Protective neighboring effect from ash trees treated with systemic insecticide against emerald ash borer

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Abstract

BACKGROUND: The emerald ash borer (EAB) (Agrilus planipennis Fairmaire) (Coleoptera: Buprestidae) is now the most destructive invasive species in North America. While biocontrol using parasitoids shows promising results in natural forests, strategies are needed to protect high-value trees against invasive EAB populations. Emamectin benzoate is a commonly used systemic insecticide for the protection of valuable trees. Methods that optimize its use allow for reduced quantities of insecticide to be released in the environment and save time and money in efforts to protect ash trees from EAB. We hypothesize that a treated tree can also offer a protective neighboring effect to nearby untreated ash trees, allowing for an optimized spatial planning of insecticide applications.

RESULTS: We sampled 896 untreated ash trees, in the vicinity of treated trees, in Maryland and Washington DC. We recorded signs of EAB infestation (canopy condition, exit holes, wood pecks, epicormic growth, and bark splits). Two subsequent yearly samplings were made of 198 and 216 trees, respectively. We also present a novel proximity index for this particular application. Results show consistent decrease in EAB infestation signs in untreated trees as proximity to treated trees increases.

CONCLUSION: Results support that a neighboring effect occurs. However, proximity to treated trees must be high for a tree to be safely left untreated. This proximity seems rare in forests, but can happen in urban/planted landscapes. Future studies should test and validate these findings, and could lead to a more precise recommended safe index tailored across multiple ash species and geographic regions.

Keywords: pest management; emamectin benzoate; invasive species; Agrilus planipennis; Fraxinus sp.

1 INTRODUCTION

Invasive forest insects are a growing problem worldwide, causing unprecedented tree mortality, biomass losses, and disrupting native ecosystem integrity.1–3 Native to north-eastern Asia, the emerald ash borer (EAB) (Agrilus planipennis Fairmaire) (Coleoptera: Buprestidae) was accidentally introduced in North America in the 1990s4 and it is now the most costly and destructive invasive insect species on the continent.5 This beetle feeds on ash trees (Fraxinus spp.) and other Oleacea6,7; while adults feed on leaves, larvae feed on phloem and cambium, creating serpentine galleries and disrupting water and nutrient transport within the tree. Unlike in its native range, in North America there are no endemic specialized natural enemies attacking EAB, but abundant highly susceptible ash species for beetle reproduction,8 and more than 8.7 billion trees are under threat.9 In the continental USA, ash trees represent more than 2.5% of all aboveground biomass.9 At local scales, tree death caused by EAB not only results in economic losses but also risks of falling limbs or branches to pedestrians for municipalities and private owners.10,11 In urban areas, ash can comprise up to 25% of a city canopy cover,12 providing a myriad of ecosystem services13 and decreasing the urban heat island effect.13 Additionally, ash trees can be of invaluable cultural and economic significance for indigenous peoples14 and for health and wellbeing in urban environments.15,16

Long-term or sustainable control of EAB will likely depend on effective biological control with specialized natural enemies introduced from the pest’s native range (northeastern Asia). Developed by the US Department of Agriculture (USDA), the EAB biological control program aims to introduce and establish co-evolved natural enemies from Asia. Currently four species of larval and egg parasitoid wasps have been introduced to the USA, and the efficiency and effectiveness of these natural enemies in controlling the invasive EAB population is under evaluation.17 While there are promising results in protection of small ash trees and saplings with the introduced natural enemies, interim strategies are needed to protect high-value trees against invasive EAB
populations in urban, suburban and managed forest systems. A variety of insecticides have been tested to preemptively protect individual trees, including methods such as soil injection, systemic bark spray, and foliage cover spray. An effective and commonly used systemic insecticide, emamectin benzoate, sold commercially in the USA as TREE-age™, is injected systemically at the base of the trunk.18,19 It must be applied every 2 or 3 years to protect an individual tree, but tree removal costs still surpass those of treating.18,20 It also has been effective in decreasing EAB populations in area-wide management practices.21–23

