Research Article

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Lethal trap trees: a potential option for emerald ash borer (*Agrilus planipennis* Fairmaire) management

Deborah G McCullough,[a,b*] Therese M Poland[c] and Phillip A Lewis[d]

Abstract

BACKGROUND: Economic and ecological impacts of ash (*Fraxinus* spp.) mortality resulting from emerald ash borer (EAB) (*Agrilus planipennis* Fairmaire) invasion are severe in forested, residential and urban areas. Management options include girdling ash trees to attract ovipositing adult beetles and then destroying infested trees before larvae develop or protecting ash with a highly effective, systemic emamectin benzoate insecticide. Injecting this insecticide and then girdling injected trees a few weeks later could effectively create lethal trap trees, similar to a bait-and-kill tactic, if girdling does not interfere with insecticide translocation. We compared EAB larval densities on girdled trees, trees injected with the emamectin benzoate insecticide, trees injected with the insecticide and then girdled 18–21 days later and untreated controls at multiple sites.

RESULTS: Pretreatment larval densities did not differ among treatments. Current-year larval densities were higher on girdled and control trees than on any trees treated with insecticide at all sites. Foliar residue analysis and adult EAB bioassays showed that girdling trees after insecticide injections did not reduce insecticide translocation.

CONCLUSIONS: Girdling ash trees to attract adult EAB did not reduce efficacy of emamectin benzoate trunk injections applied ≥18 days earlier and could potentially be used in integrated management programs to slow EAB population growth.

Keywords: emerald ash borer; *Fraxinus*; girdled trees; emamectin benzoate; bait and kill

1 INTRODUCTION

Emerald ash borer (EAB) (*Agrilus planipennis* Fairmaire), a phloem-feeding insect native to Asia, has killed tens of millions of ash (*Fraxinus* spp.) trees since it was first identified in North America in 2002.[1,2] Ash mortality rates of >95% have been documented in forested areas with a substantial ash component.[3–5] Recent economic analyses identified *A. planipennis* as the most destructive and costly invasive forest insect in North America.[6,7] Green ash (*F. pennsylvanica* Marshall) and white ash (*F. americana* L.), the most widely distributed ash species in North American forests,[8,9] are also commonly planted in landscapes within and beyond their natural range.[10] Economic costs of replacing or treating landscape ash trees in urban areas and adjacent suburbs were projected to exceed $US 20 billion by 2019.[11] These estimates do not account for ecosystem services lost when mature landscape ash trees decline and die.[12–14] Slowing EAB population growth and associated ash mortality in a given area[15,16] could save or delay expenditures of billions of US dollars that will otherwise be incurred to treat or remove landscape ash trees.[17,18]

A potential tactic to slow EAB population growth involves using girdled ash trees, which effectively serve as ‘population sinks’ in sites with low EAB densities. In its native range in China, EAB functions as a secondary pest, attacking stressed, dying or newly dead ash trees.[19,20] Similarly, in North America, adult EAB beetles are attracted to volatiles emitted by stressed ash.[21–23] Female beetles preferentially oviposit on ash trees stressed by girdling, and larval density on girdled ash can be at least 3–4 times higher than on adjacent healthy ash.[24–27] Moreover, debarking or destroying girdled trees before larvae complete development can reduce growth of local EAB populations and slow the rate of ash mortality.[15,16,28,29]

Another management option for EAB involves the use of highly effective systemic insecticides. A product with the active ingredient emamectin benzoate, sold in the United States as TREE-äge™ (Arborjet, Inc., Woburn, MA), provided nearly 100% control of EAB for up to 3 years in large-scale field studies[30–32] (McCullough DG, unpublished data). This systemic product is injected into the base of the tree and then translocated in xylem tissue to the canopy,[33,34] minimizing applicator exposure, environmental contamination and potential effects on non-target organisms. The insecticide affects adult EAB beetles, which must feed on ash leaves throughout their 3–6 week lifespan, as well as neonate or

* Correspondence to: Deborah G McCullough, 243 Natural Science Building, 288 Farm Lane, East Lansing, MI 428824, USA. E-mail: mccullo6@msu.edu

a Department of Entomology, Michigan State University, East Lansing, MI, USA

b Department of Forestry, Michigan State University, East Lansing, MI, USA

c United States Department of Agriculture, Forest Service, Northern Research Station, East Lansing, MI, USA

d United States Department of Agriculture, Animal and Plant Health Inspection Service, Buzzards Bay, MA, USA

early-instar larvae.[31,32] Treating all trees or even a portion of ash trees in a given area with emamectin benzoate is a highly effective option, not only for protecting valuable landscape trees but also for slowing local EAB population growth.[15,16,28,29]

