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EMERALD ASH BORER (COLEOPTERA: BUPRESTIDAE) HOST EXPANSION; TRADING AN OPTIMAL HOST FOR ENEMY FREE SPACE?

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EMERALD ASH BORER (COLEOPTERA: BUPRESTIDAE) HOST EXPANSION;
TRADING AN OPTIMAL HOST FOR ENEMY FREE SPACE?

THESIS

A thesis submitted in partial fulfillment of the
requirements for the Degree of Master of Science in the
College of Agriculture, Food and the Environment at the University of Kentucky

By

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Lexington, Kentucky

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2018

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ABSTRACT OF THESIS

EMERALD ASH BORER (COLEOPTERA: BUPRESTIDAE) HOST EXPANSION; TRADING AN OPTIMAL HOST FOR ENEMY FREE SPACE?

Emerald ash borer (EAB), *Agrilus planipennis* (Coleoptera: Buprestidae), is an aggressive invader from Asia that has killed millions of trees in North America. Recently EAB has been documented developing in a novel host, white fringetree, *Chionanthus virginicus*. I evaluated larval performance in two common ash species and white fringetree by infesting excised bolts with emerald ash borer eggs. In addition I evaluated several plant characteristics to determine which most influence larval development. I also conducted choice and no choice assays using the classical biological control agent, *Tetrastichus planipennisi* (Hymenoptera: Eulophidae), to assess its ability to locate larval EAB in the different host plants. I found significantly lower survival rates of EAB larvae in white fringetree compared to white ash, *F. americana*. Larval phloem consumption and larval growth were lower in fringetree than in both ash tested. In choice and no choice assays *T. planipennisi* failed to parasitize larvae in fringetree. Failure of *T. planipennisi* to parasitize larvae within fringetree has implications for the efficacy of this classical biological control agent. Coupled with the use of white fringetree as a reservoir host, the enemy free space provided to EAB through use of this alternate host may have repercussions for EAB invasion dynamics.

Keywords *Agrilus planipennis*, biological control, enemy free space, host shift, *Tetrastichus*, *Chionanthus*

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For my grandparents Sanford and Phyllis Olson, and Bernard and Barbara Hens

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“In the Sacred Heart every treasure of wisdom and knowledge is hidden. In that divine heart beats God’s infinite love for everyone, each one of us individually.”

- Pope St. John Paul II

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CHAPTER 1

Introduction

Ash trees *Fraxinus spp.* have long been a major component of North American forests. They have been highly prized for their use in high tensile strength lumber for centuries (Sterrett 1917). Likewise, green ash *F. pennsylvanica* has been extensively used in urban environments as a street tree due to its rapid growth and resistance to many native pests (Arbor Day Foundation, 2018). Culturally significant also is the black ash (*F. nigra*), which is used by many Native American tribes for basket weaving (Jourdan 2013). Alongside these cultural and economic uses, ash trees are of vital ecological importance. Many forested areas of Eastern North America are comprised heavily of this genus (MacFarlane and Meyer 2005). In the last few decades however, this genus has come under threat from an invasive wood boring beetle native to eastern Asia.

Emerald ash borer (EAB), *Agrilus planipennis*, is a phloem and cambium feeding buprestid which was first discovered in North America near Detroit in 2002 in what was initially thought to be large scale cases of ash yellows, a phytoplasmic bacteria *Candidatus fraxinii* (Hair 2001). Although initial efforts focused on eradication and containment, numerous satellite populations began to appear throughout the range of native ash most likely due to nursery stock and firewood transport (Mercator et al. 2011). Currently, infestations have been confirmed in 31 states and 3 Canadian provinces leading to billions of dollars in losses both economic and environmental (Kovacs et al. 2010).

All species of the genus *Fraxinus* native to North America are susceptible to EAB, unlike its coevolved host Manchurian ash *F. mandshurica*, which is typically only colonized when already damaged or stressed (Wei et al. 2004; Rebek et al. 2008). Although some reports have noted the persistence of blue ash *F. quadrangulata* following the invasion of EAB, it along with other ash species of North America is still susceptible and has seen extensive decline (Tanis and McCullough 2012).

A number of native predators and parasitoids have been observed to be utilizing EAB larvae as a host and food source. *Spathius floridanus* (Hymenoptera: Braconidae) and *Atanycolus spp* (Hymenoptera: Braconidae), two native parasitoid wasps, have been noted to be occasional parasitoids of the larva (Bauer et al. 2004). Likewise woodpecker populations have been observed feeding on the larvae and have shown population increases following the invasion of EAB (Lindell et al. 2008). However, these native controls are often only opportunistic and do not provide sufficient control of the invasive beetle.

Application of systemic insecticides has been shown to be effective in treating and protecting trees from EAB (Smitley et al. 2010). These include injections of emamectin benzoate, basal bark sprays of dinotefuran, and soil drenches of imidacloprid. Although insecticides can provide protection from EAB, they often need to be reapplied every 1-2 years, and large scale treatment is both cost and time prohibitive (Vannatta et al. 2012). Additionally, concerns have been raised about the effects on non-target organisms such as pollinators with application of neonicotinoids (Copping 2013).

Several biological control agents have been approved for release by the United States Department of Agriculture, Animal and Plant Health Inspection Service (USDA

APHIS). Taken from the native range of EAB these include, larval parasitoids *Spathius galinae* (Hymenoptera: Braconidae), *S. agrili* (Hymenoptera: Braconidae), and *Tetrastichus planipennisi* (Hymenoptera: Eulophidae), along with the egg parasitoid *Oobius agrili* (Hymenoptera: Encyrtidae) (USDA APHIS 2018). *Spathius agrili* has achieved only minimal success in many of its released ranges due to a likely intolerance to colder northern temperatures (Duan et al. 2012). *S. galinae* has only been approved for release since 2015, and has not had adequate time to be more thoroughly studied. Due to its size of ~1mm, *O. agrili* has been hard to detect, however it is believed to be established in some areas (Jennings et al. 2014). Overwhelmingly, the major success of biological control in this case has been *T. planipennisi* which has been recorded at release sites and spreading out from initial points of release, and may be helpful with future ash regeneration (Duan et al. 2013).

Until 2014 it was widely believed that the only species of plants that were susceptible to EAB in North America were members of the genus *Fraxinus* (Lyons et al. 2009). Thus, the progress of the invasion was believed to be well understood as EAB populations were expected to behave in a predictable manner. That is that they would increase rapidly coming into contact with abundant host material in the invaded range, but would begin to see population crashes as available hosts dwindled and became scarce. These population dynamics would then allow slow mortality approaches to be utilized (McCullough and Mercader 2012).

In 2014, EAB was observed utilizing a novel host, white fringetree *Chionanthus virginicus* (Lamiales: Oleaceae), in Southern Ohio (Cipollini 2015). Initial research on the novel interaction confirmed the plant as a host, and subsequent investigations found

infestations in several other states (Cipollini and Rigsby 2015; Peterson and Cipollini 2017). The full impact that this host shift could have on the invasion dynamics of EAB remains unknown.

My research investigates novel host interactions with emerald ash borer, its parasitoid *T. planipennisi*, and white fringetree. I had two objectives for the work summarized in chapter one. The first was to determine the suitability of white fringetree as a novel host plant for emerald ash borer larvae. My second objective was to evaluate the ability of *T. planipennisi* to parasitize emerald ash borer in this new host and to evaluate its host location abilities. My studies were conducted in both field and laboratory settings in Central Kentucky. Suitability assays were conducted on the University of Kentucky campus with material collected from Fayette and Crawford Counties.