The use of emamectin benzoate, however, does not come without disadvantages. The drilling wounds for application can damage and even kill the tree if not properly done.18 Although the chemical compound is known for degrading quickly under sunlight, making it relatively safe for nontarget organisms, the study of Burkhard et al.24 found the degradation half-life of emamectin benzoate in leaf macromolecules on the ground to be 20 days and, submersed in water, 94 days. The study concluded that the insecticide is safe, with little to no traces in water and nontarget organisms. However, the study should be viewed with caution, as it had a limited sampling size and was funded and conducted by the manufacturer (Revive™, Syngenta Crop Protection AG, Switzerland). Published studies on the effects on ground water and nontarget organisms are available for other commonly used insecticides for EAB management, but not to date for emamectin benzoate.19 It may affect pollinating insects in the vicinity of treated trees15 and, as with most insecticides, it can be toxic for the personnel transporting, handling or applying.25 Consequently, methods that optimize the use of emamectin benzoate via trunk injection are desirable, allowing for reduced quantities of insecticide to be released in the environment and saving time and money in public and private efforts to protect ash trees from EAB.

In the present study, we hypothesize that a tree treated with emamectin benzoate can offer a protective neighboring effect to nearby untreated ash trees, allowing for an optimized spatial planning of insecticide application. Since adult EAB feed on ash leaves up to 2 weeks before laying eggs, and alternate between bouts of egg-laying and feeding,20 the treatment potentially kills adult beetles that may lay eggs on other trees in the vicinity of the treated tree. Additionally, little is known on the persistence of the insecticide in the surroundings of a treated tree.19 In natural systems, antherbivore defenses are known to confer association resistance to neighboring plants, through mechanisms such as forcing post-contact dispersion or transmission of volatile signals.26 Our study addressed the following questions: (i) Do treated ash trees confer protection from emerald ash borer to untreated neighboring trees?; (ii) Is there a recommended proximity to a treated tree for which a tree can remain safely untreated?; and (iii) Does the protective neighboring effect, if it occurs, decay with time?

2 MATERIALS AND METHODS

2.1 Study sites and tree sampling

We sampled ash trees within five managed forest parks in the State of Maryland, USA: Cylburn Arboretum (39.35°N, 76.65°W), Gunpowder State Park (39.35°N, 76.35°W), Martinak State Park (38.86°N, 75.83°W), Patapsco Valley State Park (39.29°N, 76.78°W) and Susquehanna State Park (39.60°N, 76.15°W), and the National Arboretum in District of Columbia (38.91°N, 76.96°W) (Fig. 1). Major stands of white ash (F. americana L.) along with some green ash (F. pennsylvanica Marshall) dominated all sites except the National Arboretum, where green ash is the most common, along with other common northeastern deciduous species.

A total of 1051 ash trees were sampled, of which 117 were treated with emamectin benzoate. Treated trees were selected a priori by managers or by the Maryland Department of Agriculture (MDA) for their perceived importance, either for stature or location or both (e.g. proximity to campsites and trails). Trees were

Figure 1. Location of five sampling sites in the State of Maryland and one in Washington DC (National Arboretum).
treated once in the spring or early summer with the recommended medium rate of commercially sold Tree-Age G4 before sampling. For every treated tree, five concentric rings were established using a measuring tape at radii of 5, 10, 15, 20, and 25 m. Every untreated ash tree within each ring with diameter at breast height (DBH) greater than 2.5 cm was assessed for five criteria to estimate EAB infestation: canopy condition, number of wood pecks, number of exit holes, number of bark splits, and number of epicormic shoots. Canopy condition was visually assessed as five classes of canopy thinning, from 0–20% of thinning as class 1 to 80–100% as class 5. For analysis, class 1 was considered to be healthy canopy and classes from 2 to 5 were collapsed to one category as unhealthy. The remaining four criteria were assessed on the basal 2 m of each tree. Assessment data were collected from June to September each year, 2017–2019. A total of 896 untreated ash trees were surveyed in 2017, followed by 192 in 2018 and 216 in 2019. In both 2018 and 2019 surveys, sampling effort was reduced to four untreated trees for each treated tree. Out of the 896 trees sampled in the first year, 136 were assessed in each of the three sampling years.