We hypothesized that injecting ash trees with emamectin benzoate and then girdling the trees a few weeks later could effectively create lethal trap trees for EAB if the girdling did not interfere with translocation of the insecticide. Lethal trap trees, which employ the attract-annihilate strategy of behavioral pest management, have been used for a wide range of insect pests,[35] including several bark beetles.[36–40] Adult EAB beetles attracted to volatiles emitted by girdled ash trees would be controlled if they fed on leaves of treated trees, reducing oviposition on the treated tree and perhaps nearby trees. The insecticide would also prevent larvae from developing on the treated trees, reducing the number of reproductive adults emerging the following year. Therefore, combining the emamectin benzoate and girdling treatments could potentially produce a synergistic effect[41] by increasing the number of adult EAB affected by the insecticide. For this strategy to be effective, however, the insecticide must be translocated through the tree before girdling. We compared larval densities on ash trees that were girdled, injected with emamectin benzoate, girdled following emamectin benzoate injection or left as untreated controls at sites with varying levels of EAB infestation.

2 METHODS

2.1 2009 study

We selected a total of 12 blocks, each consisting of four ash trees of similar size and condition, at three different sites (48 trees in total) in May 2009. Spacing between trees within blocks ranged from 6 to 20 m. Four blocks of green ash trees were selected on the west side of an unmanaged portion of Wolverine recreation area in Genesee Co., Michigan (Wolverine West = WW). Trees were growing along the edge of wooded areas or scattered between wooded areas and exposed to full or nearly full sun. Diameter at breast height (DBH) (measured 1.3 m aboveground) of trees selected at the WW site ranged from 12.2 to 21.6 cm. Numerous trees in the area had evidence of EAB infestation, such as holes left by woodpeckers preying on EAB larvae, bark cracks over old larval galleries in the upper canopy or epicormic sprouts on large branches. Study trees had relatively healthy canopies, although three trees had woodpecker holes in the upper portion of the main leader. Six blocks of white ash trees, ranging from 10.7 to 17.3 cm DBH, were selected in a well-stocked, even-aged stand of white ash in Lincoln Brick Park, Eaton Co., Michigan (LB). Trees were growing between a prairie and a densely forested area, and canopies were exposed to full or partial sun. Two blocks of open-grown white ash trees, with DBH ranging from 13.2 to 23.9 cm, were established in a right-of-way cloverleaf at a highway intersection in Ionia Co., Michigan (CLF). Although EAB was present at the LB and CLF sites, the study trees had healthy canopies and no external evidence of infestation.

One tree in each block was randomly assigned to be (1) treated with a trunk injection of emamectin benzoate (EmBen), (2) girdled (Girdled), (3) injected with emamectin benzoate and then girdled 3 weeks later (EmBen + Girdled) (e.g. lethal trap tree) or (4) left as untreated controls (Control). Emamectin benzoate (TREE-äge™, 4%; Arborjet, Inc.) was injected into the base of trees on 2 June using the ArborJet QUIK-Jet™ delivery system at the lowest label rate (0.1 g AI per 2.5 cm DBH). To girdle trees (Girdled; EmBen + Girdled), we removed a 20 cm wide band of outer bark and phloem around the circumference of the trunk with a drawknife, 1 m aboveground, on 22 June, 21 days after the EmBen injection.

On 25 June and 23 July 2009, we used pole pruners to collect leaf-bearing shoots from 2–4 aspects of the canopy of the study trees at the CLF and WW sites. In spite of using up to five extensions on the pole pruners, we were unable to reach shoots on trees at the LB site, many of which were >12 m high. Shoots from each tree were bagged, placed in coolers and returned to the Michigan State University (MSU) Forest Entomology laboratory. Foliage was stripped from shoots and frozen, then shipped in coolers via overnight mail to the USDA APHIS laboratory in Massachusetts for residue analysis (see below).

From October to November 2009, all trees were felled, and the trunk and primary branches >5 cm in diameter were bucked into 1 m long sections. Each log was carefully debarked, and the number of old galleries representing pretreatment larvae and the number of new galleries made by current-year larvae were recorded separately. The average diameter of each log, measured 2.5 cm from both ends, was used to calculate the surface area exposed on each section. The total number of pretreatment and current-year larvae were standardized per m^2 of phloem area exposed on each tree.

2.2 2010 study

We selected a total of 16 blocks, each of which included four ash trees of similar size and condition (64 trees in total) in May 2010. Trees within blocks were spaced 8–12 m apart. Eight blocks of green ash trees, with DBH ranging from 12.4 to 21.8 cm, were selected in wooded and partially wooded areas in the Maple River State Wildlife area in Clinton Co., Michigan (MR). Four blocks of white ash trees, ranging from 12.7 to 20.1 cm DBH, were selected at Lincoln Brick (LB) park in the Eaton Co. white ash stand used in the previous year. Four additional blocks of white ash trees averaging 12.7–18.3 cm DBH were selected in a woodlot on private land in Clinton Co., Michigan (D-Woodlot = DW). All study trees had healthy canopies and no obvious evidence of EAB infestation, although we noted a few of the other ash at each site had woodpecker holes or bark cracks over old larval galleries.