In Chapter 2, I investigate the novel host interactions between EAB, *T. planipennisi*, and white fringetree. Performing a feeding assay within bolts, I measured the suitability of fringetree compared to white and blue ash for EAB larval consumption and growth. After evaluating plant characteristics such as nitrogen and carbon content, callus tissue formation, and density. I discovered several possible explanations for the sub-suitability of white fringetree as a host for EAB. I conducted choice and no choice assays with *T. planipennisi* exposed to EAB parasitized stems of white ash, blue ash, and white fringetree. I also evaluated *T. planipennisi* in a series of laboratory choice tests to investigate its host finding behavior. I observed that *T. planipennisi* is unwilling or unable to utilize EAB in fringetree, and that *T. planipennisi* uses a combination of olfactory and visual cues for selecting its host, but is repelled by fringetree stem tissue combined with a leaf visual cue. Based on my data I predict that EAB has potentially

found enemy free space and escaped dwindling resources by utilizing white fringetree, and that this could greatly alter its invasion dynamics.

In Appendix A, I investigate the uptake, distribution, and efficacy of two common pesticides used in EAB control at two different rates, full and half label rates. I treated small diameter green ash with imidacloprid and dinotefuran several weeks before infesting them with EAB. The leaf, stem, and root tissue of the trees was later evaluated for residues via liquid chromatography mass spectrometry (LCMS). I observed different concentrations of insecticide between the tissues of treatments, confirming previous research regarding translocation. I also noted no significant difference between half and full dose trees in both insecticide residue concentration and control of EAB larvae. I predict that this will have implications for ongoing management, and that lower doses may pose less of a risk to non-target organisms.

I investigate the interactions of emerald ash borer with a novel host and the implications that it may have for current and future management operations, particularly biological control. My research provides a better understanding of the many interactions involved in a host species shift. This knowledge will help to inform the management practices of EAB into the future as it continues to expand and alter its invasion dynamics.

CHAPTER 2

Host range expansion may provide enemy free space for the emerald ash borer

Introduction

Both biotic and abiotic forces serve to structure ecological communities (Strong et al. 1984; Verhoef and Morin 2010). These forces affect geographic distributions (Davis et al. 1986; Huston and DeAngelis 1994; Meier et al. 2010), resource availability (Huston and DeAngelis 1994; Davis et al. 2000), competitive interactions (Huston 1997; Davis et al. 2005), and also influence trophic relations (Jeffries and Lawton 1984; Hunter and Price 1992; Stamp 2001; Wisz et al. 2013). Fluctuating resources and natural enemies both influence invasiveness and have emerged as leading suppositions in invasion ecology. As organisms are freed from the constraints of population regulation imposed by limited resources (Davis et al 2000, Davis and Pelsor 2001) and natural enemies (Jeffries and Lawton 1984), they are subsequently able to increase their geographic range. In contrast, the abiotic constraints dictating community structure and acting on a newly arriving non-native species are often similar between donor and recipient regions (Elton 1958; Ruiz et al. 2000; Richardson and Pyšek 2006). Host range expansion or host switching is one mechanism that may allow introduced organisms to overcome these abiotic constraints. Host range expansion can buffer the depletion of optimal host material (Sax et al. 2007) and contribute to enemy free space (Gratton and Welter 1999; Drake 2003; Murphy 2004), ultimately leading to increases in population growth and to geographic range expansion.

Since its introduction to North America in the mid-1990s (Haack et al. 2002; Siegert et al. 2014), emerald ash borer (*Agrilus planipennis* Fairmaire, EAB) has invaded the eastern portion of the United States and three Canadian provinces (Herms and McCullough 2014; USDA APHIS Map 2018) causing extensive mortality and loss of ash (*Fraxinus* spp.). The ash borer larva develops underneath the bark of the host consuming cambial tissue before pupating and emerging as an adult (Cappaert et al. 2005). Ash species native to North America show little to no resistance to the borer (Rebek et al. 2008), and endemic natural enemies are unable to sufficiently curtail population growth (Duan et al. 2009; Duan et al. 2012). As a result, EAB infestation leads to ash mortality in 1 – 4 years by effectively girdling the tree (Rebek et al. 2008; Klooster et al. 2014). Invasion by EAB has led to a widespread loss of native ash species in urban and wildland forests, altering forest composition and structure (Flower et al. 2013; Levin-Nielsen and Rieske 2015; Klooster et al. 2014), affecting native biodiversity (Lindell et al. 2008; Gandhi and Herms 2010; Perry and Herms 2016) and trophic relationships (Duan et al. 2012; Davidson and Rieske 2015; Savage and Rieske 2018). Widespread ash mortality impacts management plans (Looney et al. 2017) and restoration efforts (Burr and McCullough 2014), and causes extraordinary economic losses (Klooster et al. 2014; Herms and McCullough 2014).

Systemic insecticides can kill EAB (Smitley et al. 2010), but the prevalence and extent of ash in urban and wildland forests makes this management approach impractical. Because native parasitoids are unable to effectively suppress EAB, a concerted effort was made to identify classical biological control agents for importation to help regulate populations (Liu et al. 2003). Of those classical biological control agents, *Tetrastichus*

planipennisi (Hymenoptera: Eulophidae) has been the most effective at establishing and dispersing from release sites (Duan et al. 2011; Jennings et al. 2016). Tens of thousands of *T. planipennisi* have been released for EAB management in central Kentucky forests and urban areas (Davidson and Rieske 2016; Graziosi and Rieske 2017). Host finding by *T. planipennisi* appears reliant on vibrational cues (Ulyshen et al. 2011; Chen et al. 2015), whereas olfactory cues appear less important (Chen et al. 2015). But the effectiveness of *T. planipennisi* in regulating EAB populations is limited by its short ovipositor and inability to penetrate thick bark of larger trees (Abell et al. 2012), and an apparent phenological asynchrony in portions of EAB's invaded range (Duan et al. 2014; Duan et al. 2015).

Host range studies following EAB's initial discovery suggested that all North American ash species were susceptible, and that EAB development in North America would be confined to *Fraxinus* species (Anulewicz et al. 2008; Rebek et al. 2008; Lyons et al. 2009). Although North American blue ash *F. quadrangulata* appears to possess some resistance (Tanis and McCullough 2012; Spei and Kashian 2017), it lacks the level of resistance found in Manchurian ash *F. mandshurica*, the co-evolved ancestral host of EAB (Whitehill et al. 2012; Peterson et al. 2015). In the initial host range studies, other common members of the Oleaceae were evaluated, deemed unsuitable, and determined to be of no concern in the invasion dynamics of EAB in North America (Anulewicz et al. 2008; Lyons et al. 2009).

In a surprising turn of events, in 2015 EAB was reported completing its development on another Oleaceous host, white fringetree *Chionanthus virginicus*, in south-central Ohio (Cipollini 2015). Utilization of this novel host appears to have co-

occurred with the initial EAB invasion in central Ohio (Thiemann et al. 2016) but went unnoticed. Following the initial detection of EAB in white fringetree in Ohio, EAB was soon discovered on fringetree in other areas including Lexington, KY. In spring of 2016 an adult beetle was recovered emerging from plant material collected in fall 2015 on the University of Kentucky campus.

White fringetree is a small tree or shrub with dramatic white flowers, native to the southeastern United States (Missouri Botanical Garden 2017). Horticultural varieties are widely planted as ornamentals throughout much of the range of North American *Fraxinus*, and in many areas where EAB has already invaded (USDA APHIS Map 2018). Interestingly, *C. retusus*, an Asian congeneric of *C. virginicus* that shares an ancestral native range with EAB, is resistant to the beetle (Cipollini and Rigsby 2015).

Cipollini and Rigsby (2015) determined that EAB is able to complete its life cycle from egg to adult on white fringetree. However, a full understanding of how this novel host impacts EAB development, how the novel host will affect efficacy of biological control agents, and what the consequences will be for EAB invasion dynamics in North America is lacking. Therefore, I compared host utilization by EAB of two common ash species, white and blue, *F. americana* and *F. quadrangulata*, respectively, with white fringetree, and evaluated plant characteristics that could contribute to differences in host suitability. Secondly, I evaluated the ability of *T. planipennisi* to locate and utilize EAB within white fringetree, and further evaluated parasitoid responses to olfactory and visual stimuli associated with both ash and fringetree. Because *T. planipennisi* is reliant primarily on vibrational cues to locate its EAB host within infested ash (Ulyshen et al. 2011), I hypothesized that the novel host plant would compromise EAB location by *T.*

planipennisi and contribute to enemy free space for EAB populations, with potential consequences for the EAB invasion.