2.2 Neighboring index
Ideally, the study would test the hypothesis that the signs of EAB infestation in untreated ash trees would increase as a function of distance to treated ash trees. Outside of plantation monocultures, such a design is rarely feasible. In managed forests, treated trees are often clustered, and an untreated tree may be in the proximity of several treated trees. Additionally, the size of a tree determines how much emamectin benzoate is needed, adding an interaction between the size of the treated tree and the size of the untreated tree in the vicinity. For these reasons we adapted a simple index for competition in pine trees (Hegyi 1974 *apud* O’Neal *et al.*28) as our neighboring index $I$. A given untreated tree $i$ would be under an individual index per meter given by the formula:

$$I_i = \sum \left( \frac{DBH_t}{DBH_i} \right) \frac{1}{Dist}$$

where $DBH_t$ is the diameter in centimeters of a treated tree located at a distance $Dist$ (meters), and $DBH_i$ is the diameter of the individual untreated tree. A tree with a high index is either surrounded by several treated trees or in the proximity of one large treated tree (Fig. 2).

2.3 Data analysis
To test if the neighboring index predicts signs of EAB infestation, we used zero-inflated generalized linear mixed models (GLMM) assuming a Poisson error distribution for number of exit holes, wood pecks, epicormic growth and bark splits, and a binomial

![Figure 2. Examples of different index values for nontreated trees (nTT): (a) a treated tree (TT) of equal DBH at a 1 m distance confers an index of 1 to a nontreated tree; (b) the same tree at a 2 m distance confers an index of 0.5; (c) a treated tree twice the DBH at a 1 m distance or (d) two treated trees at 1 m each confer an index of 2.](image-url)
response for healthy/unhealthy canopy condition (package \texttt{glmmTMB}, Magnusson et al.\textsuperscript{29}). Site was used as random effect to account for different stages in EAB infestation across sample locations. We use the zero-inflation component in our models due to the low EAB pressure in the all sites, leading to the majority of trees in the sample having no signs of infestation for one or more parameters. This adds ‘structural’ zero values to our sample, i.e. independent of the phenomena we are interested in, leading to biased parameter estimates if not taken into account.\textsuperscript{30} Models were checked for residual diagnostics against statistical assumptions using package \texttt{DHARMa}.\textsuperscript{31} Separate models were run for each sign of EAB infestation and for each of the three sampling

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{neighboring_index_distributions}
\caption{Distribution of neighboring index values across nontreated ash trees in six sites.}
\end{figure}
years (2017–2019). For each significant model, we calculated estimated values and the intercept of the lower boundary of the 95% confidence interval with \( y = 0 \), i.e. the index value in which the signs of EAB infestation are not significantly different from a non-infested tree. To assess whether the protective neighboring effect decays over time, we also used zero-inflated GLMM models with the same distributions and residual diagnostics, but with years since treatment (0 to 1, 2, and 3) as predicting factors. Only trees with a neighboring index above 0.5 were tested for neighboring effect decay.

### 3 RESULTS

Neighboring indexes were generally low (below 0.5) (Fig. 3) for most untreated trees, but locations with more clustered distribution of ash had trees with higher values. Zero-inflated GLMM models show a relatively consistent decrease in EAB infestation signs with higher values of the neighboring index (Table 1). While the larger sample of 2017 shows decreases in exit holes, wood pecks, and epicormic growth, as well as higher occurrence of non-infested canopy condition (Fig. 4), the smaller 2018 and 2019 samples produced less consistent results. In the 2018 sample, both epicormic growth and bark splits had a positive relationship with index. Canopy condition was the only variable across all three samples that showed consistently less EAB infestation in untreated trees under a high neighboring index. All models were rerun excluding the few trees with very high index values (four trees with index above three, all from Gunpowder State Park) to test whether these points were biasing our results, and all relationships and significance were consistent. At an index of 3.61, canopy condition was not significantly higher than 0 (Table 1; lower 95% CI = 0 column), indicating this value as a safe index at which a tree could be left untreated. For the other parameters, infestation signs did not significantly differ from 0 at index values of 0.80 for exit holes, 1.55 for wood pecks, and 1.75 for growth. Repeated sampling of untreated ash trees in the vicinity of trees treated with emamectin benzoate (neighboring index \( \geq 0.5 \)) in 2018 and 2019 (2 and 3 years after treatment) indicated a significant increase in the number of exit holes and wood pecks in both intervals relative to 2017 (0 to 1 year after treatment) (Table 2 and Fig. 5). The number of epicormic growths was slightly higher 3 years after treatment and, contrastingly, the same interval showed a slight decrease in the number of trees with an unhealthy canopy.