As in the previous year, trees were randomly assigned to one of the four treatments (Control, Girdled, EmBen, EmBen + Girdled). Two trees per block were injected with the low rate of TREE-äge using the same methods as in the previous year (emamectin benzoate, 4%, 0.1 g AI per 2.5 cm DBH) using the ArborJet QuickJet™ device on 20 May. One injected tree (EmBen + Girdled) and one non-injected tree (Girdled) in each block were girdled on 8–9 June, using the same methods as in 2009.

We wrapped a 30 cm wide band of plastic wrap tightly around the trunk of all study trees, approximately 1.3–1.5 m high, and then covered the plastic wrap with a thick coat of Tree Tanglefoot Insect Barrier (Contech Inc., Grand Rapids, Michigan) on 4 June. The sticky bands were checked at 2 week intervals to collect EAB beetles. Beetles were returned to the Michigan State University (MSU) Forest Entomology laboratory, soaked in ethanol (75%) to remove the Tanglefoot and then examined under a microscope to confirm species.

On 7 July, leaf-bearing shoots were collected from 2–4 aspects of the canopy of the study trees using pole pruners, as in 2009. Leaves were bagged, placed in coolers and returned to the MSU Forest Entomology laboratory. We were unable to reach shoots on several very tall trees, particularly at the LB site. We collected foliage from only two trees at LB (one control, one girdled tree). At the DW site, we were able to collect foliage from 13 of the 16 trees, but did not collect leaves from two girdled and one EmBen + Girdled tree. At the MR site, we could not reach leaves on nine of the 32 trees (five girdled, two EmBen and two EmBen + Girdled trees). A leaf from each of two shoots collected from opposite sides of the tree was set aside for bioassays with adult beetles. We inserted the petiole of a leaf into a water pic to slow desiccation, and then placed the leaf into a petri dish (15 cm diameter). Three EAB adults reared from infested ash logs were placed in each dish (six beetles per tree) and allowed to feed. Beetles were 3–4 days old when placed into petri dishes, and equal numbers of males and females were assigned to each tree. Beetles were observed periodically, and mortality was recorded 24 h (day 1) and 72 h (day 3) later. Remaining leaves not used in bioassays were stripped from the woody shoots, frozen and then shipped via overnight mail to the USDA APHIS laboratory in MA for residue analysis (see below).

Trees were felled, sectioned and debarked from October through December 2010 to assess larval density, using the same methods as in 2009. Density of pretreatment larval galleries and current-year larvae were again recorded and standardized per m^2 of exposed surface as before.

2.3 Foliar residues

Foliar residues of emamectin benzoate in the composite samples collected in June and July 2009 and in July 2010 were quantified using commercially available 96-well plate enzyme-linked immunosorbent assay (ELISA) kits (kit number 3100176052; Horiba, Ltd, Kyoto, Japan). Because we could not reach foliage on trees at the LB site, residues were quantified for the injected trees (EmBen, EmBen + Girdled) at the CLF and WW sites in 2009 and at the MR and DW sites in 2010. Foliage from 3–6 Control or Girdled trees per site was also analyzed. Leaf samples remained frozen until ready for analysis, and then were separated from stems and petioles and stored in paper bags at room temperature for several days until dry and brittle. Dried leaves were compressed and broken by hand, then placed into a 1.9 L stainless steel vessel atop a two-speed commercial blender. Leaf material was blended at high speed for approximately 30 s to homogenize the sample and break up the leaf tissue into a fine powder. Vessels were thoroughly cleaned after each use to avoid cross-contamination between samples. To extract the insecticide, a 0.5 g sample of the ground leaf material was weighed into a 50 mL plastic centrifuge tube and then extracted in 10 mL of pure methanol for 3 h on a table-top shaker. Samples in tubes were spun down in an Eppendorf 5810 high-speed centrifuge (Eppendorf, New York, NY) at 6000 rpm for 10 min, and the supernatant was diluted a minimum of 20× to avoid matrix effects from the kit on account of the methanol. Samples were then run on the assay kits according to the manufacturer's specifications. Individual samples were run in duplicate, and samples were reassessed if the resulting value exceeded the standard curve or if individual samples varied by more than 15% between the duplicate wells. Sample values were averaged for each tree and adjusted to achieve a value in parts per million.