Methods

EAB Development

Two white ash, two blue ash, and four stems of white fringetree, were harvested on June 21, 2016 from trees in Fayette Co. KY for bioassays to compare EAB larval survival, growth, and cambial tissue consumption. Upon removal stems were placed immediately in tap water to limit desiccation, and in the laboratory they were sectioned into 30 cm lengths and labeled. Stems with signs of emerald ash borer or other damage were discarded. Since stems varied in diameter (2–5 cm) both within and between species, bolts of each species were grouped into large and small size classes.

Twenty-four hours later, five EAB eggs attached to pieces of paper coffee filters (Kroger, Cincinnati OH) were evenly spaced along the length of each experimental bolt approximately 5 cm from one end, and secured using 2.5 cm wide strips of parafilm (Bernis NA, Neenah, WI) (Duan et al. 2013). One comparably sized bolt of each species (white ash, blue ash, and white fringetree) infested with five EAB eggs was randomly selected and placed in 4 cm of sand moistened with tap water in $13.5 \times 13.5 \times 30$ cm transparent plastic containers (OXO, NY, NY) and covered with fine mesh secured by a rubber band. There was one bolt of each species infested with five EAB eggs in each container, for a total of 39 bolts in 13 containers (N = 195 eggs).

After 6 weeks, each bolt was carefully debarked using a knife and the fate of each individual EAB larva was recorded. All living EAB larvae were collected and aged by

measuring head capsule width (Wang et al. 2005). Larval galleries were measured by wrapping each experimental bolt in translucent plastic and tracing over the galleries with a black marker. Gallery tracings were digitally photographed and ImageJ (Rasband NIH) software was used to calculate the total area of cambial tissue consumed on each bolt.

Host Plant Characteristics

Host characteristics that may affect host suitability for EAB were assessed by evaluating nitrogen and carbon content of the cambium and measuring stem density. For nitrogen and carbon analysis, three samples were collected from six trees of white ash and fringetree, and four trees of blue ash using a 10 mm cork borer to penetrate through the bark, removing a plug of tissue. Bark was removed from sample plugs using a razor blade, leaving only cambial and phloem tissue, which was then weighed, dried at 40°C for 3 days, and analyzed using a combustion assay (Flash Elemental Analyzer 1112 Thermo Fisher Scientific Inc., Waltham, MA). All trees showed no signs of EAB attack or dieback.

To evaluate wood density (density = mass · volume⁻¹), four stems of similar diameter of each species were sectioned into 3 cm pieces. To quantify the volume of each 3 cm section (N = 12 per species), the amount of water displaced in a 100 mL beaker per section was used to determine total volume using Archimedes Principle ($V_{\text{object}} = \text{mass of water displaced, given Density}_{\text{H}_2\text{O}} = 1.0$). Samples were dried at 45°C for 24 h. Mass was then measured (Mettler Toledo AB204) and divided by volume to calculate density for each stem section.

Host Plant Response to Wounding

Host response to wounding *in situ* normally caused by EAB larvae feeding, was simulated by measuring callus tissue formation following mechanical wounding using a 1 cm diameter cork borer and hammer (Dunn et al. 1990). Six stems of white ash and fringetree, and four of blue ash were selected from sites in Fayette Co., KY, and three wounds were made through the bark and into the cambial and phloem tissues at a height of 1 m around the stem circumference, separated by 120°. Wounds were then digitally photographed with a 12 megapixel camera at a distance of 10 cm at 4 week intervals from April through October. Photographs of the wounds and associated callus tissue formation were quantified using ImageJ (Rasband NIH), and compared within and between plant species.

Host Plant Effects on a Classical Biological Control Agent

Choice test: Bolts of white ash, blue ash, and white fringetree were infested with EAB as described above, and one bolt of each species were placed in 13.5 × 13.5 × 30 cm assay containers (N = 6). After 5 weeks to allow for adequate EAB larval development, four mated female *T. planipennis* were introduced into each assay container, which were placed in a growth chamber at 25°C and 14:10 L:D. After 6 days, the wasps were removed and bolts were held at 25°C for an additional 21 days to allow for maturation of parasitoid larvae. The bolts were then debarked to quantify parasitization, and all intact larvae of EAB were measured.

No-choice test: Nine white fringetree were located in Lexington KY, and three stems of each were inoculated with eight EAB eggs as described above. In the field eggs were

covered with fine mesh secured with duct tape to prevent predation. After 6 weeks to allow EAB egg hatch and larval development, cages of fine mesh were built around each intact, infested stem, and two mated female *T. planipennisi* were introduced. Females were fed a diluted honey mixture every ~48 hours. Assays were monitored for 21 days, after which the stems were cut and surviving female wasps were collected. Stems were debarked, larvae measured, and all mortality of larvae recorded.

Responses to olfactory and visual cues

Olfactory and visually-mediated responses of *T. planipennisi* were evaluated using a glass Y-tube olfactometer approximately 27 cm in length in combination with excised plant material and EAB larvae (Graziosi and Rieske 2013). Air was pumped through activated charcoal, a HEPA-CAP 36 microfiber filter (Whatman: Clifton NJ, USA), and deionized water at a rate of 0.05 Liters/Minute, before being introduced to the odor sources. The Y-tube setup was contained in a box which minimized ambient light and provided a steady even light source from two 15 watt fluorescent bulbs. Ambient temperature was raised to 27°C with the inclusion of a heating pad (K+H Manufacturing, Colorado Springs, CO). Female, mated *T. planipennisi* were obtained from a USDA APHIS rearing facility via overnight shipment, then placed into transparent containers with honey until use. Wasps were approximately one week in age. For each trial a naive *T. planipennisi* female was introduced to the system and allowed 10 minutes to make a choice by moving at least 3 cm up one arm; each completed trial consisted of 20 female wasps that made a definitive choice. Methodology was adapted from (Graziosi and Rieske 2013; Chen et al. 2016) Used wasps were discarded after trials were completed

and all trials were run between 0800 and 1700 hours EDT from November 2017 to February 2018. Y- tubes were switched out after every trial and washed in acetone and dried completely before reuse. Wasps were introduced to the system via a central stem. Tissue samples and blanks within the system were also changed with respect to location on a regular basis.

The olfactory stimuli used were excised stem tissue from green ash, *F. pennsylvanica*, excised stem tissue from white fringetree, or EAB larvae that had been removed from white ash and rinsed in deionized water. Excised stem tissue was obtained by cutting small sections of small diameter branches ~1 cm, with a pair of sanitized shears. All materials were collected in Lexington KY on the morning of the assay. Each of these olfactory stimuli was evaluated against a blank in separate assays.

Visual stimuli consisted of ash leaves collected daily from greenhouse-grown green or tropical ash *F. uhdei*, based on the availability of material in winter months. Visual stimuli were placed along the outside wall of the Y-tube so that they were not visible from the opposite arm, which contained no scent cues.

Olfactory stimuli of excised ash and fringetree stems were combined with the visual stimuli of ash leaves against a blank. Due to limitations in tissue availability, tropical ash leaves were combined with green ash, and a separate assay combined the visual cue with fringetree tissue. A trial was considered complete when 20 female wasps had made a choice.

Statistical Analyses

The effects of host plant (white ash, blue ash, white fringetree) on EAB egg hatch, larval survival and development, and EAB host utilization (cambium consumption) were evaluated using a 1-way randomized analysis of variance (SAS 9.3); a Tukey-Kramer test was used to evaluate differences among means.

