence of fluxing across all locations 11 mpt was 12%, although no active fluxing was observed. The average wound closure visual rating

Table 1. Summaries of 3 TBZ (thiabendazole) efficacy trials showing percentage of canopy with beech leaf disease and percentage of canopy with fine twig dieback. BLD (beech leaf disease).

Study location	Treatment	% canopy with BLD ¹				% canopy fine twig dieback ²			
		2022	2023	2024	<i>P</i> -value ³	2022	2023	2024	<i>P</i> -value ⁴
Aurora, OH	TBZ (<i>n</i> = 10)	70.5 (8.4) a ⁵	21.0 (8.3) b	16.9 (8.0) b	0.0001	12.0 (4.7) a	11.7 (2.2) a	10.9 (2.4) a	0.9704
	Nontreated (<i>n</i> = 10)	58.0 (10.6) a	37.0 (10.2) a	44.4 (10.4) a	0.4053	7.5 (2.4) a	17.7 (4.3) a	18.7 (4.5) a	0.0917
Chardon, OH	TBZ (<i>n</i> = 10)	n/a	59.5 (6.6) a	9.3 (4.1) b	< 0.0001	n/a	20.5 (3.8) a	14.0 (3.1) a	0.1964
	Nontreated (<i>n</i> = 10)	n/a	69.6 (4.9) a	61.5 (8.6) a	0.4241	n/a	17.0 (2.8) a	17.0 (4.2) a	0.7556
Hillsborough, NJ	TBZ (<i>n</i> = 10)	n/a	48.0 (7.0) b	80.0 (8.5) a	0.0093	n/a	26.0 (2.7) a	7.4 (1.5) b	< 0.0001
	Nontreated (<i>n</i> = 10)	n/a	36.7 (3.7) b	70.3 (6.5) a	0.0002	n/a	25.0 (1.9) b	48.8 (8.0) a	0.0088

¹ Average visual estimate of the percent canopy with beech leaf disease symptoms over time where the standard error of the mean for each year is in parentheses.

² Average visual estimate of the percent canopy with fine twig dieback over time where the standard error of the mean for each year is in parentheses.

³ *P*-value is based on a repeated measure analysis of variance (ANOVA)(Aurora, OH) or paired *T*-test (Chardon, OH, and Hillsborough, NJ) for treatment groups independently at each location across all years where the estimated percent canopy with beech leaf disease symptoms was the response variable.

⁴ *P*-value is based on a repeated measure analysis of variance (ANOVA)(Aurora, OH) or paired *T*-test (Chardon, OH, and Hillsborough, NJ) for treatment groups independently at each location across all years where the estimated percent canopy with fine twig dieback was the response variable.

⁵ Letters following each year represent levels that are statistically different based on a Tukey's HSD (Aurora, OH) or Student's *T*-test (Chardon, OH, and Hillsborough, NJ) post-hoc means separations for both % canopy with BLD and fine twig dieback independently for each treatment group at each location.

Table 2. Quantification of Lcm (*Litylenchus crenatae mccannii*) extracted from dormant buds of Arbotect 20-S treated and nontreated trees across all study locations approximately 11 months post-treatment. TBZ (thiabendazole); SEM (standard error of the mean).

Study location	Treatment	<i>n</i>	Lcm/g (SEM) ¹	<i>P</i> -value ²
Aurora, OH	TBZ	10	179 (108) a ³	0.3061
	Nontreated	10	1,264 (660) a	
Chardon, OH	TBZ	10	9 (4) b	0.0004
	Nontreated	10	869 (383) a	
Hillsborough, NJ	TBZ	10	946 (177) b	0.0161
	Nontreated	12	3,118 (851) a	

¹ Average number of Lcm per gram of dormant bud tissue 11 months post treatment where the SEM is in parentheses.

² *P*-value based on one-way analysis of variance conducted for each location independently and where treatment was a fixed effect.

³ Levels not connected by the same letter are significantly different for each location independently.

Table 3. Frequency of injection site damage in root flares per tree at all locations from all TBZ-treated trees. TBZ (thiabendazole); mpt (months post-treatment); nt (not tested).