2.4 Statistical analyses

Variables were tested for normality using the Shapiro–Wilk test and residual plots. Two-way ANOVA was used to assess differences among treatments, sites and the interaction of the two factors on tree DBH, emamectin benzoate residues in foliage and density of pretreatment and current-year larvae in 2009 and 2010. When ANOVA results were significant ($P < 0.05$), Tukey's least-squares multiple comparison test was applied. Larval density values were $\log(x + 1)$ transformed to normalize data for analyses. Differences in adult EAB mortality on day 1 and day 3 of the foliar bioassay were not normalized by transformations. These variables were tested with Friedmann's non-parametric ANOVA followed by non-parametric multiple comparison tests when ANOVA results were significant ($P < 0.05$).[42] All analyses were conducted using SAS statistical analysis software v.9.2.[43]

3 RESULTS

3.1 2009 study

Tree DBH differed among sites ($F = 8.87$; df = 2, 36; $P = 0.0007$), but was similar among trees assigned to different treatments ($F = 0.71$; df = 3, 36; $P = 0.55$) in 2009. The interaction of site and treatment was not significant ($F = 0.57$; df = 6, 36; $P = 0.75$). Diameter of trees was similar at the Wolverine West (WW) and Cloverleaf (CLF) sites, where DBH averaged (± SE) 17.8 ± 0.77 and 18.0 ± 1.09 cm respectively. Trees at the Lincoln Brick (LB) site were tall but smaller in diameter, averaging 14.1 ± 0.63 cm DBH. The average DBH of trees assigned to different treatments ranged from 15.0 ± 3.00 cm (Girdled) to 16.5 ± 1.12 cm (EmBen + Girdled).

3.1.1 Foliar residues

Girdling trees 18 days after injecting the emamectin benzoate did not appear to interfere with translocation of the insecticide. Residues of emamectin benzoate did not differ between leaves collected from EmBen and EmBen + Girdled trees in June ($F = 0.48$; df = 1, 9; $P = 0.508$) or July ($F = 0.29$; df = 1, 0; $P = 0.602$). Foliar residues from EmBen and EmBen + Girdled trees averaged 1.73 ± 0.41 mg kg^{-1} and 2.35 ± 0.75 mg kg^{-1} respectively in June, and 0.94 ± 0.26 mg kg^{-1} and 3.91 ± 1.58 mg kg^{-1} respectively in July. Residue levels in leaves of injected trees (EmBen, EmBen + Girdled) at the CLF and WW sites were also similar in June ($F = 0.29$; df = 1, 9; $P = 0.602$) and July ($F = 1.51$; df = 1, 9; $P = 0.251$). Residue levels averaged 1.70 ± 0.43 ppm and 2.21 ± 0.60 ppm in June and 4.28 ± 2.36 ppm and 1.50 ± 0.53 ppm in July at the CLF and WW sites respectively. As expected, no emamectin benzoate was detected in foliage from Control or Girdled trees that were not injected.

3.1.2 Larval densities

The density of pretreatment larval galleries on trees felled in October 2009 was generally low, which was not surprising given the healthy appearance of the study trees. The average density of galleries from larvae that fed prior to 2009 was 11.5 ± 3.87, 9.8 ± 2.46 and 3.7 ± 0.67 at the CLF, WW and LB sites respectively, and differences among sites were marginally insignificant ($F = 3.07$; df = 2, 36; $P = 0.059$). The density of pretreatment larvae did not differ among trees assigned to different treatments ($F = 0.55$; df = 3, 36; $P = 0.65$), nor was the interaction between site and treatment significant ($F = 0.82$; df = 6, 36; $P = 0.56$). The pretreatment density averaged 10.0 ± 3.02, 6.15 ± 2.08, 6.7 ± 2.54 and 5.5 ± 1.54 on the Control, Girdled, EmBen and EmBen + Girdled trees respectively.

Our primary interest was in the density of larvae that hatched and began feeding in summer 2009, following the spring injections with emamectin benzoate and girdling. Girdling and insecticide application significantly affected density of the

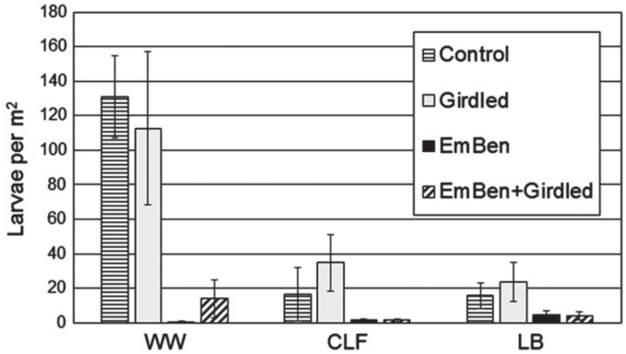

Figure 1. Number of current-year *A. planipennis* larvae per m^2 in 2009 in *Fraxinus* spp. trees left as untreated controls (Control), girdled (Girdled), injected with emamectin benzoate (EmBen) or injected with emamectin benzoate and then girdled (EmBen + Girdled) at the Wolverine West (WW), Cloverleaf (CLF) and Lincoln Brick (LB) sites ($n = 12$ trees per treatment). Larval densities were higher in Control and Girdled trees than in EmBen and EmBen + Girdled trees ($P < 0.0001$) and in trees at the WW site than in trees at the LB and CLF sites ($P = 0.002$).