To evaluate cambial carbon and nitrogen content and stem density as measures that might affect host suitability among tree species, the mean of three values was calculated for each tree and a one-way ANOVA was performed to evaluate differences. A Tukey-Kramer test was performed to evaluate differences between means. Host plant response to wounding was analyzed by comparing wounds and callus tissue formation over time, and examining simple effects of species at each time point. Tukey-Kramer tests were employed for multiple comparisons. Because EAB parasitization in both the choice and no-choice assays was low, rates were not evaluated statistically. Parasitoid responses to olfactory and visual stimuli were assessed using a chi square analysis, assuming an expected outcome of 50% for each choice.

Results

EAB egg hatch was approximately 60% and did not differ between the three tree species (white ash = 61.5 ± 4.8 ; blue ash = 56.9 ± 5.5 ; fringetree = 63.1 ± 3.8). However, larval survival was greatest on white ash and lowest on fringetree; blue ash was intermediate and did not differ between the two (Fig. 2.1a). Larval growth, as measured by head capsule width, was greater for EAB reared from *Fraxinus* compared to those reared from *Chionanthus*, but there was no difference in larval growth between the two

ash species (Fig. 2.1b). Similarly, cambium consumption was lowest for EAB reared on *Chionanthus* (Fig. 2.2) but the ash species did not differ from one another.

Carbon content differed among the three tree species, and was highest in fringetree and lowest in blue ash (Table 2.1), but neither nitrogen or C:N differed among the three. Wood density was also highest in fringetree (Table 2.1), with no difference between the two ash species.

There was no difference among the three species in their responses to wounding, as measured by callus tissue formation, 4 weeks following the wounding event (Fig 2.3). However, the rate of callus tissue formation in blue ash and fringetree began increasing relative to white ash, and by week 8 callus tissue formation in white ash diverged significantly from that of blue ash and fringetree. Formation of callus tissue in white fringetree differed from white ash at 8, 12, and 16 weeks, with blue ash values not significantly different from white fringetree.

In the choice assay with *T. planipennisi*, 33% (6 of 18) of the bolts available with EAB larvae were parasitized. All EAB parasitization occurred in *Fraxinus*, with 80% occurring in blue ash, 20% in white ash, and none in white fringetree. In the no choice assay there were no signs of EAB parasitism in fringetree indicated by parasitized larva. Although EAB larval mortality was extremely high (85%), all larvae recovered were at the critical 3rd or 4th instar, when they are highly susceptible to *T. planipennisi* parasitization (Duan et al. 2011). Regardless, the parasitization rate of EAB in white fringetree was zero.

In my assessment of olfactory and visual responses, *T. planipennisi* females responded positively to ash tissue odor combined with an ash leaf visual cue, though the

response was only weakly significant ($P = 0.07$). *Tetrastichus planipennisi* did not respond positively to ash tissue alone, the visual cue alone, an EAB larva, or fringetree tissue alone. Interestingly, a combination of fringetree odor with a visual cue of ash leaves yielded a strong preference for the blank control (Figure 2.4), suggesting repellency.

Discussion

Adaptations to exploit additional host plants is just one mechanism whereby an insect herbivore can increase its invasibility. Host range expansion potentially increases the abundance and/or prevalence of available host material, which in turn increases the likelihood of geographic range expansion (Sax et al. 2007). Emerald ash borer, an invader of unprecedented impact (Herms and McCullough 2014), has been reported completing its development in the novel host white fringetree, effectively increasing its reported host range in North America (Cipollini 2015; Peterson and Cipollini 2017). The extent to which this increase in host options could influence the EAB invasion remains unknown.

An additional mechanism by which non-native organisms increase their invasiveness is by exploiting enemy free space (Gratton and Welter 1999). In the case of EAB, lack of effective natural enemies was key to its initial invasion success in North America (MacQuarrie and Scharbach 2015), and classical biological control has subsequently been the focus of concerted management efforts (USDA APHIS 2010; Duan et al. 2013; Davidson and Rieske 2015). I evaluated EAB utilization of white fringetree in the context of its effectiveness as a larval host plant and to evaluate its influence on enemy free space, focusing on *T. planipennisi*, a central component of

classical biological control efforts. I sought to more fully understand the relationship between EAB and its novel white fringetree host, and to determine whether *T. planipennisi* can locate and utilize larval EAB developing in fringetree relative to those developing in ash hosts. Ultimately my goal is to evaluate how expansion of the host range through utilization of white fringetree may affect EAB invasion dynamics in eastern North America.

I confirm previous findings that EAB developing in white fringetree shows a lower overall performance than EAB reared in *Fraxinus* hosts (Cipollini and Rigsby 2015), and equate these findings to nutritional and physical attributes of the host plants. Insect growth is closely tied to the nitrogen content of host material (Mattson 1980). My analyses of N and C:N ratios from within cambial tissue suggest that nitrogen is unlikely to be contributing to the differences we observed in insect performance, since neither N or C:N levels differed among the three hosts tested.

My analysis did demonstrate, however, that white fringetree contains higher levels of carbon in its cambial tissue, has greater stem density, and also has a more rapid response to wounding than does the highly preferred white ash, all of which could contribute to EAB's relatively poor performance in white fringetree. Higher overall stem density and a rapid response to wounding could potentially impede gallery formation and slow larval development. Studies on the closely related bronze birch borer, *A. anxius*, which has a life history and feeding habits similar to EAB (Muilenburg and Herms 2012), have shown that rapid wound responses in hosts slows larval feeding (Muilenburg et al. 2013). These studies also suggest that a rapid wound response could help hosts encapsulate feeding larvae, thus leading to failed emergence and insect mortality (Miller

et al 1991; Muilenburg et al. 2013). The higher stem density and more rapid wound response in white fringetree could act as defense mechanisms against EAB. Rapid response to wounding equates to faster plant growth and may aid in defending plants, in lieu of metabolically expensive chemical defenses (Loehle 1988).

Chemical defenses may also play a role. *Fraxinus* spp. are rich in constitutive and induced phenolic defenses (Villari et al. 2015) which may affect insect development. While minor differences in chemical defenses are apparent in North American ash, they share a number of defensive compounds. North American ash with the exception of blue ash, are all classified as highly susceptible to EAB. To date there has been no research on white fringetree; clearly this warrants further investigation (Cipollini et al. 2011; Hill et al. 2012; Villari et al. 2015).

Parasitoids form the basis of the classical biological control program for EAB in North America (McCullough and Herms 2014). Parasitoids require input from multiple sensory systems to locate their hosts, and they use various cues to locate cryptic hosts in situations where they would otherwise remain hidden (Vinson 1976; Vinson and Williams 1991). Such is the case for parasitoids locating host insects feeding within novel host plants. While olfactory cues may be involved, tactile and vibratory cues are likely the most significant for *T. planipennisi* locating EAB larvae feeding in ash (Ulyshen et al. 2011; Chen et al 2015). Given that stem density and carbon content are greater in fringetree relative to ash, these differences could affect tactile and vibratory properties of the plant, which in turn could present significant barriers for *T. planipennisi* host seeking behavior. *Tetrastichus planipennisi* has successfully established and readily disperses from release points (Duan et al. 2013), and shows promise in regulating EAB populations

in regenerating forests following extensive EAB-induced ash mortality (Margulies et al. 2017). However, our findings suggest that the efficacy of *T. planipennisi* in EAB-infested white fringetree is questionable. *Tetrastichus planipennisi* appears unable to recognize EAB larvae within fringetree, suggesting that creation of enemy free space may be one mechanism by which utilization of white fringetree could facilitate EAB's range expansion into new hosts. This release from a key population regulator could allow EAB to further exploit this novel host, and further complicate future management efforts in North America.