Study location	mpt	# sites ¹	# sites cracked ²	# sites fluxing ³	Wound closure rating ⁴
Aurora, OH	11	15.4 (1.6)	2.9 (0.8)	1.5 (0.5)	nt
	22	15.4 (1.6)	3.1 (0.6)	0.1 (0.1)	2.7 (0.1)
Chardon, OH	11	12.8 (1.7)	3.1 (1.0)	2.5 (0.8)	1.1 (0.1)
Hillsborough, NJ	11	17.6 (1.9)	2.6 (0.6)	1.3 (0.7)	1.7 (0.1)

¹ Average # of injection sites in TBZ-treated trees where the standard error of the mean is in parentheses.

² Average # of injection sites that showed exterior crack in TBZ-treated trees where the standard error of the mean is in parentheses.

³ Average # of injection sites that showed exterior fluxing in TBZ-treated trees where the standard error of the mean is in parentheses.

⁴ Average consensus wound closure rating, where 1 = injection sites not closing, 2 = injection sites actively closing, and 3 = injection sites fully closed. The standard error of the mean is in parentheses.

per tree was 1.4 ± 0.1 at the Chardon, OH, and Hillsborough, NJ, sites 11 mpt. At the Aurora, OH, site, wound closure was measured again after 22 mpt, and the average percentage of cracked injection sites per tree was 20%, the average number of fluxing sites per tree was 0.7%, and the average wound closure rating per tree was 2.7 ± 0.1 .

DISCUSSION

This research demonstrates that root flare injections with TBZ can provide beneficial therapy to beech trees suffering from BLD. There was significant visual improvement of the beech trees treated with TBZ at all 3 sites. Trees treated with TBZ root flare injections

on 2022 August 7 and 2023 July 11 in Aurora and Chardon, OH, respectively, had a significant reduction in the percent canopy showing BLD symptoms in 2023 and/or 2024, suggesting that one treatment, even at the “low rate” (Aurora, OH), can substantially reduce symptoms (Figure 1A-B). At the Aurora, OH, site, this was further supported by observing no change in fine twig dieback progression in the TBZ-treated trees, while there was a trend of increased canopy fine twig dieback in nontreated control trees. While the disease severity (percent canopy with BLD) of all trees increased at the Hillsborough, NJ, site, trees injected with the “low rate” of TBZ had canopy density that was significantly greater than nontreated

control trees (Figure 1C-E). This was due to a significant reduction in fine twig dieback in TBZ-treated trees and a significant increase in fine twig dieback in nontreated controls. Fine twig dieback symptom expression is presumed to be associated with bud abortion caused by *Lcm* (Vieira et al. 2023).

We suspect the disparities in the data across the study locations may be due to the variable treatment timing. Reed et al. (2020) showed that *Lcm* disperses from symptomatic leaves to maturing buds as early as late July, peaking with the highest population of mobile nematodes being extracted from leaves in September. *Lcm* begin to interact and cause damage within developing buds as soon as they enter the bud sheaths in the late summer/early fall (Vieira et al. 2023). It is unknown if thiabendazole can move into beech bud tissue prior to dormancy, but these data suggest that it does not move in significant concentrations where the nematodes are developing.

Significant reductions in the percentage of canopies with BLD at the Aurora and Chardon, OH, sites may be due to treatment application occurring prior to the peak of *Lcm* dispersion, where treatments were applied on 2022 August 7 and 2023 July 11. Contrary to this, the percentage of canopy with BLD symptoms increased at the Hillsborough, NJ, site, where treatments were applied on 2023 August 30, possibly well into the peak dispersal period. This late treatment in NJ could have resulted in successful establishment of *Lcm* in the maturing buds prior to treatment deployment. This theory is supported by our dormant bud nematode counts at the site, where nematodes in buds from TBZ-treated trees (although significantly fewer than in the nontreated control buds) were still likely abundant enough to result in BLD symptoms. Despite this, the significant difference between treated and nontreated control trees at this site suggests that the treatment may have been successful in reducing the overall dispersing population of *Lcm*. This is supported by the significant reduction of fine twig dieback, or bud abortion, in the TBZ-treated trees compared to a significant increase in fine twig dieback in nontreated controls (Figure 1E and Table 1).