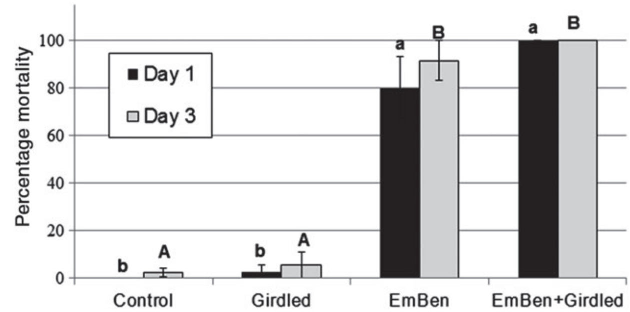

Figure 2. Percentage mortality of *A. planipennis* adults caged in 2010 with leaves from *Fraxinus* spp. trees left as untreated controls (Control), girdled (Girdled), injected with emamectin benzoate (EmBen) or injected with emamectin benzoate and then girdled (EmBen + Girdled). Letters indicate significant differences among treatments on day 1 and day 3 of the bioassay ($P < 0.0001$).

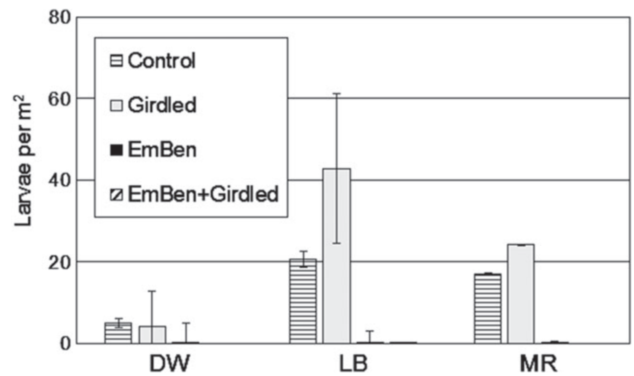

Figure 3. Number of current-year *A. planipennis* larvae per m^2 in 2010 in *Fraxinus* spp. trees left as untreated controls (Control), girdled (Girdled), injected with emamectin benzoate (EmBen) or injected with emamectin benzoate and then girdled (EmBen + Girdled) at the D-Woodlot (DW), Lincoln Brick (LB) and Maple River (MR) sites ($n = 16$ trees per treatment). Larval densities were higher in Control and Girdled trees than in EmBen and EmBen + Girdled trees ($P < 0.0001$) and in trees at the LB and MR sites than in trees at the DW site ($P < 0.009$).

current-year larvae ($F = 16.67$; df = 3, 36; $P < 0.0001$) (Fig. 1). The average densities of larvae on Girdled trees and untreated Control trees were 4–10 times higher than the larval densities on the EmBen trees and the EmBen + Girdled trees (Fig. 1), which did not differ from each other. Seven of the EmBen or EmBen + Girdled trees had no live larvae, while every Control and Girdled tree was infested.

The density of current-year larvae also differed significantly among the three sites in 2009 ($F = 5.73$; df = 2, 36; $P = 0.007$) (Fig. 1), and the interaction of site and treatment was significant ($F = 2.44$; df = 3, 36; $P = 0.0438$). These results reflect the dramatic increase in EAB density across the entire WW site between 2008 and 2009. Virtually all phloem on girdled and control trees at the WW site was consumed by EAB larvae in 2009, and these trees, like most of the other ash in the vicinity, were severely declining or dying by the end of the summer. While EAB population levels increased from 2008 to 2009 at the CLF and LB sites, 2009 larval densities at these sites remained considerably lower than at the WW site (Fig. 1). At the CLF and LB sites, the density of current-year larvae on Control and Girdled trees in 2009 was approximately 2 and 4 times as high as the density of pretreatment larvae respectively. The high EAB larval densities at the WW site obscured differences between Control and Girdled trees at the other two sites. On average, the larval densities in 2009 were 67 and 53% higher on Girdled trees than on Control trees at the CLF and LB sites respectively.