I confirm previous findings that white fringetree is a novel, yet suboptimal, host for EAB (Cipollini 2015; Cipollini and Rigsby 2015), and suggest potential mechanisms that may be at play. The relatively high survival of EAB on white fringetree in the laboratory (~65%, see Fig. 2.1a) suggests that it will not function as a dead-end host for EAB populations, but may provide an exploitable alternative host source. Given its ability to complete development from egg to adult, EAB has gained a reservoir host that may play a role in its invasion dynamics in North America by freeing it from dwindling ash hosts and availability of suitable host material. My data that suggest that by adopting and utilizing this novel host, EAB may reduce pressure from a notable natural enemy, further contributing to its invasibility. My results confirm that *T. planipennisi* is not attracted solely to ash phloem odors (Ulyshen 2011), but suggest that a combination of odor and visual cues may be important in host location for *T. planipennisi*. Interestingly, the combination of fringetree olfactory cues and visual stimuli that seemingly generate repellency of *T. planipennisi* to white fringetree support my observed lack of EAB

parasitization within this novel host plant. These findings are puzzling and clearly warrant further investigation.

The invasion of North America by EAB is unprecedented in scope and magnitude (Herms and McCullough 2014), and continues to grow in complexity. My study sheds light on how expansion of its host range seemingly generates further enemy free space, contributing to EAB's invasiveness and altering previous management plans.

Additionally, yet another oleaceous plant, olive, *Olea* spp., has been added to an expanding host list for EAB (Cipollini et al. 2017) raising concerns about the far-reaching implications of this invasive pest. Furthermore, an Asian conspecific of EAB, *A. smaragdifons*, has reportedly established in eastern North America (Hoebeke et al. 2017).

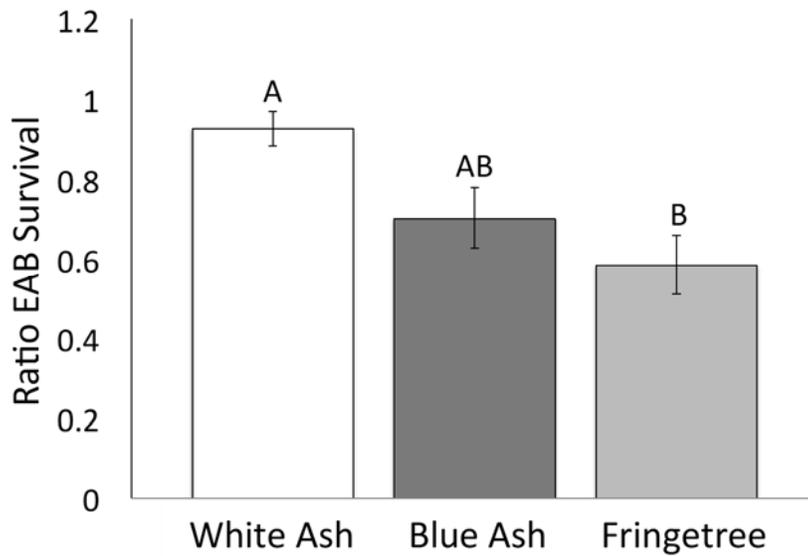
These confounding factors are likely to further contribute to the economic and ecological costs associated with the EAB invasion in North America and perhaps globally.

Table 2.1. Characteristics of white ash, blue ash, and white fringetree (N = 16) evaluated for suitability for developing emerald ash borer larvae. Means (SE) within rows followed by the same letter do not differ ($P < 0.05$). Tukey-Kramer Test

Parameter	White			F _{df} /P
	White ash	Blue ash	fringetree	
Carbon (%)	40.2(16.4) b	38.9(19.5) c	42.9(17.5) a	F _{2,15} = 83.9/ 0.001
Nitrogen (%)	0.43(0.19) a	0.50(0.25) a	0.50(0.20) a	F _{2,15} = 1.27/ 0.317
C:N	94.4(38.6) a	78.2(39.1) a	88.7(36.2) a	F _{2,15} = 3.89/ 0.210
Density (g/cm ³)	0.53(0.15) b	0.50(0.15) b	0.60(0.17) a	F _{2,11} = 9.10/ 0.002

Figure 2.1. Performance of emerald ash borer larvae after 35d in bolts (N = 13) of white ash, blue ash and fringetree artificially infested with emerald ash borer eggs, showing a) survival [$F_{2,115} = 5.10, P < 0.01$] and b) growth, as measured by head capsule (N=39) width [$F_{2,34} = 10.20, P = 0.0001$]. Means followed by the same letter do not differ ($\alpha = 0.05$). One way randomized ANOVA, Tukey-Kramer Test.

a)



b)

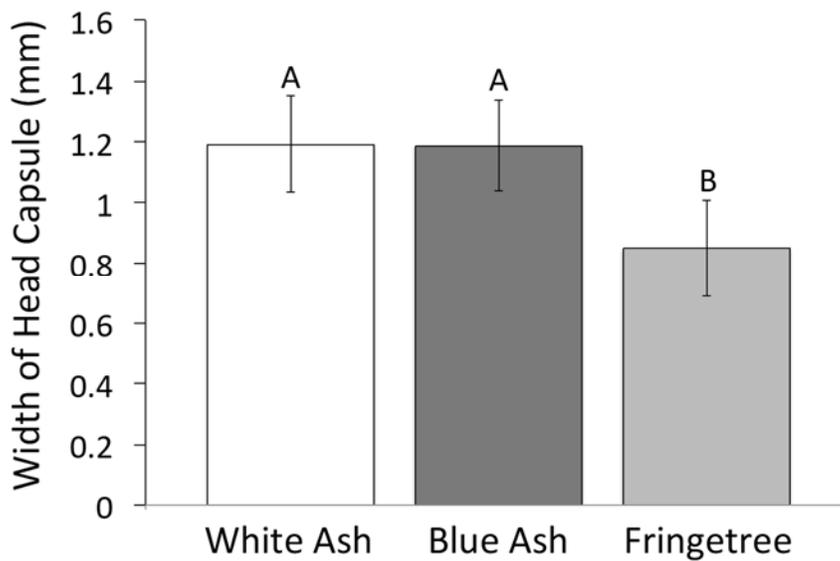


Figure 2.2. Phloem consumption by emerald ash borer larvae in artificially infested bolts of white ash, blue ash, and fringetree (N = 13) after 35 days [$F_{2,115} = 6.54$, $P < 0.01$]. Means followed by the same letter do not differ ($\alpha = 0.05$). One way randomized ANOVA, Tukey-Kramer Test.

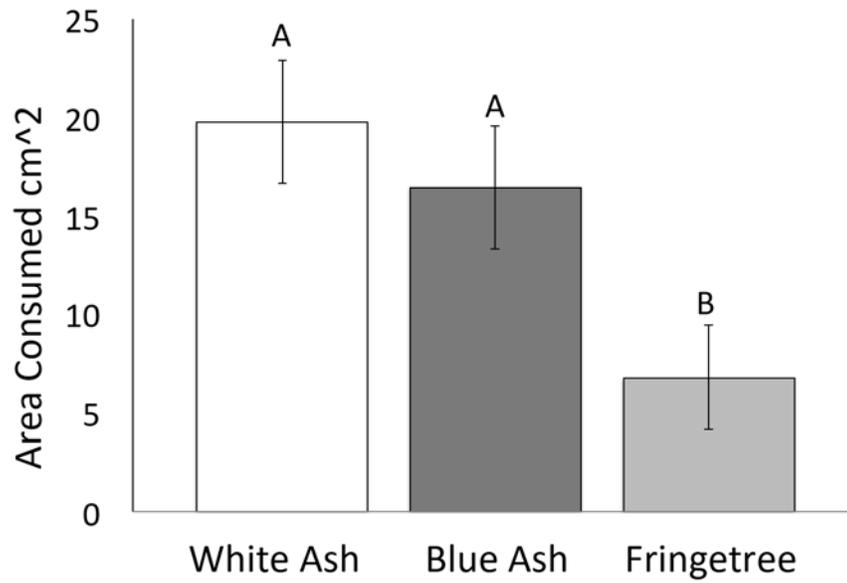


Figure 2.3. Callus tissue formation after artificial wounding on white ash, blue ash, and white fringetree (N = 12) at 4, 8, 12, and 16 week intervals [$F_{6,39} = 8.77, P = 0.001$].

Means followed by the same letter do not differ ($\alpha = 0.05$).