Beech leaf disease severity in the second growing season after root flare injections with TBZ was only evaluated at the Aurora, OH, site. Here, treated trees continued to show a significant reduction in percent canopy with BLD symptoms when compared to the

nontreated control trees (Figure 1F-G). It is difficult to determine based on these data whether this is due to TBZ residues being translocated to new leaves and buds or just a reduction in local inoculum sources within the individual trees and surrounding forest. Stennes and French (1987) showed that American elms injected with the equivalent of 1.86-g TBZ/cm DBH did not have significant distribution into new growth tissues one full growing season following injections. At the Aurora, OH, site, we applied 1.03-g TBZ/cm DBH, which would suggest that this sustained reduction in BLD was due to inoculum dynamics and not pesticide residues. However, Stennes and French (1987) also found that TBZ was distributed into twigs of American elms 2 seasons post-treatment when injected at a rate of 5.59 g/cm DBH. At the Chardon, OH, site, TBZ was applied at 4.10 g/cm DBH; therefore, it is plausible that some residues may redistribute into new flushes the following season similarly in beech. As we continue to study these trees, it will be important to determine at what point BLD symptoms return to damaging levels at both the “low” and “high” rates of thiabendazole used in this study. This will help determine retreatment intervals and expectations. Further, although we did not quantify these specifically, there were differences in uptake time between the “high” and “low” rates of TBZ used here due to the total volume being 4× in the “high rate”. We anecdotally observed an estimated uptake time of 10 to 15 minutes for the “low rate” solution to move into the tree, while the “high rate” solution moved into the tree in 30 to 45 minutes.

While there was some damage associated with the injection sites (Figure 2), the frequency was quite low across all study locations. It is difficult to determine what negative physiological effects occur from external, visual observations, but no acute or chronic phytotoxicity in the canopy was observed at any of the study locations. Cracking and fluxing, indicative of wound damage, occurred at a rate of 19% and 12%, respectively, across all locations 11 mpt. Following 22 mpt, at the Aurora, OH, site, we observed similar levels of cracking at a frequency of 20%, but fluxing was trending lower. Furthermore, the injection sites were closing rapidly after 22 mpt with a consensus visual rating of 2.7, indicating that most injection sites were actively closing, with most fully closed. Given the low-pressure injection system and large dilution of material (1:20 to 1:40), this treatment can