3.2 2010 Study

Tree diameter did not vary among sites ($F = 1.73$; df = 2, 58; $P = 0.18$) or treatments ($F = 0.33$; df = 3, 52; $P = 0.80$), nor was the interaction significant ($F = 0.95$; df = 6, 52; $P = 0.47$). Trees averaged (± SE) 15.8 ± 0.54, 16.8 ± 0.53 and 17.0 ± 0.38 cm DBH at the D-Woodlot (DW), Lincoln Brick (LB) and Maple River (MR) sites respectively. Trees assigned to the Control, Girdled, EmBen and EmBen + Girdled treatments had an average DBH of 16.8 ± 0.58, 16.4 ± 0.49, 17.0 ± 1.22 and 16.4 ± 0.54 cm respectively.

3.2.1 Adult EAB bioassays

The mortality of adult EAB beetles differed among treatments on day 1 ($F = 68.04$; df = 7, 28; $P < 0.0001$) and day 3 ($F = 92.37$; df = 7, 28; $P < 0.0001$) of the July bioassay (Fig. 2). Very few of the adult EAB beetles caged with leaves from Control and Girdled trees died during the bioassay. On day 1, a single beetle caged with foliage from a girdled tree at the LB site was dead; all other beetles caged with foliage from Control or Girdled trees were alive. By day 3, less than 5% of the beetles caged with leaves from Control or Girdled trees had died (Fig. 2). Beetles on Control and Girdled trees fed actively, consuming much of the leaf in the petri dish and producing abundant frass.

In contrast, leaves from the EmBen and the EmBen + Girdled trees were highly toxic to beetles (Fig. 2). There was no indication that beetles were repelled by leaves from trees treated with the insecticide, nor did we observe any behavioral differences among beetles caged with leaves from different treatments or sites. On average, ≥80% of beetles caged with leaves from EmBen or EmBen + Girdled trees were dead within 24 h, and >90% of beetles had died by day 3 of the bioassay, including all beetles caged with leaves from the EmBen + Girdled trees. In nearly all petri dishes with leaves from either EmBen or EmBen + Girdled trees, beetles died after only one or a few bites. Beetle mortality did not differ between sites on either day 1 ($P = 0.45$) or day 3 ($P = 0.97$).

3.2.2 Foliar residues

Analysis of emamectin benzoate residues in foliage collected in July 2010 from the EmBen and EmBen + Girdled trees at the DW and MR sites indicated that the girdling did not reduce translocation of the insecticide to the canopy. Residue levels in leaves from EmBen and EmBen + Girdled trees were similar, averaging 5.78 ± 1.40 ppm and 5.65 ± 1.23 ppm respectively ($P = 0.84$). Residue levels differed between treated trees at the two sites ($F = 5.19$; df = 2, 15; $P = 0.0194$) and were consistently higher in trees at the MR site (7.07 ± 1.13 ppm) than at the DW site (3.02 ± 1.02 ppm).

3.2.3 Beetle captures

The sticky bands, which were approximately 1.3–1.4 m aboveground on the trunks of the trees, captured relatively few EAB beetles. This was not unexpected, given that our study trees had virtually no foliage-bearing branches on the lower 6–8 m of the trunk. Beetle activity was likely concentrated in the upper canopy where leaves were available for feeding, rather than on the lower portion of the trunk where beetles were more likely to encounter the sticky bands. We captured a total of 88 adult beetles: 18 on control trees, 38 on girdled trees, ten on EmBen trees and 22 on EmBen + Girdled trees. Sticky bands captured at least one beetle on eight of the control and girdled trees, seven of the EmBen + Girdled trees but only one of the EmBen trees.

3.2.4 Larval densities

The density of pretreatment EAB larvae that fed prior to 2010 did not differ among the three sites ($F = 0.49$; df = 2, 52; $P = 0.61$) or among trees assigned to different treatments ($F = 1.69$; df = 3, 52; $P = 0.18$), nor was the interaction significant ($F = 0.48$; df = 6, 52; $P = 0.82$). Pretreatment larval densities were low at all sites, averaging 4.8 ± 1.58, 9.7 ± 3.30 and 9.0 ± 2.22 larvae m^{-2} at the DW, LB and MR sites respectively. Similarly, pretreatment larval densities averaged 11.1 ± 3.45, 10.3 ± 3.55, 5.3 ± 2.15 and 5.9 ± 1.98 larvae m^{-2} for Control, Girdled, EmBen and EmBen + Girdled trees respectively.