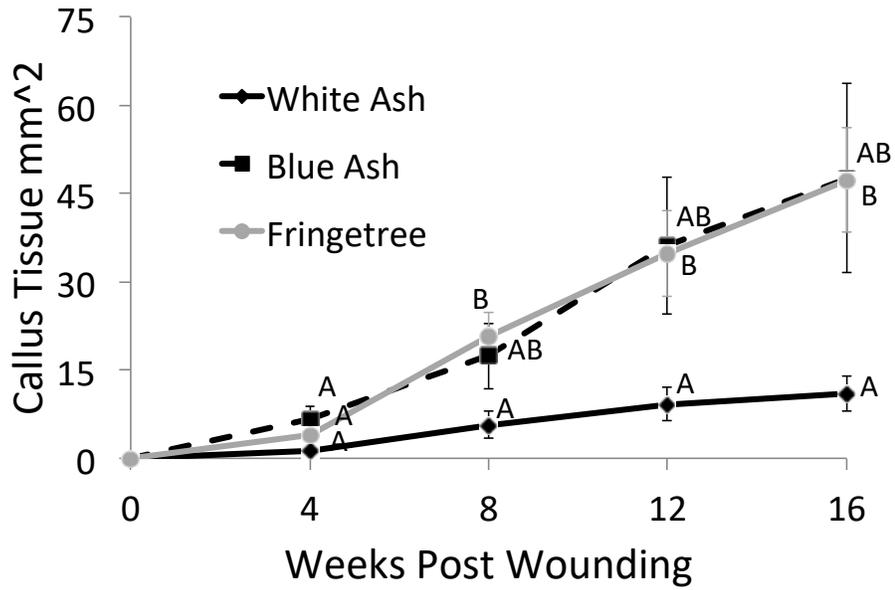
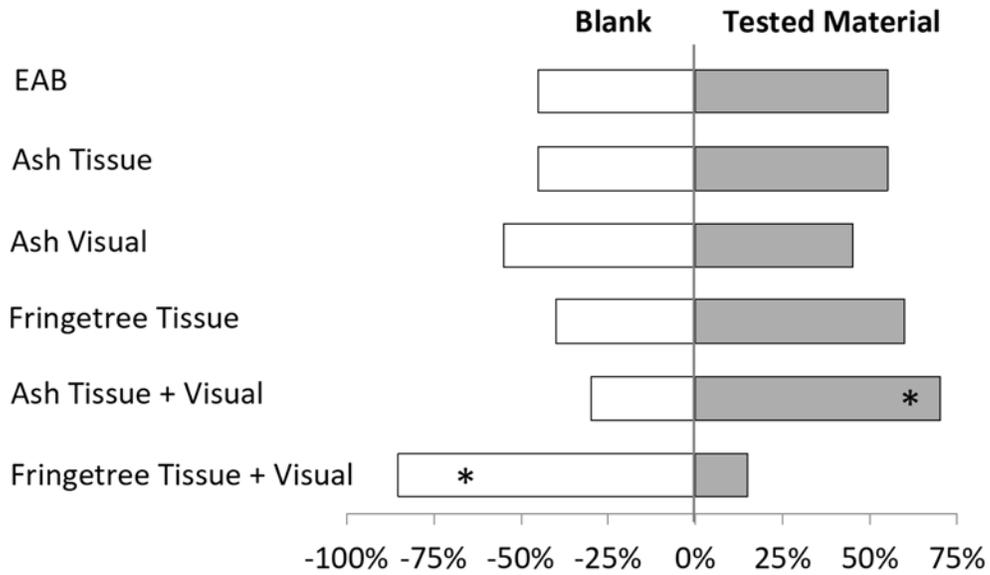


Figure 2.4. Y-tube choice test results for *T. planipennisi*. N = 20 for all trials, difference indicated by *. Ash tissue + visual cue: P = 0.07; fringetree tissue + visual cue: P = 0.001.



APPENDIX A

Another look at systemic insecticide applications for emerald ash borer suppression

Introduction

Emerald ash borer, *Agrilus planipennis* (EAB, Coleoptera: Buprestidae), an invasive pest of forest, shade, and ornamental ash, *Fraxinus* spp., has caused extensive tree mortality throughout its invaded range in North America (APHIS 2018), and is expected to cause \$10 billion in losses by the year 2019 (Kovacs et al. 2011).

EAB produces one generation per year. In summer, adults mate and females oviposit about 70 eggs on stems and large branches of ash hosts (Rutledge and Keena 2012). Eggs hatch and larvae feed on phloem, creating serpentine galleries beneath the bark. Mature larvae overwinter before pupating and emerging as adults the following summer (Cappaert et al. 2005). In high concentrations, larval feeding girdles trees and causes rapid tree mortality (Herms and McCullough 2014) (Figures A.1a; A.1b).

All North American ash are susceptible to EAB colonization (Liu et al. 2007), though white and green ash, *F. americana* and *F. pennsylvanica*, are highly preferred, and blue ash, *F. quadrangulata*, has some putative resistance (Tanis and McCullough 2012; Spei and Kashian 2017). In the eastern US, including Kentucky, ash is a significant component of wildland forests (Wharton and Barbour 1973) and is also prevalent as a street, park, and landscape tree. Because of its pervasiveness in urban and wildland forests, efforts at EAB management in the US have focused on classical biological control (Duan et al. 2012). Four hymenopteran parasitoids, *Spathius agrili* (Braconidae), *S. galinae* (Braconidae), *Tetrastichus planipennisi* (Eulophidae), and *Oobius agrili*

(Encyrtidae) discovered in EAB's native range of China, have been intensively screened and are now laboratory reared in the US for incorporation into biological control releases throughout the invaded range. Additionally, endemic natural enemies have been recruited to emerald ash borer-invaded forests (Duan et al. 2013; Davidson and Rieske 2016; Savage and Rieske 2018), and may be helping regulate EAB populations.

Classical biological control has had some success (Duan et al. 2011), and has also been deployed in concert with chemical suppression, again with some success.

Theoretically, interspersing insecticide-protected trees throughout an EAB-infested area could facilitate establishment of introduced biological control agents and lead to slower ash mortality (McCullough and Mercader 2012; Davidson and Rieske 2016; Graziosi and Rieske 2017). However, the only way to assure protection of individual trees of North American ash from EAB is through chemical means (McCullough et al. 2011).

Numerous insecticides were screened for efficacy against both larval and adult EAB following its initial discovery in the US (McCullough et al. 2005; Herms et al. 2009). Emamectin benzoate, an avermectin derivative applied through trunk injections at ~3 year intervals, is considered the gold standard (Smitley et al. 2010), and causes up to 100% EAB mortality. However, emamectin benzoate is a restricted use insecticide, requires specialized equipment to apply, and is expensive. As alternatives, imidacloprid applied as a soil drench on smaller diameter trees (< 38 cm dbh), or dinotefuran applied as a trunk spray, have been employed. Neither are as efficacious as emamectin benzoate, but still provide adequate protection from EAB mortality when applied annually (Herms et al. 2009; Smitley et al. 2015). They require no specialized equipment, are more accessible, and are therefore widely used. Both imidacloprid and dinotefuran are

translocated to the foliage, where emerging adult EAB encounter them as they undergo feeding prior to mating and oviposition (Mota-Sanchez et al. 2009). However, little is known about which plant tissues concentrate or retain these chemicals following treatments (Harrell 2006). These insecticides have also shown effective long lasting residues within other forest ecosystems such as hemlock (Benton et al. 2016). However insecticide rates and treatment frequency necessary for tree protection against EAB vary, and are influenced by tree size, canopy condition, site, soil conditions, and overall tree health. Consequently, there is some confusion among home owners and tree care specialists as to what application rates and frequency are optimal.