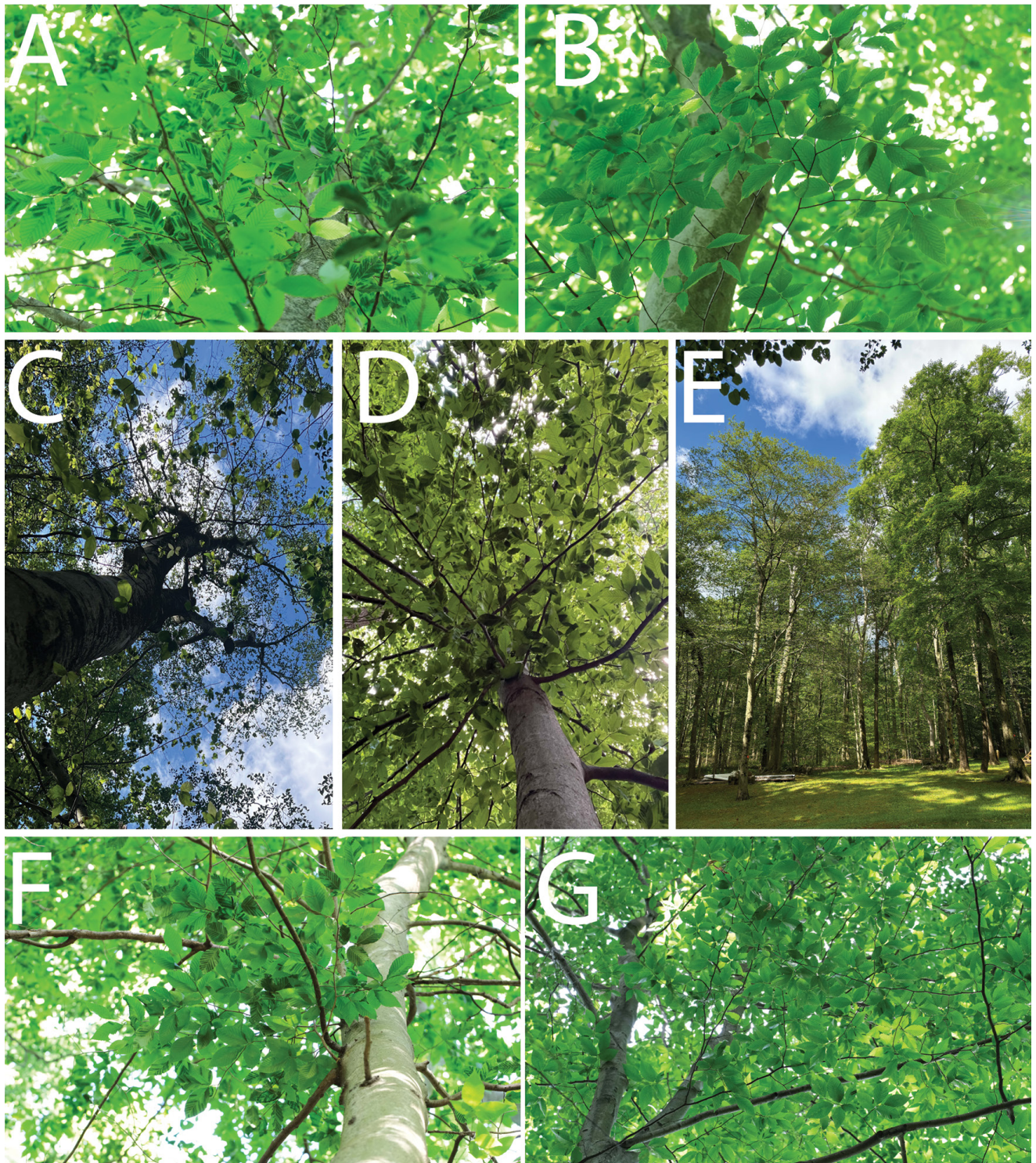


Figure 1. An illustrative representation of treatment effect of root flare injections with TBZ showing symptom reduction in beech trees compared to nontreated control trees. (A-B) Close up of leaf banding symptom in a nontreated control tree (A) and disease free canopy of a TBZ injected tree (B) 11 months post-treatment at the Chardon, OH, site where injections were applied at the “high rate”; (C-D) photograph taken up into the canopy illustrating differences in canopy density of a nontreated control tree (C) and TBZ injected tree (D) 11 months post-treatment at the Hillsborough, NJ, site where injections were applied at the “low rate”; (E) side-by-side photograph of a nontreated control tree (left) and TBZ injected tree (right) at the Hillsborough, NJ, site where injections were applied at the “low rate”; (F-G) close up of leaf banding symptom in a nontreated control tree (F) and disease free canopy of an TBZ injected tree (G) 22 months post-treatment at the Aurora, OH, site where injections were applied at the “low rate”.