### 4 DISCUSSION

We present a simple but novel index to estimate the protective neighboring effect in ash trees treated with systemic insecticide. Our results show strong evidence that the protective neighboring effect occurs but index values must be high for an untreated tree to show no signs of infestation. For the number of exit holes, wood pecks and epicormic growth, untreated trees with an index above 1.75 (e.g. a treated tree with twice the DBH at approximately 1 m distance) showed no signs of infestation. However, canopy condition is likely a more reliable and earlier sign of infestation, and only untreated trees with very high indexes (above 3.61, e.g. a tree surrounded by two other trees twice the DBH at approximately 1 m distance) were likely to show no sign of canopy depletion. Only a handful of untreated trees in our entire sample had such high indexes. There is also some evidence that the effect decays with time, as most signs of infestation increased in high-indexed untreated trees in the 3-year sampling period. Tree mortality was not tracked in our study, meaning that the ultimate fate of the untreated trees is unknown.

The mechanism by which the treatment of an ash tree with emamectin benzoate appears to marginally protect nearby untreated trees is yet to be elucidated. Studies on associational resistance show both abiotic and biotic mechanisms in which the antitherbivory defenses of a plant can benefit neighboring individuals, whether conspecifics or not. Volatile toxic

### Table 1. Zero-inflated GLMM results for signs of EAB infestation in untreated ash trees under the neighboring effect (measured by index) of nearby treated trees (*p<0.05, **p<0.01, ***p<0.001)

| Year | Exit holes | Wood pecks | Epicormic growth | Bark splits | Canopy condition |
|------|------------|------------|------------------|-------------|-----------------|
| 2017 (n = 896) | Index coefficient | Std. error | Site variance | z.i. intercept | z.i. std. error | Lower 95% CI |
| | -2.078** | 0.707 | 4.457 | 1.976** | 0.228 | 0.80 |
| | -0.913* | 0.402 | <0.001 | 2.763*** | 0.175 | 1.55 |
| | -2.182*** | 0.348 | 0.280 | 1.953*** | 0.129 | 1.75 |
| | -0.226 | 0.436 | 2.537 | 1.870*** | 0.198 | - |
| | -0.907*** | 0.261 | 2.417 | - | - | 3.61 |
| 2018 (n = 198) | Index coefficient | Std. error | Site variance | z.i. intercept | z.i. std. error | Lower 95% CI |
| | 0.757 | 0.803 | 5.362 | 1.598*** | 0.387 | - |
| | -0.321 | 0.474 | 0.616 | 2.101*** | 0.319 | - |
| | 1.135* | 0.525 | <0.001 | 1.510*** | 0.261 | - |
| | 8.037* | 3.868 | <0.001 | 2.187** | 0.969 | - |
| | -2.429** | 0.830 | 0.377 | - | - | 1.25 |
| 2019 (n = 216) | Index coefficient | Std. error | Site variance | z.i. intercept | z.i. std. error | Lower 95% CI |
| | -1.192** | 0.425 | 3.624 | 1.398*** | 0.220 | 1.47 |
| | -0.378 | 0.422 | 3.956 | 1.621*** | 0.241 | - |
| | -0.089 | 0.210 | 0.554 | 1.025*** | 0.193 | - |
| | -1.413 | 0.821 | 1.505 | 1.440*** | 0.325 | - |
| | -1.065* | 0.471 | <0.001 | - | - | - |