Unfortunately, as neonicotinoid insecticides, both imidacloprid and dinotefuran are under scrutiny due to concerns over their potential non-target effects. In particular, the effects of neonicotinoids on honey bees and other pollinators has caused alarm (Copping 2013; Goulson 2013; Vanbergen et al. 2013). Although deployment of imidacloprid soil drenches at label rates and lower for EAB management in forested situations has shown no negative effects on native hymenopteran abundance and diversity (Davidson and Rieske 2016), even short-term exposure to this chemical class has long-term implications on pollinator colony fitness (Blacquiere et al. 2012; Whitehorn et al. 2012; Larson et al. 2013). Additionally, several studies have noted significant use of ash pollen by bees early in the the growing season (Kraemer and Favi 2005; Richardson et al. 2015), raising concerns that pollinators may come into contact with systemic insecticides used for EAB suppression.

Given the scrutiny that neonicotinoids are under, it is essential that we have a full understanding of how they distribute in the plant following application. I focus here on

within-plant insecticide residues and effects on EAB survival for plants treated with imidacloprid and dinotefuran. Using imidacloprid soil drenches and dinotefuran trunk sprays applied at the full label rate and at one half the label rate, EAB survival was measured and insecticide residues were assessed within ash tissue. Specifically, the objectives were to assess: 1) tissue-specific insecticide concentrations in ash trees 5 weeks after application, 2) insecticide concentrations in trees receiving applications at label rates and at one half label rates, and 3) differences in effectiveness for EAB suppression.

Methods

This work was conducted at Taylor Fork Ecological Area, a 24.3 ha abandoned pasture in Madison Co., KY, situated at the interface of the Outer Bluegrass and Eastern Knobs Regions of Kentucky. Ash thrive on the moist and fertile soils that predominate in the Bluegrass Region (Campbell 1989), and were historically a significant component of these forests (Wharton and Barbour 1973). Taylor Fork is owned and administered by Eastern Kentucky University, and is characterized by open cane fields, trees in abandoned fencerows, small patches of early- to mid-succession woodlands, and sporadically occurring, large, open-grown trees with areas of dense regeneration. At the onset of the study EAB was present in Madison Co. but was not yet reported at Taylor Fork, and ash present showed no signs of EAB-induced stress. In summer 2016, the large ash at Taylor Fork were treated prophylactically with bark sprays of dinotefuran applied at label rates to protect against EAB.

In spring 2017 three discrete sites were established >1000 m apart, separated by distinct topographic features. Within each site three plots containing significant ash regeneration were designated, and in each plot five green ash trees (dbh ~5 cm) were selected for treatment. Using a randomized block design, selected trees in each plot received either: i) a soil drench of imidacloprid (Imidacloprid 2F Select T/I, 21.4% Imidacloprid: 1-N-nitro-2-imidazolidinimine, Prime Source LLC, Evansville IN, USA) applied at a rate of 2.64mL/ L of water, 0.94 L of total solution ('full rate'), ii) a soil drench of imidacloprid applied at a rate of 1.32mL/ L of water, 0.94 L of total solution ('half rate'), iii) a basal bark spray of dinotefuran (Safari 20SG, Valent, Walnut Creek, CA, USA) applied at a rate of 90 grams/ L of water ('full rate'), iv) a basal bark spray of dinotefuran applied at a rate of 45 grams/ L of water ('half rate'), or v) an untreated control (N=45 trees).

Seven days after treatment the trees were artificially infested with three laboratory reared emerald ash borer eggs at heights of 25 and 50 cm above ground level, for a total of six eggs per tree. Eggs were screened with fine polyester mesh to prevent predation, and larvae were allowed to hatch and develop for 30 days, after which plant material was processed.

For processing, plants were excavated, roots were clipped and placed in sample bags, and all foliage was removed from a single south-facing branch on each tree. Samples were then stored on ice for transport to the laboratory. The main stem of the tree was sectioned and phloem tissue from the top 12.7 cm was removed for chemical analysis; the remainder was used to evaluate EAB colonization success. Thus, for each

experimental tree there were root, phloem, and foliar samples to evaluate for insecticide residues, and stem sections to evaluate for EAB colonization.

Root, phloem, and foliar tissues were placed in liquid nitrogen and ground into a powder with a mortar and pestle, and stored at -20 °C prior to analysis. Samples from imidacloprid-treated trees were analyzed for the presence of imidacloprid and its metabolites, imidacloprid-olefin and imidacloprid-dihydroxy, and samples from dinotefuran-treated trees were analyzed for the presences of dinotefuran via liquid chromatography mass spectrometry (LC/MS) (Villanova University Dept of Chemistry). Limits of detection (LOD) were 1.36 ppb for imidacloprid, 1.41 ppb for imidacloprid-olefin, 6.69 ppb for imidacloprid-dihydroxy, and 0.43 ppb for dinotefuran.

When neonate larval emergence holes were evident, or larval galleries were present on the stem, EAB survival was scored as positive. Larval galleries were measured by tracing transparent film on each stem, then using ImageJ (Rasband NIH) software to quantify the amount of phloem tissue consumed by each larva.

Data were analyzed using Proc Glimmix (SAS 9.3) to determine differences in insecticide residues based on application rate (full versus half dose), as well as differences based on plant tissue (root, stem, and foliage). A one-way ANOVA was used to evaluate differences in total EAB phloem consumption (gallery length) in ash stems treated with full and half dose imidacloprid and dinotefuran, compared to controls. Differences were assessed using Tukey's HSD.

Results

There were no differences in insecticide residues in ash leaves, stems, or roots based on insecticide dose (full or half) for either imidacloprid (Table A.1a) or dinotefuran (Table A.1b). Likewise, there was no difference between plant tissues in concentrations of imidacloprid-olefin, a metabolite of imidacloprid that has shown to be efficacious in suppressing hemlock woolly adelgid, *Adelges tsugae* (Benton et al. 2016) (Table A.1c). Concentration of insecticide residue in stem tissue was below the limit of detection in several cases, effectively rendering them as a value of 0. Imidacloprid-dihydroxy, another metabolite of imidacloprid, was not consistently found within samples or at significant levels (data not shown); it is thought to be of minimal importance for insect suppression (Benton et al. 2016). However, tissue-specific differences in insecticide concentrations were evident (Table A.2). Ash root tissue had significantly higher levels of imidacloprid than either the stem and leaf tissues, while the leaf and stem tissues did not differ significantly from one another (Table A.2).

In trees treated with dinotefuran applied at the full label rate, leaf and root tissue had significantly higher levels of insecticide residue than did the stem tissue (Table A.2), but there were no differences in insecticide residues among tissue types in trees treated at half the label rate.

EAB success, measured by total gallery length, did not differ between full and half label rates of either insecticide. However, all treatments showed significantly lower rates of phloem consumption than the untreated control trees (Figure A.2).

Discussion

Systemic neonicotinoid insecticides, including imidacloprid and dinotefuran, are effective at protecting ash trees from EAB (Herms et al. 2009; Smitley et al. 2015) and are also efficacious against other forest pests (Faulkenberry et al. 2012; Benton et al. 2016). Woody plants translocate neonicotinoids from the point of application to the leaves, where it acts as a strong antifeedant (Tanis et al. 2012; Poland et al. 2015).

Both imidacloprid and dinotefuran have been shown to have lethal effects on emerald ash borer larvae (Poland et al. 2015). This study found concentrations of the insecticides in stem phloem tissue were low, consistent with previous studies. Tanis et al. (2012) suggested that more insecticide may be present in the xylem tissue as opposed to the phloem, but may leach outwards where it is encountered by feeding larvae.

This study found that the neonicotinoid insecticides that were tested were effectively translocated to foliar tissue, regardless of label rate (full or half dose), type (imidacloprid or dinotefuran), or site of application (soil drench or trunk spray). It was also found that insecticides applied at one half the label rate were effective in reducing EAB phloem consumption. Larval consumption did not differ between full and half rates of either insecticide (Fig. A.2), suggesting that homeowners and land managers utilizing these products for EAB management could potentially reduce costs by reducing insecticide rates.