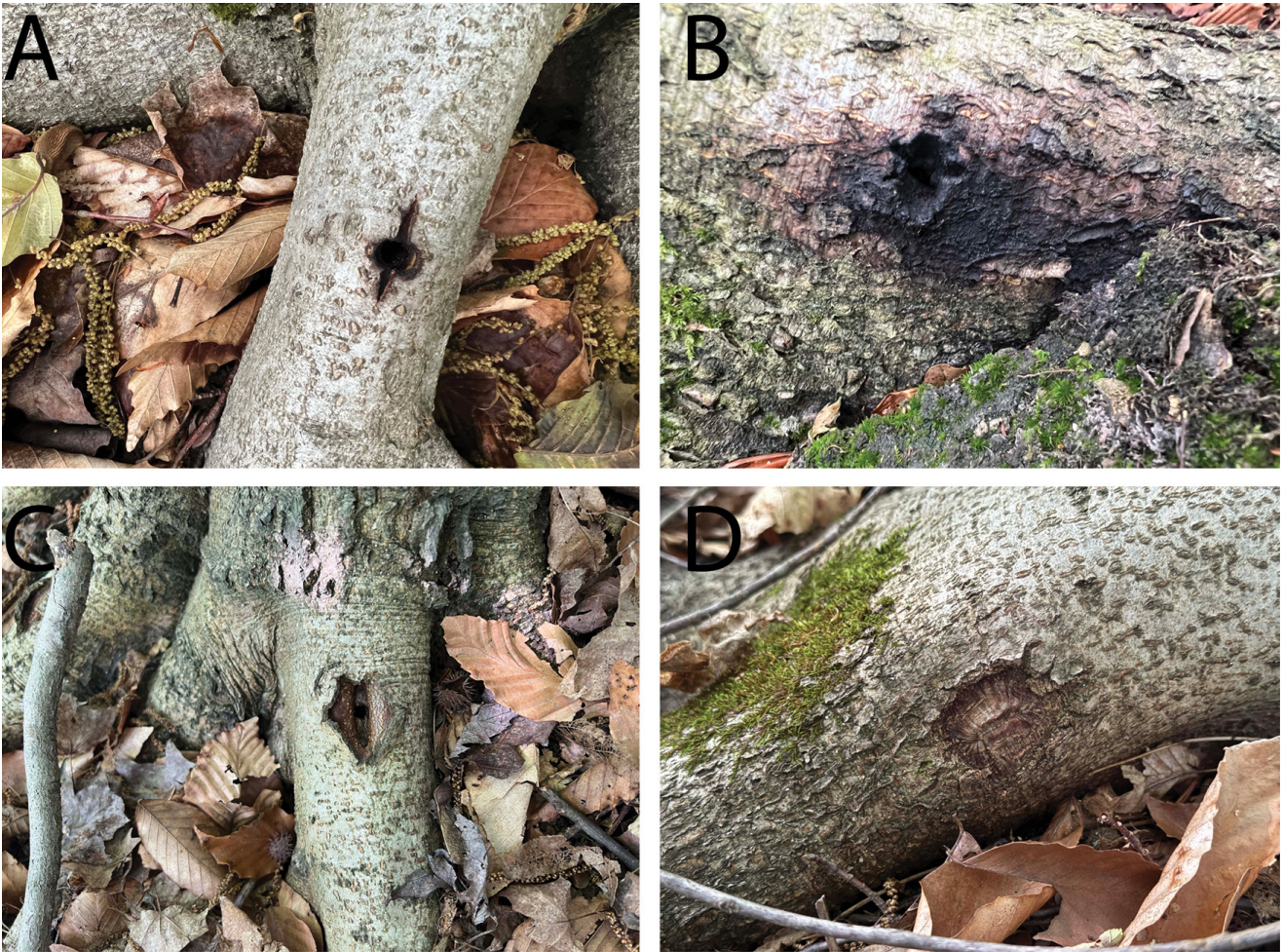


Figure 2. An illustrative representation of wound closure symptoms and visual consensus rating. (A) An example of a cracked injection site 11 months post-treatment where the visual wound closure rating was scored a 1 (not closing); (B) an example of a fluxing injection site 11 months post-treatment where the visual wound closure rating was scored a 1 (not closing); (C) an example of a cracked injection site 22 months post-treatment where the visual wound closure rating was scored a 2 (actively closing); (D) an example of a fully closed injection site 22 months post-treatment where the visual wound closure rating was scored a 3 (fully closed).

provide significant reduction in BLD symptoms and cause minimal long-term damage to the root flares. Given that the TBZ injections yielded more than one season worth of protection (i.e., Aurora, OH, site), we speculate that injections will not need to be made more often than 3 or more years or when damaging levels of BLD return.

CONCLUSIONS

This study demonstrates a novel and effective treatment of root flare injections with thiabendazole as found in Arbotect 20-S for suppression of beech leaf disease, caused by the foliar nematode *Litylenchus crenatae mccannii*. As the disease continues to spread

throughout North America, management strategies like the one developed here are critical to keeping beech trees healthy. While this treatment can be deployed and effective in natural landscapes, injecting trees can be costly, and strategizing treatments on select individuals may be warranted, but treatments of whole forests will not likely be feasible. Continued work investigating the combination of silviculture practices such as reduction in stand density in conjunction with pesticide application, similar to what is recommended for management of hemlock woolly adelgid (Vose et al. 2013; Mayfield et al. 2023), may be a potential strategy in slowing the spread of this disease in a forest setting. TBZ injection represents an

effective treatment option for large beech (> 25-cm DBH) where full coverage sprays with fluopyram are difficult, where trees are growing near bodies of water, or where pesticide drift may be of concern. This root flare injection recommendation should be implemented into a BLD integrated pest management program for beech in the United States where the use of thiabendazole for treatment of BLD is legal.