The density of current-year larvae that began feeding in 2010 was strongly affected by treatment ($F = 52.76$; df = 2, 52; $P < 0.0001$) (Fig. 3). Only four of the 32 trees injected with the emamectin benzoate insecticide, including the EmBen and EmBen + Girdled trees, had any live larvae, and none had more than four live larvae on the entire tree. The density of live larvae did not differ between EmBen and EmBen + Girdled trees, which had an average of only 0.30 ± 0.12 and 0.03 ± 0.03 larvae m^{-2} respectively. Larval densities were higher on Control and Girdled trees, which averaged 15.0 ± 3.39 and 23.9 ± 5.61 larvae m^{-2} respectively, than on the EmBen and EmBen + Girdled trees (Fig. 3).

The density of 2010 larvae also differed among sites ($F = 5.16$; df = 2, 52; $P = 0.0091$) (Fig. 3), averaging 2.4 ± 0.77, 15.0 ± 6.43 and 10.4 ± 2.31 larvae m^{-2} at the DW, LB and MR sites respectively, while the interaction was not significant ($F = 2.26$; df = 6, 52; $P = 0.515$). When only Control and Girdled trees were considered, the larval densities at the DW, LB and MR sites averaged 5.3 ± 1.5, 31.8 ± 10.3 and 20.6 ± 2.86 larvae m^{-2} respectively. Larval densities were notably low on all trees at the DW site, compared with the MR and LB sites, where larval densities did not differ (Fig. 3). Overall differences in EAB density between Girdled and Control trees were not significant, presumably reflecting the low EAB density at the DW site. At the LB and MR sites, however, larval densities were 52 and 30% higher on Girdled trees than on Control trees respectively (Fig. 3). Even at the DW site, a total of 38 EAB larvae were feeding on the four Girdled trees, compared with only nine larvae on the four Control trees.

4 DISCUSSION

Integrating two or more tactics to manage an insect pest population can result in outcomes that yield antagonistic, redundant, additive or synergistic effects.[41,44,45] Minimally, a multifaceted strategy should yield additive effects on the pest population. Targeting two life stages of a pest with insecticides or other control tactics, for example, would produce an additive effect. A synergistic effect occurs when the combination of tactics yields an outcome greater than the combined individual effects of the tactics. We hypothesized that lethal trap trees, created by injecting ash with the highly effective TREE-äge insecticide, and then girdling the trees could result in a synergistic effect on the local EAB population if the insecticide was effectively translocated throughout the tree prior to girdling.

Our hypothesis was based on adult EAB feeding behavior and the response of beetles to girdled trees. Beetles feed on ash leaves for at least a week before mating begins, females feed for another 1–2 weeks before oviposition begins and both sexes continue to feed throughout their 3–6 week lifespan.[1] Leaves from trees treated with emamectin benzoate, with or without subsequent girdling, were highly toxic to beetles in the 2010 bioassay. Within 24 h, at least 80% of the beetles caged with a leaf from an injected tree had died, and after 3 days the mortality averaged 90%. Every beetle caged with a leaf from a tree that was injected and subsequently girdled was dead within 24 h. Beetles typically took only one or two bites from leaves before succumbing. Similarly high rates and rapid mortality of adult EAB provided with foliage from trees treated with TREE-äge were reported in previous studies.[31]

Overall, the sticky bands wrapped around the trunk of our 2010 study trees captured relatively few beetles, which reflects both the low EAB densities and the height of the bands on the trees in the well-stocked, even-aged sites. Activity of adult beetles was likely concentrated in the upper portion of the canopies where foliage-bearing shoots were present, minimizing opportunities for beetles to encounter the sticky bands which were ≤1.5 m high. In addition, beetles that fed on foliage on the injected trees would have died, probably within a day, further reducing the likelihood of capture on the sticky bands. Nevertheless, girdling did appear to attract adult EAB. More than twice as many beetles were captured on the EmBen + Girdled trees (25% of the total) as on the EmBen trees (10%), while 40% of the beetles were captured on the Girdled trees and 20% on the Control trees.

Girdling trees 18–20 days after injecting the emamectin benzoate had no detectable effect on insecticide residue levels in foliage compared with trees that were injected but not girdled. When we girdled the trees in 2009 and 2010, we removed the outer bark and phloem, but were careful to minimize injury to the xylem.[24,25] Recent studies indicate systemic insecticides applied via trunk injection are transported to the canopy primarily in xylem.[33,34,46] If girdling does not impede conduction within xylem tissue, it seems likely that trees could be girdled and injected on the same day. Future studies to assess translocation rates and potential effects of injection timing, local weather or site conditions on treatment efficacy would be helpful. For example, insecticide residues in 2010 in the green ash trees at the MR site were generally higher than in the white ash trees at the DW site. This likely reflects site conditions rather than species differences, given that in a large-scale, 2 year study with substantial replication the foliar residues of emamectin benzoate were similar in green ash and white ash trees.[31] Study trees at the DW site were growing in a well-stocked, even-aged woodlot, and their canopies were only partially exposed to sun, which may have reduced uptake and translocation of the insecticide. At the MR site, the study trees were either superdominant or growing in openings and exposed to full or nearly full sun, which may have increased translocation, accounting for the higher foliar residues.