Negative coefficients mean fewer signs of infestation on higher index values. Lower 95% CI = 0 indicates at which index value the signs of EAB infestation are not significantly different from 0.
compounds, for example, can repel herbivores that would attack neighboring plants, but this is an unlikely parallel with the results we found, since emamectin benzoate degrades under UV light. Postcontact repulsion or death of the herbivore is another possible associational resistance mechanism to explain these patterns. EAB adults alternate between feeding and egg-laying, meaning that a treated tree could kill adults that would potentially infest other trees in the vicinity. It is unclear from

Figure 4. Signs of EAB infestation in untreated ash trees under the neighboring effect of nearby treated trees. A high index indicates close proximity to one or more trees treated with emamectin benzoate. Lines represent main predicted values of GLMMs and the shaded area 95% confidence intervals. Figure for data sampled in 2017.
the literature, however, how often and how far a female would move from tree to tree while engaged in this behavior. More studies on emamectin benzoate persistence in the environment and nontarget organisms could also elucidate why the neighboring effect occurs. Our results point to a noticeable neighboring effect in the immediate proximity to treated trees, along with a decay with time, which could indicate persistence in the soil or leaf litter.

### 4.1 Management recommendations

Area-wide management simulations and field surveys show that a small proportion of ash trees treated with systemic insecticide provide detectable reduction in EAB population growth and distribution, suggesting that a neighboring effect could allow for cost-effective approaches. Due to the high proximity needed to other treated trees, and the possible diminishing effect with time, we do not recommend that high-value ash trees are left untreated in urban forests like those assessed in our study. In most cases, ash density was not sufficiently high to allow for spatial planning in which trees could be safely left untreated. In high-density forests where ash predominates, however, this approach may be feasible and should be investigated. Black ash (F. nigra Marshall), for instance, can be locally dominant, and even occur in pure stands, in its natural habitats such as deciduous and deciduous-conifer wetland forests. Additionally, it is a species particularly vulnerable to EAB, showing high levels of infestation and mortality when compared to other species of ash. Systemic insecticide application planning in ash-predominant forests may be more cost-efficient if the protective neighboring effect is taken into account. Future studies in such locations can lead to more precise recommendations tailored for the species and its local relative abundance.

### AUTHOR CONTRIBUTIONS

KA, JJD, and PS conceived and designed the study. KA conducted fieldwork. RBA performed data analyses. RBA and DSG wrote the manuscript.

### Table 2.

Zero-inflated GLMM coefficients (and standard errors) for yearly progression of signs of EAB infestation in untreated ash trees in the vicinity of trees treated with emamectin benzoate (neighboring index ≥0.5). (*p<0.05, **p<0.01, ***p<0.001)

|                           | 2 years after treatment | 3 years after treatment | Site variance | z.i. intercept |
|---------------------------|-------------------------|-------------------------|---------------|----------------|
| Exit holes                | 3.585*** (1.177)        | 2.902* (1.167)          | 7.174         | 1.712*** (0.434) |
| Wood pecks                | 4.444*** (1.239)        | 4.394*** (1.240)        | 2.334         | 1.429*** (0.309) |
| Epicormic growth          | 0.504 (0.358)           | 0.743* (0.332)          | 0.534         | 1.122*** (0.254) |
| Bark splits               | −0.835 (0.705)          | −0.547 (0.547)          | 2.508         | 0.676 (0.638)   |
| Canopy condition          | −0.757 (0.473)          | −0.949* (0.471)         | 0.279         |                |

Positive coefficients mean an increase in signs of infestation.

### Figure 5.

Yearly progression of signs of EAB infestation in nontreated ash trees in the vicinity of trees treated with emamectin benzoate (neighboring index >0.5). Lines connect sequential yearly sampling of individual trees.
manuscript and KA, JJD, and PS revised and improved the manuscript. All authors reviewed and approved the final version of the manuscript.

ACKNOWLEDGEMENTS
This study was supported by Agreement AP18PPQS&T00C175 from the USDA APHIS PPQ. Many thanks to Stokes Aker, Michael Nugent, Jack Cantilli, and Alex Forde at the University of Maryland, and Heather Disque at Maryland Department of Agriculture. The authors also thank two anonymous reviewers for comments and suggestions to the manuscript.

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