LITERATURE CITED

- Bernick S, Smiley ET. 2022. *Tree injection*. 2nd Ed. Best management practices. Atlanta (GA, USA): International Society of Arboriculture. 43 p.
- Brown HD, Matzuk AR, Ilves IR, Peterson LH, Harris SA, Sarett LH, Egerton JR, Yakstis JJ, Campbell WC, Cuckler AC. 1961. Antiparasitic drugs. IV. 2-(4'-thiazolyl)-benzimidazole, a new anthelmintic. *Journal of the American Chemical Society*. 83(7):1764-1765. <https://doi.org/10.1021/ja01468a052>
- Carta LK, Handoo ZA, Li S, Kantor M, Bauchan G, McCann D, Gabriel CK, Yu Q, Reed S, Koch J, Martin D, Burke DJ. 2020. Beech leaf disease symptoms caused by newly recognized nematode subspecies *Litylenchus crenatae mccannii* (Anguinata) described from *Fagus grandifolia* in North America. *Forest Pathology*. 50(2):e12580. <https://doi.org/10.1111/efp.12580>
- Ewing CJ, Hausman CE, Pogacnik J, Slot J, Bonello P. 2019. Beech leaf disease: An emerging forest epidemic. *Forest Pathology*. 49(2):e12488. <https://doi.org/10.1111/efp.12488>
- Fearer CJ, Volk D, Hausman CE, Bonello P. 2022. Monitoring foliar symptom expression in beech leaf disease through time. *Forest Pathology*. 52(1):e12725. <https://doi.org/10.1111/efp.12725>
- Fletcher LR, Borsuk AM, Fanton AC, Johnson KM, Richburg J, Zailaa J, Brodersen CR. 2024. Anatomical and physiological consequences of beech leaf disease in *Fagus grandifolia* L. *Forest Pathology*. 54(1):e12842. <https://doi.org/10.1111/efp.12842>
- Himelick EB, Neely D. 1988. Systemic chemical control of sycamore anthracnose. *Journal of Arboriculture*. 14(6):137-141. <https://doi.org/10.48044/jauf.1988.034>
- Kelly JD, Whitlock HV, Gunawan M, Griffin D, Porter CJ, Martin ICA. 1981. Anthelmintic efficacy of low-dose phenothiazine against strains of sheep nematodes susceptible or resistant to thiabendazole, levamisole and morantel tartrate: Effect on patent infections. *Research in Veterinary Science*. 30(2):161-169. [https://doi.org/10.1016/S0034-5288\(18\)32575-X](https://doi.org/10.1016/S0034-5288(18)32575-X)
- Loyd AL, Cowles RS, Borden MA, LaMondia JA, Mitkowski N, Faubert H, Burke D, Hausman C, Volk D, Littlejohn C, Stillier A, Rigsby CM, Brantley B, Fite K. 2024. Exploring novel management methods for beech leaf disease, an emerging threat to forests and landscapes. *Journal of Environmental Horticulture*. 42(1):1-13. <https://doi.org/10.24266/0738-2898-42.1.1>
- Mayfield AE III, Jetton RM, Mudder BT, Whittier WA, Keyser TL, Rhea JR. 2023. Silvicultural canopy gaps improve health and growth of eastern hemlocks infested with *Adelges tsugae* in the southern Appalachian Mountains. *Forest Ecology and Management*. 546:121374. <https://doi.org/10.1016/j.foreco.2023.121374>
- McCullough DG, Heyd RL, O'Brien JG. 2005. Biology and management of beech bark disease: Michigan's newest exotic forest pest. Reprint. East Lansing (MI, USA): Michigan State University Extension. Bulletin E-2746. 12 p. <https://www.canr.msu.edu/uploads/files/e2746.pdf>
- McIntire CD. 2023. Physiological impacts of beech leaf disease across a gradient of symptom severity among understory American beech. *Frontiers in Forests and Global Change*. 6:1146742. <https://doi.org/10.3389/ffgc.2023.1146742>
- McLeod RW. 1973. Suppression of *Aphelenchoides composticola* and *Ditylenchus myceliophagus* on *Agaricus bisporus* by thiabendazole and benomyl. *Nematologica*. 19(2):236-241. <https://doi.org/10.1163/187529273X00358>
- NCATS (National Center for Advancing Translational Sciences). 2024. Inxight drugs: Thiabendazole hydrochloride, N3B9AKC0T9. Bethesda (MD, USA): USDHHS, NIH, NCATS. [Accessed 2024 July 25]. <https://drugs.ncats.io/substance/N3B9AKC0T9>
- Njanja JC, Wescott RB, Ruvuna F. 1987. Comparison of ivermectin and thiabendazole for treatment of naturally occurring nematode infections of goats in Kenya. *Veterinary Parasitology*. 23(3-4):205-209. [https://doi.org/10.1016/0304-4017\(87\)90006-9](https://doi.org/10.1016/0304-4017(87)90006-9)
- Reed SE, Greifenhagen S, Yu Q, Hoke A, Burke DJ, Carta LK, Handoo ZA, Kantor MR, Koch J. 2020. Foliar nematode, *Litylenchus crenatae* ssp. *mccannii*, population dynamics in leaves and buds of beech leaf disease-affected trees in Canada and the US. *Forest Pathology*. 50(3):e12599. <https://doi.org/10.1111/efp.12599>
- Skomarkova MV, Vaganov EA, Mund M, Knohl A, Linke P, Boerner A, Schulze ED. 2006. Inter-annual and seasonal variability of radial growth, wood density and carbon isotope ratios in tree rings of beech (*Fagus sylvatica*) growing in Germany and Italy. *Trees*. 20:571-586. <https://doi.org/10.1007/s00468-006-0072-4>
- Staron T, Allard C. 1964. Antifungal properties of 2-(4'-thiazolyl) benzimidazol or thiabendazol. *Phytia. Phytopharm*. 13:163-168.
- Stennes MA, French DW. 1987. Distribution and retention of thiabendazole hypophosphite and carbendazim phosphate injected into mature American elms. *Phytopathology*. 77:707-712. <https://doi.org/10.1094/Phyto-77-707>
- Stephanson CA, Ribarik Coe N. 2017. Impacts of beech bark disease and climate change on American beech. *Forests*. 8(5):155. <https://doi.org/10.3390/f8050155>
- Stipes RJ. 1973. Control of Dutch elm disease in artificially-inoculated American elms with soil-injected benomyl, captan, and thiabendazole. *Phytopathology*. 63:735-738. <https://doi.org/10.1094/Phyto-63-735>
- Tubbs CH, Houston DR. 1990. *Fagus grandifolia* Ehrh. American beech. Fagaceae Beech family. In: Burns RM, Honkala BH, technical coordinators. *Silvics of North America: Volume 2. Hardwoods*. Washington (DC, USA): USDA Forest Service. Agricultural Handbook 654. p. 325-332. <https://research.fs.usda.gov/treearch/1548>
- Vieira P, Kantor MR, Jansen A, Handoo ZA, Eisenback JD. 2023. Cellular insights of beech leaf disease reveal abnormal