Numerous studies and operational projects have consistently shown that adult EAB are attracted to and preferentially colonize ash trees stressed by girdling.[24–27,47,48] Adult EAB attraction to girdled trees likely reflects changes in volatile profiles of the stressed trees[21] and is most pronounced in areas with fairly low densities of EAB.[24,27] As EAB densities build and more trees are stressed by larval feeding, beetles are exposed to stress-related volatiles from many competing trees, obscuring attraction to girdled trees. In operational projects, establishing lethal trap trees is likely to be most effective in infestations that are relatively recent or where effective insecticides are being used to protect other, more valuable ash trees. Wounds or injuries, including EAB larval galleries, can disrupt translocation and within-tree distribution of systemic insecticides.[49] To ensure adequate insecticide translocation and optimize efficacy, trees should be treated with insecticide before high larval densities disrupt vascular tissue.[32]

Recent evidence from two large-scale studies indicates that girdling ash trees in low-density infestations can produce a spillover effect, resulting in higher than expected colonization of ungirdled trees growing near girdled trees.[29,50,51] In other words, beetles attracted to a girdled tree may still deposit some portion of their eggs on adjacent or nearby ungirdled trees. Intermixing or surrounding girdled trees with treated trees may increase the likelihood that beetles attracted to the vicinity of a girdled tree will encounter a treated tree, thereby increasing adult mortality.[29,51] Lethal trap trees may function similarly. Girdling trees injected with emamectin benzoate may not only increase attraction to the treated tree, it may also reduce larval densities on nearby untreated trees by killing adult EAB that feed on the Girdled + Injected tree.

The attract-annihilate or bait-and-kill method is by far the most widely used behavioral manipulation for pest management.[35] The underlying strategy of the method is simple: attract the pests to a location or device where as many of them as possible can be removed from the environment. It usually involves a long-distance attractant (olfacatory or visual) and a device to kill or trap the attracted pests. Well-known applications of the bait-and-kill approach include strategies to protect livestock from screwworm flies (*Cochliomyia* spp.) by distributing a pelletized formulation containing a chemical attractant (swormlure), food (dried blood), a feeding stimulant (sugar) and an insecticide.[52] Similarly, oriental fruit fly [*Bactrocera dorsalis* (Hendel)] infestations were eradicated using an attractant (methyleugenol) combined with insecticide-saturated squares of cane fiber dispensed from airplanes.[53] In forested settings, lethal trap trees consisting of pine or spruce trees that were sprayed with insecticide and then felled or baited with semiochemicals (e.g. aggregation pheromones) were used in management efforts targeting aggressive bark beetles, including the spruce beetle, *Dendroctonus rufipennis* Kirby,[37,40] the mountain pine beetle, *Dendroctonus ponderosae* Hopkins,[38] the Douglas-fir beetle, *Dendroctonus pseudotsugae* Hopkins,[39] and the southern pine beetle, *Dendroctonus frontalis* Zimmerman.[36]

While TREE-äge or other highly effective, systemic insecticides are most likely to be used for long-term protection of high-value ash, lethal trap trees could play a role in operational EAB management programs. Ash have been popular ornamental trees for decades, and are often abundant in landscapes, parks and along roads or highways. In most urban or residential areas, however, at least some of the landscape ash have outgrown their space or have other problems and need to be replaced. Many municipalities have developed plans gradually to replace ash with other species, as resources permit. Minimally, ash trees designated for removal could be girdled in spring and removed in fall, to attract EAB in the area and eliminate the larvae developing on the tree. Another option may involve girdling trees a year after TREE-äge injections, which would ensure trees contributed to EAB mortality for 2 years. Studies have consistently shown that TREE-äge remains highly effective for at least 2 years post-injection,[31,32] but whether insecticide efficacy would be reduced if trees were girdled the year after injection is not known. Injecting and girdling trees designated for removal obviously adds further cost. On the other hand, girdled trees must be debarked, burned, chipped or otherwise destroyed to prevent larvae from emerging as adults the following year. If lethal trap trees were established in areas such as windbreaks, woodlots or forests, where dead trees would not pose a hazard, they could be left in place. Eliminating the need to return and remove or destroy girdled trees could offset injection and girdling costs. Whether such costs are acceptable will depend on site-specific objectives and circumstances. Nevertheless, it seems clear that lethal trap trees could potentially play a role in EAB management programs. Understanding more about how girdling and systemic insecticides interact to affect EAB population growth over time will be needed to optimize efforts to slow the onset and progression of ash mortality.

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