Imidacloprid is metabolized by plants into by-products with varying toxicities (Nauen et al. 1998), including imidacloprid-olefin and imidacloprid-dihydroxy. Olefin has demonstrated efficacy against several pests with piercing sucking mouthparts, including aphids (Nauen et al. 1998) and adelgids, and reportedly is found in imidacloprid-treated

eastern hemlock, *Tsuga canadensis*, contributing to suppression of hemlock woolly adelgid, *Adelges tsuga* (Benton et al. 2016). This study also found high levels of olefin in imidacloprid treated green ash, suggesting that olefin may contribute to EAB larval suppression, though this was not directly evaluated. The inability to detect dihydroxy in green ash suggests that it is unimportant in the green ash – EAB system.

Reducing rates of neonicotinoids applied for EAB control could have added benefits. This class of insecticides has adverse effects on non-target organisms, most notably pollinators (Blacquiere et al. 2012; Whitehorn et al. 2012), and lower rates of application could reduce these unwanted effects (Karahan et al. 2015). These results suggest that applying these insecticides at lower doses than those recommended by the manufacturer may be effective for EAB suppression, and additionally pose lower risks to the ecosystem.

Although there has been no negative effect associated with chemical treatment of ash trees, this research shows lower doses of systemic insecticides may be effective at controlling emerald ash borer larvae. This will reduce costs and reduce potential risks of effects to non-target species. These results can assist land managers in making more informed decisions about ash treatment as the emerald ash borer continues to devastate both urban and wildland forests throughout North America.

Table A.1. Insecticide residues (ppb) 5 weeks post-treatment in leaf, stem, and root tissues of ash trees treated with A) imidacloprid applied as a soil drench at full label rate (2.64 mL · L⁻¹ H₂O) and one half label rate (1.32 mL · L⁻¹ H₂O), B) dinotefuran applied as a trunk spray at full label rate (90 g · L⁻¹ H₂O) and one half label rate (45 g · L⁻¹ H₂O). Means within tissue type within insecticide treatments followed by the same letter do not differ (Tukey's HSD, $\alpha = 0.05$), and c) imidacloprid-olefin, a metabolite of imidacloprid, applied as a soil drench at full label rate (2.64 mL · L⁻¹ H₂O) and one half label rate (1.32 mL · L⁻¹ H₂O).

a) Imidacloprid			
Ash tissue type	Full Rate	Half Rate	F _{df} / P
Leaf	54.09 (12.59) a	50.71 (18.64) a	F _{2,17} = 0.04/ 0.96
Stem	15.03 (1.98) a	9.28 (2.82) a	F _{2,17} = 0.08/ 0.94
Root	406.22 (115.04) a	371.81 (120.22) a	F _{2,17} = 0.46/ 0.65
b) Dinotefuran			
Ash tissue type	Full Rate	Half Rate	F _{df} / P
Leaf	5.12 (1.28) a	4.09 (1.14) a	F _{2,17} = 0.01/ 0.99
Stem	0.12 (0.12) a	0.13 (0.13) a	F _{2,17} = 0.00/1.0
Root	5.37 (1.46) a	7.08 (3.45) a	F _{2,17} = 0.02/ 0.98
c) Imidacloprid olefin			
Ash tissue type	Full Rate	Half Rate	F _{df} / P
Leaf	32.10 (16.41) a	2.45 (1.73) a	F _{2,17} = 3.23/0.09
Stem	6.08 (4.13) a	1.26 (1.26) a	F _{2,17} = 1.25/0.25
Root	7.63 (3.45) a	1.92 (1.92) a	F _{2,17} = 2.09/0.17

Table A.2. Insecticide residues (ppb) in ash foliage, stem, and root tissues 5 weeks following applications of imidacloprid applied as a soil drench and dinotefuran applied as a bark spray. Both treatments of imidacloprid showed significantly higher levels in root tissue. However, detectable amounts were present in all tissues. Dinotefuran full rate showed significantly higher levels in both leaf and root tissue, while half rate was nearly significant in the same regard. Means within columns followed by the same letter do not differ ($\alpha = 0.05$).

Ash tissue type	Imidacloprid		Dinotefuran	
	Full Rate	Half Rate	Full Rate	Half Rate
Leaf	54.1 (12.6) a	50.7 (18.6) a	5.1 (1.3) a	4.1 (1.1) a
Stem	15.0 (2.0) a	9.3 (2.8) a	0.1 (0.1) b	0.1 (0.1) a
Root	406.2 (115.0) b	371.8 (120.2) b	5.4 (1.5) a	7.1 (3.5) a
	F _{2,24} = 10.4/ 0.01	F _{2,24} = 8.00/ 0.01	F _{2,24} = 7.00/ 0.01	F _{2,24} = 2.8/ 0.08

Figure A.1. Emerald ash borer larvae feed on phloem beneath bark, (a) creating serpentine galleries that expand as the larvae grow, (b) eventually coalescing and completely girdling the tree.

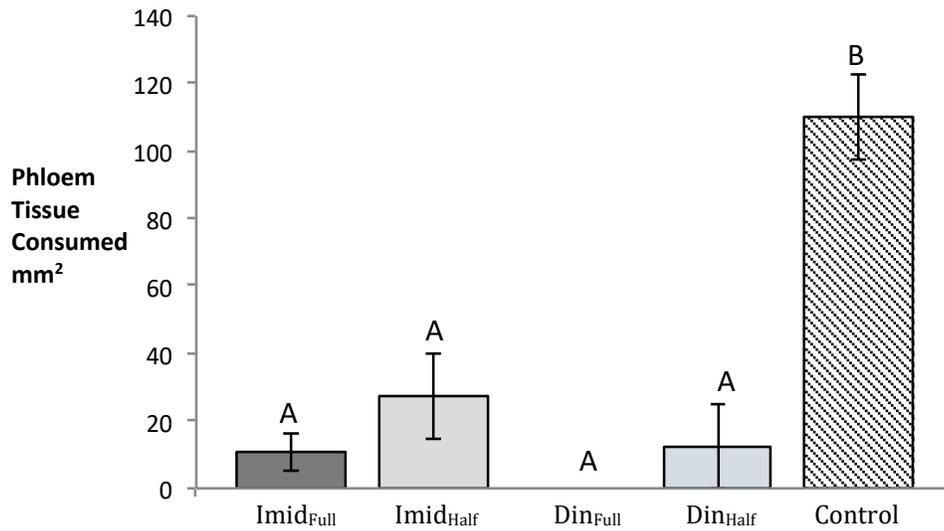
a)



b)



Figure A.2. Phloem tissue consumed (mean \pm s.e.) by EAB larvae developing in ash trees treated with full and half rates of imidacloprid (Imid_{Full}, Imid_{Half}) and full and half rates of dinotefuran (Din_{Full}, Din_{Half}), relative to an untreated control. Phloem tissue consumption in treated trees was lower than in untreated controls ($F_{4,40} = 15.76$, $P = 0.0001$), but there were no differences among treatments. Means followed by the same letter do not differ.



APPENDIX B

Location of sites where materials were collected

Blue Ash

Anderson County KY (38.0074, -84.9574)
Raven Run Nature Preserve (37.8849, -84.0412)

White Ash

Raven Run Nature Preserve, Fayette Co KY (37.8922, -84.3870)

Fringetree

University of Kentucky Campus (38.0367, -84.4997) –Rose Lane
(38.0350, -84.5042) – Chem Phys Bldg
(38.0278, -84.5082) – Garrigus Bldg
(38.0249, -84.5087) – Good Barn

University of Kentucky Arboretum (38.0140, -84.5033)
(38.0141, -84.4988)

McConnell Springs, Lexington KY (38.0557, -84.5280)

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Project Assistant, 2011-2015, Creek Connections, Allegheny College

Scholastic Honors

First Place Southern Forest Insect Work Conference Student Presentation, Melbourne FL,
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Professional Publications

Olson DG, & Rieske LK (In Review) Host range expansion may provide enemy free
space for the highly invasive emerald ash borer. *Biological Invasions*. Submitted March
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