- ectopic cell division of symptomatic interveinal leaf areas. *PLoS ONE*. 18(10):e0292588. <https://doi.org/10.1371/journal.pone.0292588>
- Vitti AJ, Neto UdRR, de Araújo FG, Santos LdC, Barbosa KAG, da Rocha MR. 2014. Effect of soybean seed treatment with abamectin and thiabendazole on *Heterodera glycines*. *Nematropica*. 44(1):74-80. <https://journals.flvc.org/nematropica/article/view/83320/0>
- Volk D, Martin D. 2023. Beech leaf disease distribution. Cleveland Metroparks. [Updated 2023 December 21]. <https://www.clevelandmetroparks.com/getmedia/0c3f7d9f-4510-4250-9a3a-2d0ed9c091f5/December-2023-BLD-Map-by-Year.pdf.ashx>
- Vose JM, Wear DN, Mayfield AE III, Nelson CD. 2013. Hemlock woolly adelgid in the southern Appalachians: Control strategies, ecological impacts, and potential management responses. *Forest Ecology and Management*. 291:209-219. <https://doi.org/10.1016/j.foreco.2012.11.002>
- Williams JC, Broussard SD. 1995. Comparative efficacy of levamisole, thiabendazole and fenbendazole against cattle gastrointestinal nematodes. *Veterinary Parasitology*. 58(1-2): 83-90. [https://doi.org/10.1016/0304-4017\(94\)00701-D](https://doi.org/10.1016/0304-4017(94)00701-D)

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Conflicts of Interest:

Cory McCurry and Mark Ware report being employed by Rainbow Ecoscience, a company that directly sells Thiabendazole (Arbotect 20-S) and provides education to tree care professionals on best practices for tree injection. The remaining authors reported no conflicts of interest.